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TOXICOLOGICAL REVIEW OF FORMALDEHYDE - INHALATION ASSESSMENT

(CAS No. 50-00-0)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

VOLUME I of IV

Introduction, Background, and Toxicokinetics

June 2, 2010

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U.S. Environmental Protection Agency
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LIST OF ABBREVIATIONS AND ACRONYMS

ACGIH	American Conference of Governmental Industrial Hygienists
ADAF	age-dependent adjustment factors
ADH	alcohol dehydrogenase
ADS	anterior dorsal septum
AGT	O^6-alkylguanyl-DNA alkyltransferase
AIC	Akaike Information Criterion
AIE	average intensity of exposure
AIHA	American Industrial Hygiene Association
ALB	albumin
ALDH	aldehyde dehydrogenase
ALL	acute lymphocytic leukemia
ALM	anterior lateral meatus
ALP	alkaline phosphatase
ALS	amyotrophic lateral sclerosis
ALT	alanine aminotransferase
AML	acute myelogenous leukemia
AMM	anterior medial maxilloturbinate
AMPase	adenosine monophosphatase
AMS	anterior medial septum
ANAE	alpha-naphthylacetate esterase
ANOVA	analysis of variance
APA	American Psychiatric Association
ARB	Air Resources Board
AST	aspartate aminotransferase
ATCM	airborne toxic control measure
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
ATS	American Thoracic Society
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the curve
BAL	bronchoalveolar lavage
BALT	bronchus associated lymphoid tissue
BBDR	biologically based dose response
BC	bronchial construction
BCME	bis(chloromethyl)ether
BDNF	brain-derived neurotrophic factor
BEIR	biologic effects of ionizing radiation
BfR	German Federal Institute for Risk Assessment
BHR	bronchial hyperresponsiveness
BMC	benchmark concentration
BMCL	95% lower bound on the benchmark concentration
BMCR	binucleated micronucleated cell rate fluoresce
BMD	benchmark dose
BMDL	95% lower bound on the benchmark dose
BMR	benchmark response
BN	Brown-Norway
BrdU	bromodeoxyuridine
BUN	blood urea nitrogen
BW	body weight
CA	chromosomal aberrations
CalEPA	California Environmental Protection Agency
CAP	College of American Pathologists
CASRN	Chemical Abstracts Service Registry Number
CAT	catalase
CBMA	cytokinesis-blocked micronucleus assay
CBMN	cytokinesis-blocked micronucleus
CDC	U.S. Centers for Disease Control and Prevention
CDHS	California Department of Health Services
CFD	computational fluid dynamics
CGM	clonal growth model
CHO	Chinese hamster ovary

CI	confidence interval
CIIT	Chemical Industry Institute of Toxicology
CLL	chronic lymphocytic leukemia
CML	chronic myelogenous leukemia
CNS	central nervous system
CO_2	carbon dioxide
COEHHA	California Office of Environmental Health Hazard Assessment
CREB	cyclic AMP responsive element binding proteins
CS	conditioned stimulus
C × t	concentration times time
DA	Daltons
DAF	dosimetric adjustment factor
DDC/DDX	DNA-DNA cross-links
DEI	daily exposure index
DEN	diethylnitrosamine
Der f	common dust mite allergen
DMG	dimethylglycine
DMGDH	dimethylglycine dehydrogenase
DNA	deoxyribonucleic acid
DOPAC	3,4-dihydroxyphenylacetic acid
DPC/DPX	DNA-protein cross-links
EBV	Epstein-Barr virus
EC	effective concentration
ED	effective dose
EHC	Environmental Health Committee
ELISA	enzyme-linked immunosorbent assay
EPA	U.S. Environmental Protection Agency
ERPG	emergency response planning guideline
ET	ethmoid turbinates
FALDH	formaldehyde dehydrogenase
FDA	U.S. Food and Drug Administration
FDR	fecundability density ratio
FEF	forced expiratory flow
FEMA	Federal Emergency Management Agency
FEV1	forced expiratory volume in 1 second
FISH	fluorescent in situ hybridization
FSH	follicle-stimulating hormone
FVC	forced vital capacity
GALT	gut-associated lymphoid tissue
GC-MS	gas chromatography-mass spectrometry
GD	gestation day
GI	gastrointestinal
GO	gene ontology
G6PDH	glucose-6-phosphate dehydrogenase
GPX	glutathione peroxidase
GR	glutathione reductase
GM-CSF	granulocyte macrophage-colony-stimulating factor
GSH	reduced glutathione
GSNO	S-nitrosoglutathione
GST	glutathione S-transferase
HAP	hazardous air pollutant
Hb	hemoglobin
HCl	hydrochloric acid
HCT	hematocrit
HEC	human equivalent concentration
5-HIAA	5-hydroxyindoleacetic acid
hm	hydroxymethyl
HMGSH	S-hydroxymethylglutathione
HPA	hypothalamic-pituitary adrenal
HPG	hypothalamo-pituitary-gonadal
HPLC	high-performance liquid chromatography
HPRT	hypoxanthine-guanine phosphoribosyltransferase
HR	high responders
HSA	human serum albumin
HSDB	Hazardous Substances Data Bank
Hsp	heat shock protein
HUVEC	human umbilical vein

	endothelial cell
HWE	healthy worker effect
I cell	initiated cell
IARC	International Agency for Research on Cancer
ICD	International Classification of Diseases
IF	interfacial
IFN	interferon
Ig	immunoglobulin
IL	interleukin
I.P.	intraperitoneal
IPCS	International Programme on Chemical Safety
IRIS	Integrated Risk Information System
K_m	Michaels-Menton constant
KM	Kaplan-Meier
LD_{50}	median lethal dose
LDH	lactate dehydrogenase
LEC	95% lower bound on the effective concentration
LED	95% lower bound on the effective dose
LHP	lymphohematopoietic
LI	labeling index
LM	Listeria monocytogenes
LMS	linearized multistage
LLNA	local lymph node assay
LOAEL	lowest-observed-adverse-effect level
LPS	lipopolysaccharide
LR	low responders
LRT	lower respiratory tract
MA	methylamine
MALT	mucus-associated lymph tissues
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCS	multiple chemical sensitivity
MCV	mean corpuscular volume
MDA	malondialdehyde
MEF	maximal expiratory flow
ML	myeloid leukemia
MLE	maximum likelihood estimate
MMS	methyl methane sulfonate
MMT	medial maxilloturbinate
MN	micronucleus, micronuclei
MNNG	N-methyl-N'-nitro-N-nitrosoguanidine
MOA	mode of action
MoDC	monocyte-derived dendritic cell
MP	macrophage
MPD	multistage polynomial degree
MPS	mononuclear phagocyte system
MRL	minimum risk level
mRNA	messenger ribonucleic acid
MVE-2	Murray Valley encephalitis virus
MVK	Moolgavkar, Venzon, and Knudson
N cell	normal cell
NaCl	sodium chloride
NAD+	nicotinamide adenine dinucleotide
NADH	reduced nicotinamide adenine dinucleotide
NALT	nasally associated lymphoid tissue
NATA	National-Scale Air Toxics Assessment
NCEA	National Center for Environmental Assessment
NCHS	National Center for Health Statistics
NCI	National Cancer Institute
NEG	Nordic Expert Group
NER	nucleotide excision repair
NGF	nerve growth factor
NHL	non-Hodgkin's lymphoma
NHMRC/ARMCANZ	National Health and Medical Research Council/Agriculture and Resource Management Council of Australia and New Zealand

NNK	4-(methylnitrosamino)-1-(3-pyridyl)-butanone
N^6-hmdA	N^6-hydroxymethyldeoxyadenosine
N^4-hmdC	N^4-hydroxymethyldeoxycytidine
N^2-hmdG	N^2-hydroxymethyldeoxyguanosine
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NIOSH	National Institute for Occupational Safety and Health
NLM	National Library of Medicine
NMDA	N-methyl-D-aspartate
NMU	N-methyl-N-nitrosourea
NNK	nitrosamine, 4-(methylnitrosamino)- 1-(3-pyridyl)-1-butanone
NO	nitric oxide
NOAEL	no-observed-adverse-effect level
NPC	nasopharyngeal cancer
NRBA	neutrophil respiratory burst activity
NRC	National Research Council
NTP	National Toxicology Program
OR	odds ratio
OSHA	Occupational Safety and Health Administration
OTS	Office of Toxic Substances
OVA	ovalbumin
PBPK	physiologically based pharmacokinetic
PC	Philadelphia chromosome
PCA	passive cutaneous anaphylaxis
PCMR	proportionate cancer mortality ratio
PCNA	proliferating cell nuclear antigen
PCR	polymerase chain reaction
PCV	packed cell volume
PECAM	platelet endothelial cell adhesion molecule
PEF	peak expiratory flow
PEFR	peak expiratory flow rates
PEL	permissible exposure limit
PFC	plaque-forming cell
PG	periglomerular
PHA	phytohemagglutinin
PLA2	phospholipase A2
PI	phagocytic index
PLM	posterior lateral meatus
PMA	phorbol 12-myristate 13-acetate
PMR	proportionate mortality ratio
PMS	posterior medial septum
PND	postnatal day
POD	point of departure
POE	portal of entry
PTZ	pentilenetetrazole
PUFA	polyunsaturated fatty acids
PWULLI	population weighted unit length labeling index
RA	reflex apnea
RANTES	regulated upon activation, normal T-cell expressed and secreted
RB	reflex bradypnea
RBC	red blood cells
RD_{50}	exposure concentration that results in a 50% reduction in respiratory rate
REL	recommended exposure limit
RfC	reference concentration
RfD	reference dose
RGD	regional gas dose
RGDR	regional gas dose ratio
RR	relative risk
RT	reverse transcriptase
SAB	Science Advisory Board
SCC	squamous cell carcinoma
SCE	sister chromatid exchange
SCG	sodium cromoglycate
SD	standard deviation
SDH	succinate dehydrogenase; sarcosine dehydrogenase

SEER	Surveillance, Epidemiology, and End Results
SEM	standard error of the mean
SEN	sensitizer
SH	sulfhydryl
SHE	Syrian hamster embryo
SI	sensory irritation
SLMA	spontaneous locomotor activity
SMR	standardized mortality ratio
SNP	single nucleotide polymorphism
SOD	superoxide dismutase
SOMedA	N^6-sulfomethyldeoxyadenosine
SOMedG	N^2-sulfomethyldeoxyguanosine
Sp1	specificity protein
SPIR	standardized proportionate incidence ratio
SSAO	semicarbazide-sensitive amine oxidase
SSB	single strand breaks
STEL	short-term exposure limit
TBA	tumor bearing animal
TH	T-lymphocyte helper
THF	tetrahydrofolate
TK	toxicokinetics
TL	tail length
TLV	threshold limit value
TNF	tumor necrosis factor
TP	total protein
TRI	Toxic Release Inventory
TRPV	transient receptor potential vanilloid
TWA	time-weighted average
TZCA	thiazolidine-4-carboxylate
UCL	upper confidence limit
UDS	unscheduled DNA synthesis
UF	uncertainty factor
UFFI	urea formaldehyde foam insulation
ULLI	unit length labeling index
URT	upper respiratory tract
USDA	U.S. Department of Agriculture
VC	vital capacity
VOC	volatile organic compound
WBC	white blood cell
WDS	wet dog shake
WHO	World Health Organization
WHOROE	World Health Organization Regional Office for Europe

FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic inhalation exposure to formaldehyde. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of formaldehyde.

In Chapter 6, *Major Conclusions in the Characterization of Hazard and Dose Response*, EPA has characterized its overall confidence in the qualitative and quantitative aspects of hazard and dose response by addressing knowledge gaps, uncertainties, quality of data, and scientific controversies. The discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

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1. INTRODUCTION

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard and dose-response assessment of formaldehyde. IRIS Summaries may include oral reference dose (RfD) and inhalation reference concentration (RfC) values for chronic and other exposure durations, and a carcinogenicity assessment.

The RfD and RfC, if derived, provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a nonlinear (presumed threshold) mode of action. The RfD (expressed in units of mg/kg-day) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation RfC (expressed in units of mg/m^3) is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal of entry [POE]) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). Reference values are generally derived for chronic exposures (up to a lifetime), but may also be derived for acute (≤24 hours), short-term (>24 hours up to 30 days), and subchronic (>30 days up to 10% of lifetime) exposure durations, all of which are derived based on an assumption of continuous exposure throughout the duration specified. Unless specified otherwise, the RfD and RfC are derived for chronic exposure duration.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral and inhalation exposure may be derived. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates may be derived from the application of a low-dose extrapolation procedure. If derived, the oral slope factor is a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, an inhalation unit risk is a plausible upper bound on the estimate of risk per $\mu g/m^3$ air breathed.

Development of these hazard identification and dose-response assessments for formaldehyde has followed the general guidelines for risk assessment as set forth by the National Research Council (NRC) (1983). EPA Guidelines and Risk Assessment Forum Technical Panel Reports that may have been used in the development of this assessment include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Recommendations for and Documentation*

of Biological Values for Use in Risk Assessment (U.S. EPA, 1988), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000a), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000c), *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002a), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), *Science Policy Council Handbook*: *Peer Review* (U.S. EPA, 2006a), and *A Framework for Assessing Health Risks of Environmental Exposures to Children* (U.S. EPA, 2006b).

The literature search strategy employed for this compound was based on the Chemical Abstracts Service Registry Number (CASRN) and at least one common name. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. This assessment includes a comprehensive review of literature through April 2009. As periodic literature searches are conducted by EPA for the formaldehyde assessment, additional literature identified through December 2009 is included where that literature was determined to be critical to the assessment. This included a few articles which were identified through PubMed© searches and publically available as "e-publications" in 2009, but have final publication dates of 2010.

2. BACKGROUND

This chapter provides an overview of the physical and chemical characteristics of formaldehyde. Also provided in this chapter are a description of the production, uses, and sources of formaldehyde and information regarding environmental levels and human exposure. A description of the toxicokinetics and toxicodynamic processes involved in formaldehyde toxicity for the inhalation, oral, and dermal routes can be found in Chapter 3 (Toxicokinetics).

2.1. PHYSICOCHEMICAL PROPERTIES OF FORMALDEHYDE

Formaldehyde (CASRN 50-00-0) is the first of the series of aliphatic aldehydes and is a gas at room temperature. Its molecular structure is depicted in Figure 2-1. It is noted for its reactivity and versatility as a chemical intermediate. It readily undergoes polymerization, is highly flammable, and can form explosive mixtures with air. It decomposes at temperatures above 150°C.

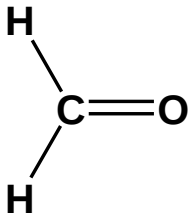

Figure 2-1. Chemical structure of formaldehyde.

At room temperature, pure formaldehyde is a colorless gas with a strong, pungent, suffocating, and highly irritating odor. Formaldehyde is readily soluble in water, alcohols, ether, and other polar solvents. A synopsis of its physicochemical properties is given in Table 2-1.

2.2. PRODUCTION, USES, AND SOURCES OF FORMALDEHYDE

Formaldehyde has been produced commercially since the early 1900s and, in recent years, has been ranked in the top 25 highest volume chemicals produced in the U.S. (National Toxicology Program [NTP], 2002). In 2003, 4.33 million metric tons of formaldehyde were produced in the U.S. (Global Insight, 2006). In 2000, worldwide formaldehyde production was estimated to be 21.5 million metric tons (International Agency for Research on Cancer [IARC], 2006).

Table 2-1. Physicochemical properties of formaldehyde

Name	Formaldehyde
International Union for Pure and Applied Chemistry name	Formaldehyde
Synonyms	Formic aldehyde Methanal Methyl aldehyde Methylene oxide Oxomethane Oxymethylene
Chemical Abstracts Service Index name	Formaldehyde
Chemical Abstracts Service Registry Number	50-00-0
Formula	HCHO
Molecular weight	30.03
Density	Gas: 1.067 (air = 1) Liquid: 0.815 g/mL at −20°C
Vapor pressure	3,883 mm Hg at 25°C
Log K_{ow}	−0.75 to 0.35
Henry's law constant	3.4×10^{-7} atm-m^3/mol at 25°C 2.2×10^{-2} Pa-m^3/mol at 25°C
Conversion factors (25°C, 760 mm Hg)	1 ppm = 1.23 mg/m^3 (v/v) 1 mg/m^3 = 0.81 ppm (v/v)
Boiling point	−19.5°C at 760 mm Hg
Melting point	−92°C
Flash point	60°C; 83°C, closed cup for 37%, methanol-free aqueous solution; 50°C closed cup for 37% aqueous solution with 15% methanol
Explosive limits	73% upper; 7% lower by volume in air
Autoignition temperature	300°C
Solubility	Very soluble in water; soluble in alcohols, ether, acetone, benzene
Reactivity	Reacts with alkalis, acids and oxidizers

Sources: American Conference of Governmental Industrial Hygienists (ACGIH) (2002); International Programme on Chemical Safety (IPCS) (2002); Agency for Toxic Substances and Disease Registry (ATSDR) (1999); Gerberich and Seaman (1994); Walker (1975).

Formaldehyde is a chemical intermediate used in the production of some plywood adhesives, abrasive materials, insulation, foundry binders, brake linings made from phenolic resins, surface coatings, molding compounds, laminates, wood adhesives made from melamine resins, phenolic thermosetting, resin curing agents, explosives made from hexamethylenetetramine, urethanes, lubricants, alkyd resins, acrylates made from trimethylolpropane, plumbing components from polyacetal resins, and controlled-release fertilizers made from urea formaldehyde concentrates (IPCS, 1989). Formaldehyde is used in smaller quantities for the preservation and embalming of biological specimens. It is also used as a germicide, an insecticide, and a fungicide in some products. It is found (as an ingredient or impurity) in some cosmetics/personal hygiene products, such as some soaps, shampoos, hair preparations, deodorants, sunscreens, dry skin lotions, and mouthwashes, mascara and other eye makeup, cuticle softeners, nail creams, vaginal deodorants, and shaving cream (IPCS, 2002; ATSDR, 1999).

Formaldehyde is commonly produced as an aqueous solution called formalin, which usually contains about 37% formaldehyde and 12−15% methanol. Methanol is added to formalin to slow polymerization that leads eventually to precipitation as paraformaldehyde. Paraformaldehyde has the formula $(CH_2O)_n$ where *n* is 8 to 100. It is essentially a solid form of formaldehyde and therefore has some of the same uses as formaldehyde (Kiernan, 2000). When heated, paraformaldehyde sublimes as formaldehyde gas. This characteristic makes it useful as a fumigant, disinfectant, and fungicide, such as for the decontamination of laboratories, agricultural premises, and barbering equipment. Long-chain polymers (e.g., Delrin plastic) are less inclined to release formaldehyde, but they have a formaldehyde odor and require additives to prevent decomposition (U.S. EPA, 2008).

The major sources of anthropogenic emissions of formaldehyde are motor vehicle exhaust, power plants, manufacturing plants that produce or use formaldehyde or substances that contain formaldehyde (i.e., adhesives), petroleum refineries, coking operations, incineration, wood burning, and tobacco smoke. Among these anthropogenic sources, the greatest volume source of formaldehyde is automotive exhaust from engines not fitted with catalytic converters (NEG, 2003). The Toxic Release Inventory (TRI) data for 2007 show total releases of 21.9 million pounds with about half to the air and half to underground injection (EPA TRI Explorer, http://www.epa.gov/triexplorer/) (U.S. EPA, 2009a).

Formaldehyde is formed in the lower atmosphere by photochemical oxidation of hydrocarbons or other formaldehyde precursors that are released from combustion processes (ATSDR, 1999). Formaldehyde can also be formed by a variety of other natural processes such

as decomposition of plant residues in the soil, photochemical processes in sea water and forest fires (National Library of Medicine, 2001).

During smog episodes, indirect production of formaldehyde may be greater than direct emissions (Fishbein, 1992). Grosjean et al. (1983) estimated the relative contributions of direct emissions and atmospheric photochemistry to levels of formaldehyde and other carbonyls in Los Angeles. They found that photochemical production predominates over direct emissions in controlling formaldehyde levels in Los Angeles air. Using two models, their data were translated into formaldehyde photochemical production rates of 12−161 tons per day.

Oxidation of methane is the dominant source of formaldehyde in regions remote from hydrocarbon emissions (Staffelbach et al., 1991). Based on atmospheric measurements at a rural site in Ontario, Canada and principal component analysis, Li et al. (1994) estimated that formaldehyde production by atmospheric photochemical oxidation of hydrocarbons is approximately 16 times that from primary emissions.

The input of formaldehyde into the environment is counterbalanced by its removal by several pathways. Formaldehyde is removed from the air by direct photolysis and oxidation by photochemically produced hydroxyl and nitrate radicals. Measured or estimated half-lives for formaldehyde in the atmosphere range from 1.6 to 19 hours, depending upon estimates of radiant energy, the presence and concentrations of other pollutants, and other factors (ATSDR, 1999). Given the generally short daytime residence times for formaldehyde, there is limited potential for long-range transport (IPCS, 2002). In cases where organic precursors are transported long distances, however, secondary formation of formaldehyde may occur far from the anthropogenic sources of the precursors.

Formaldehyde is released to water from the discharges of both treated and untreated industrial wastewater from its production and from its use in the manufacture of formaldehyde-containing resins (ATSDR, 1999). Formaldehyde is also a possible drinking-water disinfection by-product from the use of ozone and/or hydrogen peroxide. In water, formaldehyde is rapidly hydrated to form a glycol, and the equilibrium favors the glycol.

2.3. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

General population exposure to formaldehyde can occur via inhalation, ingestion and dermal contact. Each of these pathways and associated media levels are discussed below. Formaldehyde exposure can also occur occupationally via three main scenarios:

- The production of aqueous solutions of formaldehyde (formalin) and their use in the chemical industry (e.g., for the synthesis of various resins, as a preservative in medical laboratories and embalming fluids, and as a disinfectant).
- Release from formaldehyde-based resins in which it is present as a residue and/or through their hydrolysis and decomposition by heat (e.g., during the manufacture of wood products, textiles, synthetic vitreous insulation products, and plastics). In general, the use of phenol-formaldehyde resins results in much lower emissions of formaldehyde than those of urea- based resins.
- The pyrolysis or combustion of organic matter (e.g., in engine exhaust gases or during firefighting) (IARC, 2006).

Industries with the greatest potential for exposure include health services, business services, printing and publishing, manufacture of chemicals and allied products, manufacture of apparel and allied products, manufacture of paper and allied products, personal services, machinery (except clerical), transport equipment, and furniture and fixtures (IARC, 1995).

2.3.1. Inhalation

The most current ambient air monitoring data for formaldehyde come from EPA's air quality system database (EPA's AirData Web site: http://www.epa.gov/air/data/index.html) (U.S. EPA, 2009b). These data have been collected from a wide variety of sources, including state and local environmental agencies, but have not been collected from a statistically based survey. The most recent data, for the year 2007, come from 188 monitors located in 33 states as shown in Figure 2-2 (U.S. EPA, 2008). The annual means for these monitors range from 0.7−45.03 μg/m^3 (0.56−36.31 ppb) and have an overall average of 3.44 μg/m^3 (2.77 ppb). The annual means are derived by EPA by averaging all available daily data from each monitor. Table 2-2 shows a breakout of the data by land use category based on the annual means from each monitor for 2005, 2006, and 2007. The land use is established on the basis of the most prevalent land use within 0.25 miles of the monitor. The mobile category (land near major highways or interstates such that it is primarily impacted by mobile sources) has the highest mean levels, and agricultural lands have the lowest.

Under the National-Scale Air Toxics Assessment (NATA) program, EPA has conducted an emissions inventory for a variety of hazardous air pollutants (HAPs), including formaldehyde (U.S. EPA, 2006c). The NATA uses the emissions inventory data to model nationwide air concentrations/exposures (U.S. EPA, 2006c). The results of the 1999 ambient air concentration modeling for formaldehyde suggest that county median air levels range from 0 to 6.94 μg/m^3 (0−5.59 ppb) with a national median of 0.56 μg/m^3 (0.45 ppb) (see Figure 2-3). Similar results

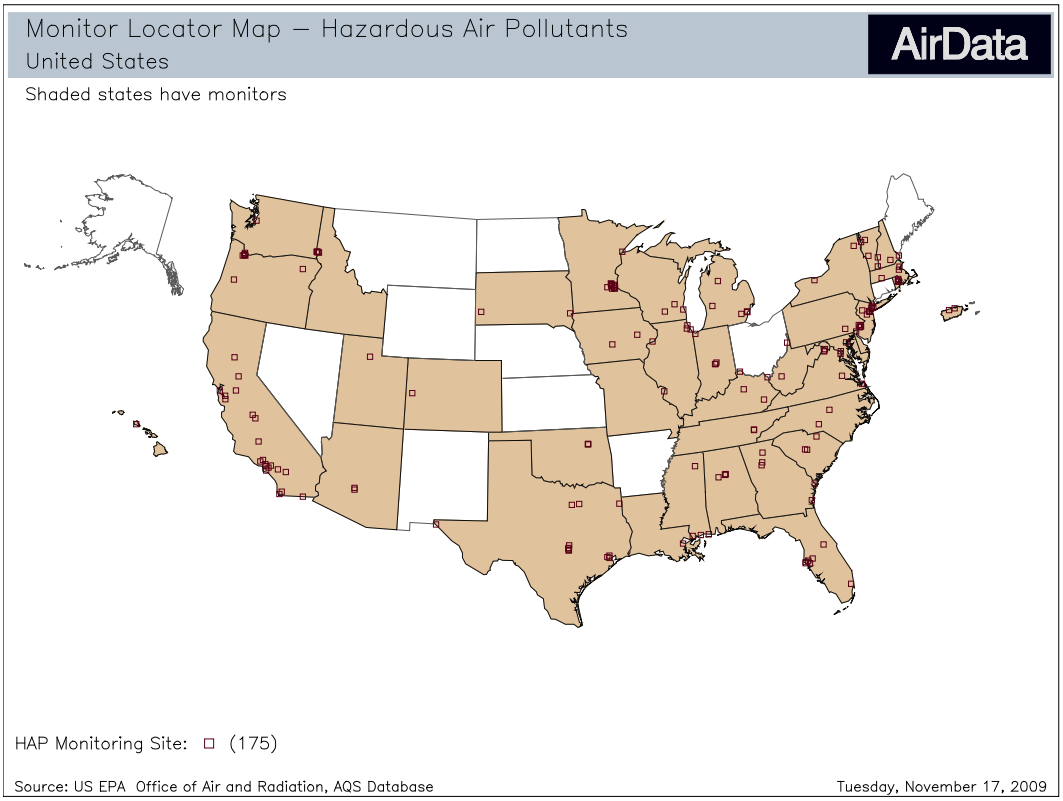

Figure 2-2. Locations of hazardous air pollutant monitors.
Dasgupta et al. (2005) measured formaldehyde levels in 5 U.S. cities during 1999−2002. Samples were collected over approximately a one month period in the spring or summer. Mean levels were 5.05 ppb in Nashville, TN; 7.96 ppb in Atlanta, GA; 4.49 ppb in Houston, TX; 3.12 ppb in Philadelphia, PA; and 2.63 in Sydney, FL.

Table 2-2. Ambient air levels by land use category

	Formaldehyde exposure by category[a]					
	Agriculture	**Commercial**	**Forest**	**Industrial**	**Mobile[b]**	**Residential**
Number of data points	17	166	19	61	16	282
Mean ± standard deviation	2.08 ± 0.98	3.26 ± 2.76	2.79 ± 2.17	6.28 ± 14.45	6.84 ± 7.28	2.75 ± 1.71
Minimum	0.34	0.20	0.40	0.14	2.02	0.17
Maximum	4.34	20.61	7.33	74.72	23.39	12.35

[a]Values are µg/m^3.
[b]"Mobile" is ambient air in locations primarily impacted by mobile sources.
Source: AirData for 2005, 2006, and 2007 (U.S. EPA, 2009b).

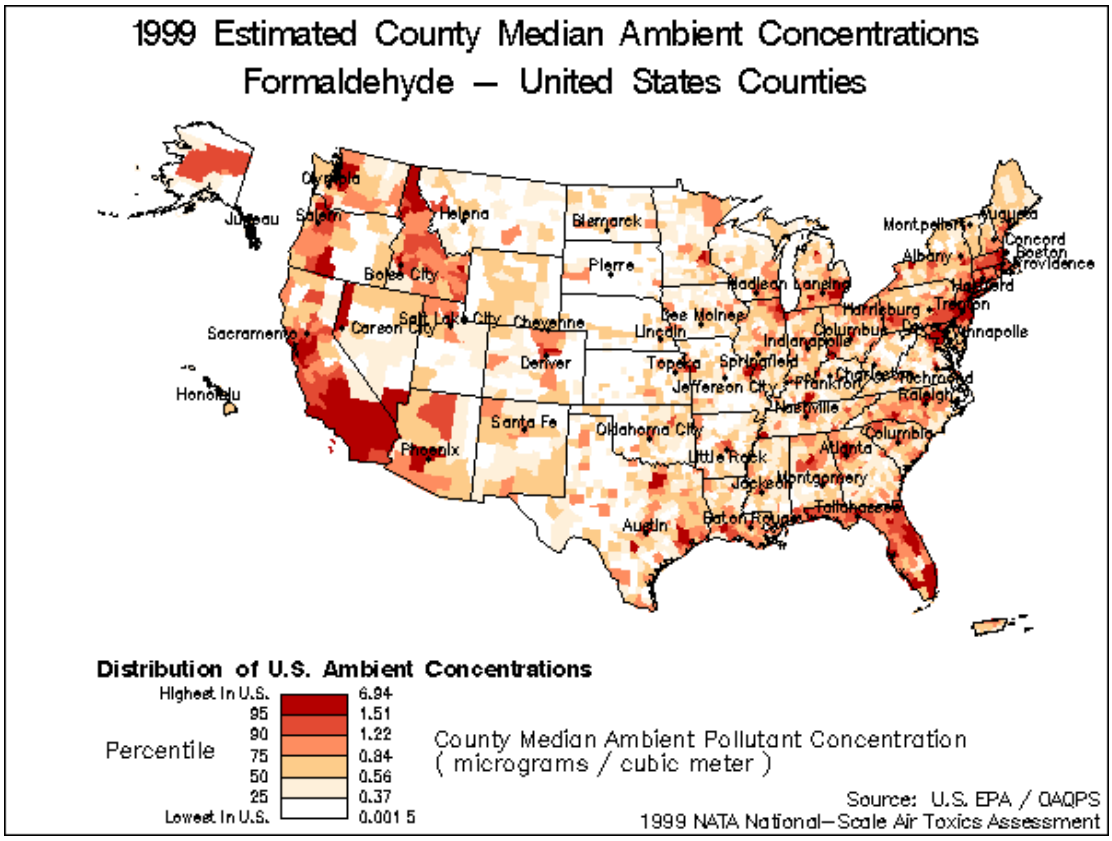

Figure 2-3. Modeled ambient air concentrations based on 1999 emissions.

were found for the year 2002: county concentrations ranged from 0.12 to 9.17 µg/m^3 (0.097−7.38 ppb) with median of 0.78 µg/m^3 (0.63 ppb). NATA has not provided updated concentration maps for 2002. The 1999 map shows the highest levels in the far west and northeastern regions of the U.S. While these modeling results can be useful, it is important to consider their limitations. Some of the geographical differences result from differences in methods used by states supplying the data. For example, the high levels indicated for Idaho result from the large amount of wood burned during forest fires and the relatively high emission factor that Idaho uses (compared with other states) to estimate formaldehyde emissions from forest fires. A comparison of modeling results from NATA to measured values at the same locations is presented in EPA (2006c). For 1999, it was found that formaldehyde levels were underestimated at 76% of the sites (n = 68). One possible reason why the NATA results appear low compared to measurements is that the modeling has not accounted for secondary formation of formaldehyde in the atmosphere.

In general, ambient levels of formaldehyde in outdoor air are significantly lower than those measured in the indoor air of workplaces or residences (ATSDR, 1999; IARC, 1995).

Indoor sources of formaldehyde in air include volatilization from pressed wood products, carpets, fabrics, insulation, permanent press clothing, latex paint, and paper bags, along with emissions from gas burners, kerosene heaters, and cigarettes (NLM, 2001). In general, the major indoor air sources of formaldehyde can be described in two ways: (1) those sources that have the highest emissions when the product is new with decreasing emission over time, as with the first set in the examples above; and (2) those sources that are reoccurring or frequent such as the second set of examples above. Gilbert et al. (2006) studied 96 homes in Quebec City, Canada and found elevated levels in homes with new wood or melamine furniture purchased within the previous 12 months. A summary of indoor data is provided in Table 2-3. Results vary depending on housing characteristics and date of study.

Salthammer et al. (2010) present a thorough review of formaldehyde sources and levels found in the indoor environment. Based on an examination of international studies carried out in 2005 or later they conclude that the average exposure of the population to formaldehyde is 20 to 40 $\mu g/m^3$ under normal living conditions. They used the diagram shown in Figure 2-4 to summarize data they found on the range of formaldehyde air concentrations (in ppb) in different environments.

Data on formaldehyde levels in outdoor and indoor air were collected under Canada's National Air Pollution Surveillance program (IPCS, 2002; Health Canada and Environment Canada, 2001). The effort included four suburban and four urban sites sampled in the period 1990−1998. A Monte Carlo analysis applied to the pooled data (n = 151) was used to estimate the distribution of time-weighted 24-hour air exposures. This study suggested that mean levels in outdoor air were 3.3 $\mu g/m^3$ (2.7 ppb) and mean levels in indoor air were 35.9 $\mu g/m^3$ (29.2 ppb) (Health Canada and Environment Canada, 2001). The simulation analysis also suggested that general population exposures averaged 33−36 $\mu g/m^3$ (27−30 ppb).

Since the early to mid 1980s, manufacturing processes and construction practices have been changed to reduce levels of indoor formaldehyde emissions (ATSDR, 1999). A 2008 law enacted by the California Air Resource Board (CARB. 2008, Final Regulation Order: Airborne Toxic Control Measure to Reduce Formaldehyde Emissions from Composite Wood Products; http://www.arb.ca.gov/regact/2007/compwood07/fro-final.pdf) has limited the amount of formaldehyde that can be released by specific composite wood products (i.e., hardwood plywood, particle board, and medium density fiberboard) sold, supplied, or manufactured for use in California. For this reason the mean indoor air levels presented by Health Canada and Environment Canada (2001) (based on samples collected from 1989−1995) may overestimate

Table 2-3. Studies on residential indoor air levels of formaldehyde (nonoccupational)

Citation	No. of samples	Target population/house type	Mean (µg/m^3)	Range (µg/m^3)
Gold et al., 1993		Complaint homes[1] Older conventional homes	<60	24−960
Hare et al., 1996		Newly built homes	91	
Hare et al., 1996		30 days after installing pressed wood	42−540	
Gammage and Hawthorne, 1985	>1,200 131 >500 260	Homes with UFFI Homes without UFFI Complaint mobile homes Newer mobile homes Older mobile homes	60−144 30−84 120−1080 1032 300	12−4080 12−204 0−5040
Hawthorne et al., 1986a, b	18 11 11 40	Conventional homes 0−5 yr Conventional homes 5−15 yr Conventional homes >15 yr Conventional homes overall	96 48 36 72	 24−480
U.S. EPA, 1987	560	Noncomplaint, conventional, randomly selected Noncomplaint, mobile homes, randomly selected	32−109 109−744	6−576 12−3480
Health Canada and Environment Canada, 2001	151	Residential (Canadian) noncomplaint homes	35	?−148
Zhang et al., 1994a, b	6	Residential, carpeted, nonsmoking homes	66	42−89
Gilbert et al., 2006	96	Residential (Canadian)	29.5	9.6−90.0
Shah and Singh, 1988	315	Residential and commercial	59	23−89
Stock, 1987	43	Conventional homes	84	96−216
Krzyzanowski et al., 1990	202	Conventional homes	31	

[1] The "complaint" homes are ones where the occupants have complained about formaldehyde irritant symptoms.
Note: 1 ppb = 1.2 µg/m^3.

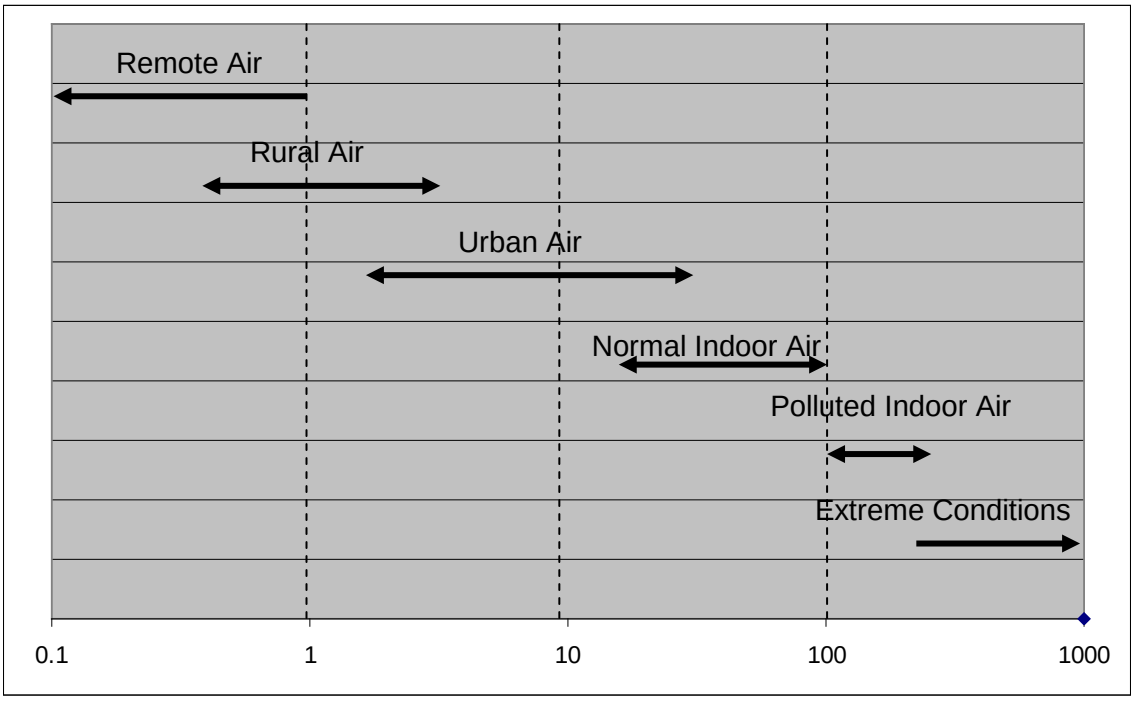

Figure 2-4. Range of formaldehyde air concentrations (ppb) in different environments.

Source: Salthammer et al. (2010).

current levels. In addition, the Canadian indoor air data may overestimate formaldehyde levels in U.S. homes, because many residential homes in Canada use wood burning stoves more frequently and have tighter construction (due to colder winters), leading to less dilution of indoor emissions. The outdoor air levels, however, appear to have remained fairly constant over recent years, and the median outdoor level from the Canadian study (2.8 µg/m^3) (2.3 ppb) is very similar to the median of the U.S. monitoring data (2.83 µg/m^3) (2.3 ppb) in 1999.

Even though formaldehyde levels in construction materials have declined, indoor inhalation concerns still persist. For example, recent studies have measured formaldehyde levels in mobile homes/trailers (these terms are used interchangeably here to refer to homes with wheels that are designed to be moved). ATSDR (2007) reported on air sampling in 96 unoccupied trailers provided by the Federal Emergency Management Agency (FEMA) used as temporary housing for people displaced by Hurricane Katrina. Formaldehyde levels in closed trailers averaged 1,250 ± 828 µg/m^3 (mean ± standard deviation [SD]) (1.04 ± 0.69 ppm), with a range of 12−4,390 µg/m^3 (0.01−3.66 ppm). The levels decreased to an average of 468 ±

324 μg/m^3 (0.39 ± 0.27 ppm), with a range of 0.00−1,960 μg/m^3 (0.00−1.63 ppm) when the air conditioning was turned on. Levels also decreased to an average of 108 ± 96 μg/m^3 (0.09 ± 0.08 ppm), with a range of 12−588 μg/m^3 (0.01−0.49 ppm) when the windows were opened. ATSDR (2007) found an association between temperature and formaldehyde levels; higher temperatures were associated with higher formaldehyde levels in trailers with the windows closed. They also noted that different commercial brands of trailers yielded different formaldehyde levels.

In December 2007 and January 2008, the Centers for Disease Control and Prevention (CDC) measured formaldehyde levels in a stratified random sample of 519 FEMA-supplied occupied travel trailers, park models, and mobile homes ("trailers") (CDC, 2008). At the time of the study, sampled trailers were in use as temporary shelters for Louisiana and Mississippi residents displaced by hurricanes Katrina and Rita. The geometric mean level of formaldehyde in sampled trailers was 95 μg/m^3 (77 ppb), and the range was 3.7−730 μg/m^3 (3−590 ppb).

2.3.2. Ingestion

Limited U.S. data indicate that concentrations in drinking water may range up to approximately 10 μg/L in the absence of specific contributions from the formation of formaldehyde by ozonation during water treatment or from leaching of formaldehyde from polyacetyl plumbing fixtures (IPCS, 2002). In the absence of other data, one-half this concentration (5 μg/L) was judged to be a reasonable estimate of the average formaldehyde in Canadian drinking water. Concentrations approaching 100 μg/L were observed in a U.S. study assessing the leaching of formaldehyde from domestic polyacetal plumbing fixtures, and this concentration was assumed to be representative of a reasonable worst case (IPCS, 2002).

Formaldehyde is a natural component of a variety of foodstuffs (IARC, 1995; IPCS, 1989). However, foods may be contaminated with formaldehyde as a result of fumigation (e.g., grain fumigation), cooking (as a combustion product), and release from formaldehyde resin-based tableware (IARC, 1995). Also, the compound has been used as a bacteriostatic agent in some foods, such as cheese (IARC, 1995). There have been no systematic investigations of levels of formaldehyde in a range of foodstuffs that could serve as a basis for estimation of population exposure (Health Canada and Environment Canada, 2001). According to the limited available data, concentrations of formaldehyde in food are highly variable. In the few studies of the formaldehyde content of foods in Canada, the concentrations were within a range of <0.03−14 mg/kg (Health Canada and Environment Canada, 2001). Data on formaldehyde levels in food have been presented by Feron et al. (1991) and IPCS (1989) from a variety of studies, yielding the following ranges of measured values:

- Fruits and vegetables: 3−60 mg/kg
- Meat and fish: 6−20 mg/kg
- Shellfish: 1−100 mg/kg
- Milk and milk products: 1−3.3 mg/kg

Daily intake of formaldehyde was estimated by IPCS (1989) to be in the range of 1.5−14 mg for an average adult. Similarly, Fishbein (1992) estimated that the intake of formaldehyde from food is 1−10 mg/day but discounted this on the belief that it is not available in free form. Although the bioavailability of formaldehyde from the ingestion of food is not known, it is not expected to be significant (ATSDR, 1999). Using U.S. Department of Agriculture (USDA) (1979) consumption rate data for various food groups, Owen et al. (1990) calculated that annual consumption of dietary formaldehyde results in an intake of about 4,000 mg or approximately 11 mg/day.

2.3.3. Dermal Contact

The general population may have dermal contact with formaldehyde-containing materials, such as some building products and cosmetics (see Section 2.2 for the details on these products). Generally, though, dermal contact is more of a concern in occupations that involve handling concentrated forms of formaldehyde, such as those occurring in embalming and chemical production.

3. TOXICOKINETICS

This chapter presents chemical specific information on the toxicokinetics of formaldehyde which helps to inform the potential for health effects from formaldehyde exposure. As a water soluble and reactive gas (see Chapter 2), the chemical reactions of formaldehyde at the site of first contact in biological systems is important to understanding its toxic potential. Therefore, before a discussion of the absorption, distribution, and metabolism of formaldehyde (which normally comprises the heart of the toxicokinetic discussion of an agent) a section is provided which discusses some key issues regarding formaldehyde's reactivity. Section 3.1 provides information regarding the hydration of formaldehyde in biological aqueous systems and the equilibrium which exists between free formaldehyde and methylene glycol. Additional information is provided on what is known of the nature of chemical reactions of free formaldehyde with proteins. These discussions are provided to give context to the following Sections of Chapter 3.

Sections 3.2 and 3.3 present the available studies which describe the absorption and distribution of formaldehyde, including animal studies of radiolabeled formaldehyde. The influence of formaldehyde's reactivity at the site of first contact and effects on the mucociliary apparatus are presented here as well, as these effects may modify the uptake of formaldehyde. Metabolism of formaldehyde is presented in Section 3.4, but the endogenous production of formaldehyde from normal metabolic processed, as well as metabolism of other xenobiotics. The last section of Chapter 3 present the available models which apply to the toxicokinetics of formaldehyde—in this case primarily modeling of the flux of formaldehyde through tissues at the sight of first contact using computational fluid dynamics models.

3.1. CHEMICAL PROPERTIES AND REACTIVITY

Formaldehyde (HCHO) is the smallest aldehyde (30 g/mol) and is a gas at room temperature. It is highly water soluble and reactive. In water, less than 0.1% of formaldehyde exists unhydrated, with the majority reported to be in the hydrated form, methylene glycol ($CH_2(OH)_2$) (Priha et al., 1996). Formaldehyde reacts readily with high and low molecular weight biological constituents.

3.1.1. Hydration of Formaldehyde

In aqueous solution formaldehyde exists in equilibrium with its hydrated form methanediol (CH_2OH_2) ($K_d = 5.5 \times 10^{-4}$). The equilibrium favors methanediol at physiological

temperature and pH (>99.9%) and is readily reversible. In biological systems, as free formaldehyde is removed from aqueous solution through binding with serum proteins and cellular components, the equilibrium is reestablished by dehydration of methanediol to free formaldehyde. The reversible nature of this hydration reaction describes how a pool of free formaldehyde may be sustained in biological systems.

3.1.2. Binding of Formaldehyde to Proteins

Formaldehyde is a reactive molecule that is likely to react with both low molecular weight cellular components (e.g., reduced glutathione[GSH]) as well as high molecular weight components. Unlike deoxyribonucleic acid (DNA), which has some additional barriers to exposure (i.e., nucleus), extracellular and intracellular proteins are obvious targets for interacting with formaldehyde. Formaldehyde is a well-known cross-linking agent that is used in the fixation of tissues, preparation of vaccines, and study of protein-protein interactions (Metz et al., 2006). However, the exact nature of the protein modifications used for these purposes is not yet fully characterized (Metz et al., 2006, 2004). Figure 3-1 provides a general reaction scheme for formaldehyde-mediated modifications of amino acids. In step 1, formaldehyde reacts with primary N-terminal amines to form a labile methylol adduct. This adduct can undergo dehydration (step 2) to form an imine, or Schiff base ($–N=CH_2$). Metz et al. (2004) examined the types of formaldehyde-protein reactions that are likely to occur in vivo by synthesizing several identical polypeptides with one varying amino acid (X) within the sequence VEL**X**VLL (V = valine, E = glutamate, L = Leucine, X = varying amino acid). Several peptides with reactive amino acids did not exhibit modifications, suggesting that the peptide sequence/structure affects the ability of formaldehyde to react with amino acids. Peptides that were modified indicated formation of methylol adducts (see Figure 3-1, step 1) or a mixture of methylol and imine adducts (see Figure 3-1, step 2).

Mucus is composed of water, electrolytes, polysaccharides, and about 0.5% soluble proteins (Priha et al., 1996; Bogdanffy et al., 1987). Bogdanffy et al. (1987) showed that although human nasal mucus can bind 70% of 100 mM formaldehyde, irreversible binding of [^{14}C]-formaldehyde to serum albumin (the major protein in mucus) was shown to be insignificant after a 1-hour incubation. Irreversible binding (50% or more) did not occur until after about 7 hours of incubation. These data suggest that the protein content of mucus may not provide a significant formaldehyde irreversible sink. Nonetheless, the solubility of formaldehyde in mucus along with mucus flow and ingestion likely indicate that much of the inhaled dose is removed—perhaps as much as 42% in rodents (IARC, 2005; Schlosser, 1999).

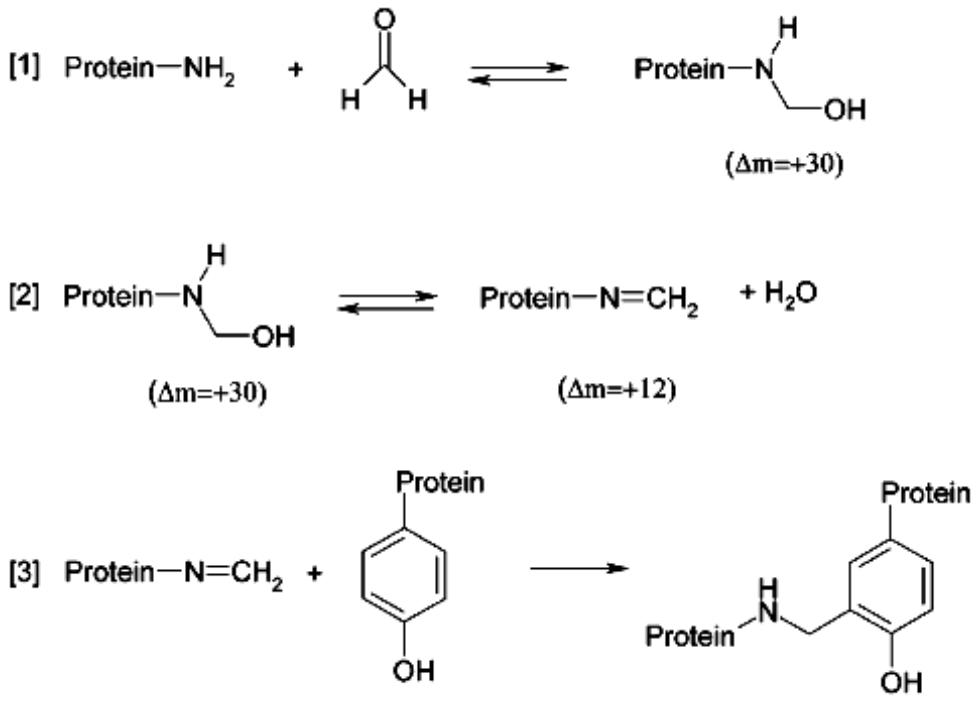

Figure 3-1. Formaldehyde-mediated protein modifications.

Note: Formaldehyde reacts with primary *N*-terminal amines to form a methylol adduct [1], which increases the molecular weight by 30 Da (Δm). This labile adduct can rearrange to form an amine, or Schiff base [2], that results in an increase in MW of 12 Da. Schiff bases can react with certain amino acids to form intra- or intermolecular methylene bridges [3]. The two amino acids depicted in step 3 may be within the same protein or possibly from two different proteins.

Source: Metz et al. (2004).

In general, formaldehyde interacts with proteins. Studies carried out in cell culture media containing serum and formaldehyde have shown that such mixtures are quite labile. For example, during a 60-minute incubation of formaldehyde with complete cell media (i.e., with fetal calf serum) at 38°C, gas chromatography-mass spectrometry (GC-MS) exhibited very different peak profiles at different points during the incubation (Proctor et al., 1986). In contrast, GC-MS chromatograms of cell media containing formaldehyde but no serum proteins appeared relatively unchanged throughout the incubation. Compared to cell culture medium alone, complete media were considered to provide a more suitable model for the hypothetical interactions that formaldehyde could undergo in vivo (including perhaps blood).

3.2. ABSORPTION

3.2.1. Oral

Oral absorption of [^{14}C]-formaldehyde (7 mg/kg) in rats resulted in 40% elimination as ^{14}C-carbon dioxide ($^{14}CO_2$), with 10% excretion in urine, 1% excretion in feces, and much of the remaining 49% retained within the carcass, presumably due to metabolic incorporation (IARC, 1995; Buss et al., 1964).

3.2.2. Dermal

Jeffcoat et al. (1983) reported on the disposition of various doses of [^{14}C]-formaldehyde dermally administered to rats, guinea pigs, and monkeys. Very little (<1% of the applied dose) of the radiolabel was found in the major organs excised during necropsy. As noted by the authors, the disposition of formaldehyde when administered via the dermal route was markedly different to that observed when the compound was administered intravenously or intraperitoneally. In the latter cases, there was much evidence of metabolic activity, and substantial portions of the load were expired as CO_2. The difference appeared to be the result of a reaction of dermally applied formaldehyde with macromolecules at or near the skin surface or of its evaporation. In general, portions of the load that succeed in entering the circulation probably do so bound to macromolecules or by incorporation of the radiolabel via the one-carbon pool. Likewise, Bartnik et al. (1985) who applied [^{14}C]-formaldehyde to the shaved backs of rats concluded that the overwhelming majority of the formaldehyde load remained sequestered in the outer layers of skin at or near the site of application. At the end of the various measurements, approximately 70% of the dose was found in the treated skin, with a marked localization of the remaining radioactivity in the uppermost layers. This fraction of the load was considered to be permanently sequestered, most likely as a result of irreversible binding to macromolecular components.

3.2.3. Inhalation

Studies indicate that the majority of inhaled formaldehyde is absorbed in the upper respiratory tract (URT) but that the extent of the scrubbing in this region varies significantly across species. In dogs, nearly 100% of nasally inhaled formaldehyde is absorbed (Egle, 1972). Lower respiratory tract (LRT) studies designed to collect formaldehyde via a tube inserted into the lower trachea revealed that nearly 95% of formaldehyde was absorbed during the first pass through the upper respiratory tract (Egle, 1972), an effect observed with multiple ventilation rates. The rat nasal passages also scrub nearly all of the inhaled formaldehyde (on average ~97%) (Morgan et al., 1986). In computational dosimetry modeling based on anatomically

realistic representation of the human nasal airways from a single individual, approximately 90% of inhaled formaldehyde was predicted to be absorbed in the nose at resting inspiration. As the inspiratory rate increased, this fraction decreased to about 70% at light exercise and to 58% at heavy exercise conditions (see Figure 1 in Kimbell et al. [2001b]). The normal human breathing mode during heavy exercise is oronasal (with ~54% of airflow being oral) (ICRP 66, 1994). Consequently, it is estimated that during heavy exercise breathing (50 L/minute) the flux of formaldehyde into tissue (or rate of mass transported per mm^2 of tissue surface area) in the first six to eight generations of the tracheobronchial airways is comparable to that in the nasal region (Overton et al., 2001).

It is important to note that the computer simulations mentioned above are based on anatomical representations of a single individual. Significant anatomical variations occur in human nasal airways. For example, the nasal volumes of 10 adult nonsmoking subjects between 18 and 50 years of age in a study in the U.S. varied between 15 and 60 mL (Santiago et al., 2001), and disease states can result in considerable further variation (Singh et al., 1998).

Species differences in kinetic factors have been argued to be the key determinants of species-specific lesion distributions for formaldehyde and other reactive inhaled gases. Airway geometry is an important determinant of inhaled-formaldehyde dosimetry in the respiratory tract and its differences across species. These issues will be discussed in a later section on dosimetry modeling.

3.2.3.1. *Formaldehyde Uptake Can Be Affected by Effects at the Portal of Entry*

Certain formaldehyde-related effects have the potential to modulate its uptake and clearance. The mucociliary apparatus of the upper respiratory tract is the first line of defense against airborne toxins. Comprising a thick mucus layer (epiphase), hydrophase, and a ciliated epithelium, the mucociliary apparatus may entrain, neutralize, and remove particulates and airborne chemicals from inspired air. As reviewed by Wolfe (1986), airborne pollutants and reactive gases have been shown to decrease mucus flow rates in several animal models (Mannix et al., 1983; Iravani, 1974; Carson et al., 1966; Dalhamn, 1956; Cralley, 1942). Degradation in the continuity or function of this mucociliary apparatus could result in a lower clearance of inhaled pollutants at the portal of entry.

Morgan et al. (1983) first reported defects in mucociliary function in F344 rats exposed to 15 ppm formaldehyde 6 hours/day for 1−9 days. Mucostasis occurred in several regions in all rats after a single 15 ppm exposure. Ciliastasis occurred with greater frequency and across more regions of the nasoturbinate in subsequent days of exposure. The authors observed that mucostasis preceded ciliastasis in most cases, and vigorous ciliary activity was noted in areas

without mucus flow. Morgan et al. (1984a) also studied formaldehyde effects on the mucociliary apparatus of isolated frog palates in vitro. Mucostasis was evident as mucus became stiff and eventually rigid with increasing formaldehyde concentration and time of exposure. Ciliary beat continued even after mucostasis, but ciliastasis ultimately occurred when exposure reached 4 and 9 ppm.

When a rodent is exposed to an irritant, its inhaled dose and pattern of deposition can be profoundly affected by reflex bradypnea, a protective reflex seen in rodents but not in humans. Reflex bradypnea can occur when the trigeminal nerve is exposed to a sufficient concentration of an irritant, such as formaldehyde. It is manifest as markedly decreased activity or prostration, reduced metabolism, hypothermia (as much as 5ºC), significantly reduced respiratory rate and minute volume, and altered blood and brain chemistry. Because of their small size, rodents are able to rapidly lower their metabolism and body temperature and therefore their oxygen demand. The consequence is that their inhaled dose of an irritating chemical is dramatically lowered. Reflex bradypnea is quantified as the RD_{50}, which is the concentration of a chemical that results in a 50% decrease in respiratory rate. It can take as much as two hours for rodents to fully recover from the effects of reflex bradypnea. The clinical manifestations of reflex bradypnea can easily be misconstrued as toxicity. None of the studies described in this assessment took into account the fact that reflex bradypnea may have confounded the results. Reflex bradypnea is discussed in depth in Section 4.2.1.1.

Sensory irritation studies suggest that formaldehyde activates the trigeminal nerve by activating nociceptors through the modification of receptor amino acids, possibly including thiol groups. Cassee et al. (1996) measured sensory irritation to formaldehyde, acetaldehyde, and acrolein in male Wistar rats, following a 30-minute nose-only exposure. Formaldehyde and acrolein elicited similar responses, whereas acetaldehyde was far less irritating. The authors suggested that the differences in sensitivity to the aldehydes might be explained by differences in physicochemical properties and by regional differences in activities of detoxifying enzymes for each chemical. In addition, it has been suggested that acetaldehyde might interact with sensory nerves via an amino group (Steinhagen and Barrow, 1984), whereas the receptor-binding site for formaldehyde and acrolein is believed to be a thiol group. Differential binding sites for sensory irritants in the trigeminal nerve have been reported (Nielsen, 1991).

Sensory irritation effects are discussed in depth in Chapter 4 but are noted here because stimulation of the trigeminal nerve by formaldehyde can result in significantly lower pulmonary ventilation, and formaldehyde exposure in rodents at concentrations that approach the RD_{50}. Barrow et al. (1983) have estimated the “inhaled dose” equivalent to an exposure concentration of 15 ppm in mice and rats used in the chronic formaldehyde bioassays by Kerns et al. (1983)

and Monticello and Morgan (1994). Their results indicate that, because mice are observed to decrease their minute volume by approximately 75% as compared to 45% in rats, a twofold greater inhaled dose would be expected in rats versus mice. This difference may be relevant to the increased incidence of squamous cell carcinoma of the nasal cavity in F344 rats as compared to B6C3F1 mice. Chang et al. (1983) estimated a reduction of 25% in the minute volume of F344 rats. Yokley et al. (2008) have recently published a model that accounts for physiological changes in ventilation rate induced by sensory irritation in rats. Thus, the "standard" minute volumes used for rats and mice need to be adjusted downward when calculating dosimetric adjustment factors for extrapolation of adverse effects to humans (Thompson et al., 2008). This question is further discussed in the section on modeling the dosimetry.

Another effect that modulates dosimetry is the dynamic tissue remodeling of nasal airways that occurs as a consequence of exposure to reactive gases. For example, formaldehyde dosimetry is influenced by the occurrence of squamous metaplasia, an adaptive tissue conversion to squamous that occurs in nasal epithelium exposed to toxic levels of formaldehyde. The metaplasia has been observed to occur in rats at exposure concentrations of 3 ppm and higher (Kimbell et al., 1997b). Squamous epithelium is known to absorb considerably less formaldehyde than other epithelial types (Kimbell et al., 1997b). Overall, the highest flux levels of formaldehyde in the simulations of the rat nose in Kimbell et al. (2001a) are estimated in the region just posterior to the nasal vestibule. A consequence of squamous metaplasia would be to "push" the higher levels of formaldehyde flux toward the more distal regions of the nose (Kimbell et al., 1997b). Subramaniam et al. (2008) discussed this issue further in the context of uncertainties in the modeling of formaldehyde dosimetry.

3.3. DISTRIBUTION

3.3.1. Transport of Methylene Glycol

In biological systems, formaldehyde is known to exist in equilibrium with its hydrated form, as methanediol (CH_2OH_2) ($K_d = 5.5 \times 10^{-4}$) at physiological temperatures and pH (>99.9%) in the body and is readily reversible. When free formaldehyde is removed from aqueous solution through binding with serum proteins and cellular components, the equilibrium is reestablished by dehydration of methanediol to free formaldehyde. Thus, a pool of free formaldehyde may be sustained in biological systems due to the reversible nature of this hydration reaction.

There is strong and consistent evidence in biological testing systems in vitro that treating cells with formaldehyde in an aqueous media results in significant cytotoxicity, cell proliferation, clastogenic effects and clear evidence of mutational events (see Section 4.3). Similarly, animal

bioassays where formaldehyde is administered in drinking water report portal of entry toxicity including hyperplasia, increased cell proliferation, focal lesions and tumors (see Section 4.2.1). It should be noted that URT tissues are covered by an aqueous mucous layer, through which formaldehyde must pass to react the cellular components of the URT. It has been postulated that formaldehyde transports through this mucous layer and the underlying tissues as methanediol (Georgieva et al., 2003).

The dynamic equilibrium between the hydrated and unhydrated forms of formaldehyde in biological systems is well understood. Since the hydration reaction favors methanediol, it is expected that exogenous formaldehyde which reaches the blood will primarily exist as methanediol and is subject to physiological elimination. As free, unhydrated formaldehyde continues to react with serum proteins and cellular components, the blood levels of methanediol are expected to reduce as it is dehydrated to maintain equilibrium. Although some attempts to measure significant changes in free formaldehyde levels in blood after inhalation exposure have not been successful, the half-life in blood has been measured after i.v. injection at approximately 2 minutes (McMartin et al., 1979). Additionally, the detection of antibodies to formaldehyde-hemoglobin adducts and formaldehyde-albumin adducts in exposures workers, smokers and laboratory animals exposed via inhalation provides direct evidence that formaldehyde is able to react with serum albumin and hemoglobin in biological systems (Li et al., 2007; Varro et al., 1997; Grammer et al., 1993; Dykewicz et al., 1991; Thrasher et al., 1990, Grammer et al., 1990). These data support the hypothesis that exogenous formaldehyde may reach and transport through the blood. If so, formaldehyde (or methanediol) may reach sites distal to the portal of entry.

3.3.2. Formaldehyde-GSH Conjugate as a Method of Systemic Distribution

Formaldehyde is primarily metabolized by alcohol dehydrogenase (ADH3) which uses the formaldehyde-glutathione hemiacetal adduct as the substrate. Sanghani et al. (2000) have shown that due to high circulating concentrations (50-fold) of glutathione in human blood, the S-(hydroxymethyl)glutathione (HMGSH) adduct, the nonenzymatic product of formaldehyde with glutathione is the major form of formaldehyde seen in vivo (Sanghani et al., 2000). It is likely that the reversibly bound HMGSH may be transported to different tissues through circulation, but, specific experimental evidence is lacking.

3.3.3. Levels in Blood

Inhalation studies in several species indicate that exposure to formaldehyde does not result in elevated levels in blood. These studies were carried out over a wide range of exposure concentrations and durations. Rats exposed to 14 ppm formaldehyde for 2 hours exhibited no

increase in blood formaldehyde levels [2.25 ± 0.07 μg/(g blood) in treated animals compared with 2.24 ± 0.07 μg/(g blood) in control animals] when measured by GC-MS using a stable isotope dilution technique (Heck et al., 1985, 1982). Similarly, mean formaldehyde blood levels in humans (n = 6) exposed to 1.9 ppm formaldehyde for 40 minutes in a walk-in chamber (2.77 ± 0.28 μg/g blood) were not statistically different from measurements in the same population before exposure (mean of 2.61 ± 0.14 μg/g) (Heck and Casanova-Schmitz, 1984). The variability in the levels was large. At the individual level, the data showed both increase and decrease in blood levels relative to pre-exposure levels, which was attributed by the authors as plausibly due to temporal variations in baseline levels in humans, particularly since the experiment did not control food intake prior to exposure. Studies in rhesus monkeys have revealed endogenous formaldehyde levels (2.4 μg/g blood) comparable to humans and that levels were also unaltered following exposure to 6 ppm formaldehyde via inhalation 6 hours/day for 4 weeks, measurements being taken at both 7 minutes and 45 hours post final exposure (Casanova et al., 1988).

It is important to keep in mind that the GC-MS method is not capable of detecting irreversibly bound formaldehyde; for example, formaldehyde levels detected by this method, even in the anterior nasal mucosa of rats exposed to 6 ppm of formaldehyde, were not elevated over control levels. Furthermore, the GC-MS method does not differentiate between free and reversibly bound adducts of formaldehyde (Heck et al., 1982). Thus, measured levels represent total formaldehyde concentration that includes free formaldehyde as well as reversibly bound adducts. Based on the known Michaelis-Menten constant, Km, for formaldehyde dehydrogenase with respect to the GSH adduct formation, Heck et al. (1982) estimated under certain assumptions that free formaldehyde comprised only about 1−2% of the total formaldehyde measured by their method. Furthermore, as shown by Metz et al. (2006, 2004), formaldehyde reactions with primary amino and thiol groups can, in a second step, react with many other amino acids to form stable methylene bridges. Presumably, such reactions would not be detectable by using the methods employed by Heck et al. (1982).[4] Thus, the limited interpretation of GC-MS measurements of blood levels suggests that formaldehyde does not appreciably reach the blood,

[4] Additionally, note that, although Heck et al. (1982) demonstrated that formaldehyde concentration can be accurately measured from glutathione and tetrahydrofolate adducts, similar experiments were not performed by using protein samples or cellular extracts (i.e., in the presence of various amino acids). In addition, standard curves for predicting formaldehyde concentration in tissues were generated in aqueous solutions rather than biological samples.

is rapidly metabolized or interacts with macromolecules when it escapes metabolism, or is otherwise undetected.

Results from an earlier experiment using radiolabeled formaldehyde in rats are consistent with the conclusion based on the GC-MS measurements of no appreciable increase in blood levels of formaldehyde. Following a 6-hour exposure of F344 rats to 15 ppm of [^{14}C]-formaldehyde (Heck et al., 1983), the concentrations of ^{14}C in the nasal mucosa were 28-fold higher than those in the blood. The observed half-life of the terminal phase of the radioactivity was long (55 hours); on the other hand, it is known that the half-life of free formaldehyde in the rat blood is very short. Therefore, the authors concluded that the radioactivity was likely due to modification of macromolecules or metabolic incorporation rather than slow metabolic clearance of formaldehyde. The terminal decline of the radioactivity in the packed cell fraction of the blood was much slower and observed to be consistent with incorporation into erythrocytes.

In the same paper, Heck et al. (1983) report on the similarity in the pharmacokinetics of radiolabeled formaldehyde and radiolabeled formate in the rat blood, supporting their hypothesis that oxidation of formaldehyde to formate and subsequent incorporation of this compound through one-carbon metabolism were major factors in the disposition of formaldehyde. Studies by Gottschling et al. (1984) have also established that the main product of metabolic clearance of formaldehyde is formate, which is either further metabolized to CO_2 and water, incorporated into the one-carbon pool, and/or eliminated in the urine as a sodium salt at about 13 mg/L urine.

3.3.4. Levels in Various Tissues

The radiolabeling studies indicated high levels of ^{14}C in the rat nasal mucosa (equivalent concentrations of ^{14}C-formaldehyde in the nasal mucosa of rats naïvely exposed to 15 ppm ^{14}C-formaldehyde were 2,148 ± 255 nmol/g compared with 76 ± 11 nmol/g in plasma). In contrast, the GC-MS studies did not detect elevated formaldehyde in this region. This is not to be interpreted as a discrepancy, because the radiolabeling study did not distinguish among radiolabeled species and thus the measured radioactivity could potentially be free or bound formaldehyde, formate, or any [^{14}C] metabolically incorporated into macromolecules.

In concurrent studies, Casanova-Schmitz et al. (1984) resolved the question as to whether the higher [^{14}C] levels in the nasal mucosa were a consequence of GSH depletion and a subsequent reduction in GSH-dependent clearance of formaldehyde. An important result in these studies was that there was no significant difference in labeling in either the nasal mucosa or in plasma between naïve F344 rats and those pre-exposed to unlabeled 15 ppm formaldehyde 6 hours/day for the 9 previous days. These findings indicated little or no apparent effect on the

disposition of formaldehyde following short-term exposure to relatively high levels of formaldehyde. In contrast, Farooqui et al. (1986) reported decreases in GSH in several tissues 3 hours after a sublethal I.P. injection of formaldehyde but not after 6 and 9 hours. Taken together, these data suggest that formaldehyde exposure does not result in long-term alterations in cellular GSH levels and that repeated inhalation exposure does not alter the dosimetry to the bloodstream or formaldehyde body burden.

Heck et al. (1983) determined the ^{14}C concentrations in different tissues in the F344 rat body by exposing rats in a head-only chamber to various concentrations (5−24 ppm) of radiolabeled formaldehyde for 6 hours. (Concentrations of ^{14}C in internal organs and tissues relative to that in plasma did not appear to vary much as exposure concentrations increased; therefore only averages over the concentration range were reported.) Except for the esophagus, levels in the heart, spleen, lung, intestines, liver, and kidney were 1−3 times higher relative to that in plasma. Labeling in the esophagus was high (fivefold relative to plasma). The authors attributed this relatively higher dose to mucociliary action in the nose and trachea. The data also indicate that the brain, testes, and erythrocytes appear to have about threefold lower ^{14}C levels than plasma. Pre-exposure to formaldehyde (for 9 days) did not alter the measured radioactivity in the nasal mucosa or plasma. Thus, it was concluded that the single exposure findings may also be qualitatively extended to chronic exposures.

The total radiolabel measured in the bone marrow (femur) of F344-rats exposed for 6 hours to 0.3−15 ppm of radiolabeled formaldehyde in the Casanova et al. (1984) experiment was high (generally within a factor of 0.5 of the total labeling in the nasal respiratory mucosa). Nearly half of the ^{14}C was contained in the DNA in this tissue presumably on account of the high rate of cell turnover in the bone marrow, indicating that the carbon derived from ^{14}C-formaldehyde was utilized for DNA synthesis (Casanova-Schmitz et al., 1984).

Chang et al. (1983) described visceral labeling (via autoradiography) in rats, following exposure to 15 ppm [^{14}C]-formaldehyde 6 hours/day for 4 days. The authors attributed this labeling to mucociliary clearance and grooming-related ingestion of formaldehyde.

In summary, following exposure to radiolabeled formaldehyde, the radioactivity was very high in the nasal mucosa but was also extensively distributed to various tissues. In particular, levels in the bone marrow were high. On the other hand, formaldehyde levels in the blood measured by GC-MS were not significantly elevated. Thus, the authors considered it unlikely that the elevated ^{14}C in various tissues was due to free formaldehyde. Instead, these levels were thought to arise from either rapid metabolic incorporation or formation of covalent adducts or incorporation via carboxylation reactions of the $^{14}CO_2$ formed during metabolism.

The data presented thus far in this section illustrate that measuring the distribution of the absorbed formaldehyde based on ^{14}C-radiolabeling and GC-MS studies alone is problematic because it is difficult to resolve (through these studies) whether it is free, reversibly bound, irreversibly bound, formate, one-carbon pool, etc. This is of significance with regard to understanding the availability of the absorbed formaldehyde. More indirect methods had to be developed to further examine the disposition of formaldehyde; however, as discussed below, the interpretation of these approaches may also not be straightforward.

3.3.4.1. *Disposition of Formaldehyde: Differentiating Covalent Binding and Metabolic Incorporation*

The motivation in presenting this section is twofold, as follows:

1. As concluded above, subsequent studies were necessary to ascertain whether measured radiolabeling in different experiments was due to formaldehyde adducts or incorporation of [^{14}C] one-carbon units of formaldehyde into macromolecules via the one-carbon pool.
2. DNA protein cross-links (DPXs) formed by formaldehyde (covalently bound in this case) have been regarded as a surrogate dose metric for the intracellular concentration of formaldehyde (Hernandez et al., 1994; Casanova et al., 1991, 1989). This is particularly relevant because of the nonlinear dose response for DPX formation due to saturation of enzymatic defenses at high concentrations (Casanova et al., 1991, 1989). Thus, the ability to measure DPX is an important development.

An important question is whether the formaldehyde disposed in the form of DPX is detected in remote tissues. A set of elegant but complex experiments involving dual isotope labeling (^{14}C and ^{3}H) was carried out to this end by the Heck and Casanova-Schmitz and their coworkers. Casanova-Schmitz et al. (1984) and Casanova-Schmitz and Heck (1983) used dual isotope labeling of formaldehyde as a way to partially distinguish between formaldehyde adducts formation and metabolic incorporation. In separate experiments, F344 rats were exposed to ^{3}H- and ^{14}C-formaldehyde at different exposure concentrations (0.3−15.0 ppm), and the ^{3}H/^{14}C ratios of different phases of DNA were measured. Only the highlights of the results and significant issues are presented here. The overall conclusions from these experiments were as follows:

- Labeling in the nasal mucosa was due to both covalent binding and metabolic incorporation.
- DPX was formed at 2 ppm and greater concentrations in the respiratory mucosa.
- In the bone marrow, formaldehyde did not bind covalently to bone marrow macromolecules at any exposure concentration. The labeling of bone marrow

macromolecules was found to be entirely due to metabolic incorporation and not due to covalent binding.

Macromolecules such as DNA and protein can be isolated from tissue homogenates by extraction into three phases: an organic phase consisting of proteins, an aqueous phase consisting of only double-stranded DNA, and an interfacial phase consisting of both DNA and protein. Single-stranded (but not double-stranded) DNA was particularly likely to form adducts. DNA from this interfacial phase can be further purified and has been shown to consist of DPXs (Casanova-Schmitz and Heck, 1983). Because both [^{14}C]-formaldehyde and [^{3}H]-formaldehyde can become incorporated into DNA and protein metabolically as well as by cross-linking, the ^{3}H/^{14}C ratio in such cross-linked material should be higher than in material that primarily contains metabolically incorporated formaldehyde. Figure 3-2 shows the labeling of tissue from the nasal respiratory mucosa and bone marrow (distal femur) in rats exposed to [^{14}C]-formaldehyde and [^{3}H]-formaldehyde vapor.

In the nasal mucosa the interfacial phase has a significantly higher ^{3}H/^{14}C ratio than the material in the aqueous phase. This suggests that interfacial DNA has significantly more ^{3}H, a phenomenon likely explained by additional [^{3}H]-formaldehyde molecules present as DPXs prior to extraction. The amount of interfacial DNA was found to have a clear dose response. These cross-links were also judged to be due to exogenous formaldehyde. Likewise, the organic phase of the nasal mucosa showed a similar increase in ^{3}H/^{14}C ratio at higher concentrations, a result that could be attributed to various inter- and intraprotein adducts (Metz et al., 2004; Trezl et al., 2003; Skrzydlewska, 1996).

In contrast, analysis of macromolecules at the distal femur location presents a different pattern (see Figure 3-2, part B). First, the interfacial phase was not detected during extraction, suggesting that there were few or no DPXs to be detected. Second, there was no increase in ^{3}H/^{14}C ratio in the organic (i.e., protein) phase as a function of dose. Therefore, it was concluded that either radiolabeled formaldehyde or formate reached the distal site and was subsequently incorporated into macromolecules. According to the mechanistic interpretation of these studies, the quantity plotted on the ordinate in Figure 3-2 (the ratio of ^{3}H/^{14}C between the tissue and the exposure gas) should approach unity as metabolism becomes saturated and more adduct formation occurs, particularly for protein. Indeed, this is what is observed (see Figure 3-2, Part A). In contrast, there is no dose effect in the femur, suggesting that the labeling at all doses in that tissue may be due to metabolic incorporation and not due to the parent formaldehyde.

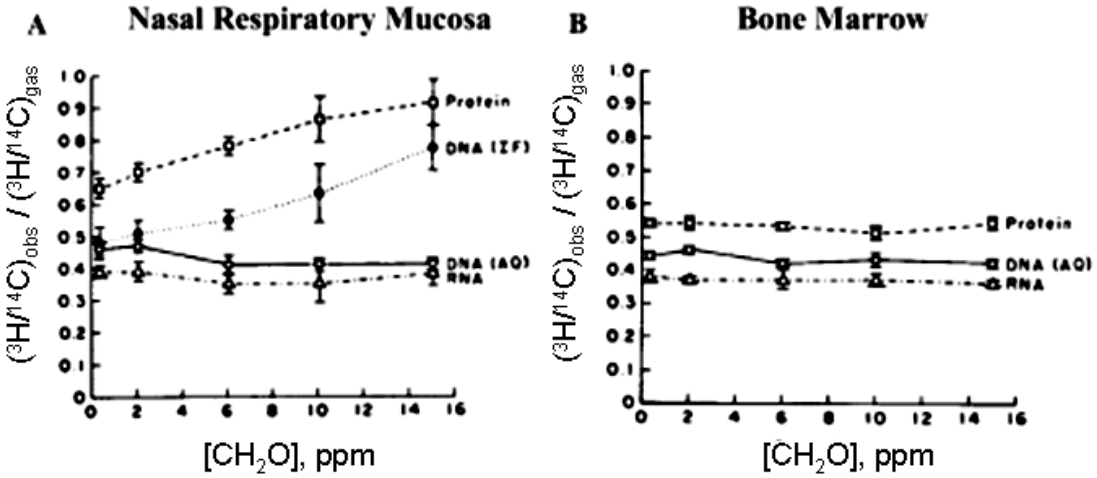

Figure 3-2. $^3H/^{14}C$ ratios in macromolecular extracts from rat tissues following exposure to ^{14}C- and ^3H-labeled formaldehyde (0.3, 2, 6, 10, 15 ppm).

Note that the small yield of interfacial (IF) phase from bone marrow tissue precluded further analysis; this is *prima facie* evidence for the lack of significant DPXs in this tissue.

Source: Casanova-Schmitz et al. (1984a).

(Note: These data were originally shown in the absence of an analysis of isotope effects on covalent binding and metabolism. Subsequent studies determined that [^{3}H]-formaldehyde is oxidized less rapidly than [^{14}C]-formaldehyde and unlabeled formaldehyde. This suggests that the $^3H/^{14}C$ ratio, and therefore the amount of formaldehyde covalently bound to tissue, is likely overestimated because more [^{3}H]-formaldehyde remains unmetabolized, i.e., free to bind [Heck and Casanova, 1987]. The authors hypothesized that this overestimate was relatively greater at the lower concentrations.)

Similar results were obtained in GSH-depleted rats (Casanova and Heck, 1987). Again, these authors observed a dose-dependent increase in the $^3H/^{14}C$ ratio in the interfacial DNA and organic fractions of disrupted cells of the respiratory and olfactory mucosa and no such increases in bone marrow. Interestingly, at 10 ppm exposure (only), GSH-depleted rats exhibited a higher $^3H/^{14}C$ ratio in the organic phase than did normal rats. Casanova and Heck (1987) posited that much of the covalent binding at 6 ppm and lower was due to binding to extracellular proteins, whereas the higher $^3H/^{14}C$ ratio in GSH-depleted rats at 10 ppm was due to more intracellular binding.

In their first experiment to measure DPX concentrations, Casanova-Schmidt et al. (1984) and Casanova and Heck (1987) used the dual isotope method ($^{3}H/^{14}C$) mentioned above. In this experiment, DPX was observed only at formaldehyde concentrations ≥2 ppm. Subsequently, Casanova et al. (1989) developed a more sensitive method using high-performance liquid chromatography (HPLC) for measuring DPX. In this method, tissue homogenates were digested with a proteolytic enzyme and extracted with a phenolic solvent. DPX was detected in the nasal mucosa of rats at formaldehyde concentrations as low as 0.3 ppm. This method was also used to measure DPX in the nasal region, the larynx, trachea and carina, and major intrapulmonary airways (airway diameters >2 mm) of rhesus monkeys exposed for 6 hours to 0.7, 2.0, and 6.0 ppm of formaldehyde. DPX was detected in the nose (including the nasopharynx) at all concentrations and at 2.0 and 6.0 ppm in the larynx, trachea, carina, and other lower airways. However, DPX was not detectable in the bone marrow of these monkeys at any concentration.

Overall, Heck and Casanova-Schmitz and their coworkers interpreted the results of these various experiments to mean that inhaled formaldehyde could not reach distant sites in the body. It may be noted in this context that Shaham et al. [1996] reported elevated DPX levels in the white blood cells of laboratory workers exposed to formaldehyde. These data are further reported in Chapter 4.)

3.4. METABOLISM

Formaldehyde is primarily metabolized by glutathione-dependent formaldehyde dehydrogenase (FALDH) and aldehyde dehydrogenases (ALDHs). Numerous studies now recognize FALDH as a member of the alcohol dehydrogenase (ADH) family, specifically ADH3 (Thompson et al., 2009; Liu et al., 2004, 2001; Hedberg et al., 2003; Høøg et al., 2003; and the references in each of these). The remainder of this report will refer to FALDH as ADH3.

3.4.1. In Vitro and In Vivo Characterization of Formaldehyde Metabolism

Formaldehyde is oxidized to formate by two metabolic pathways (see Figure 3-3). The first pathway involves conversion of free formaldehyde to formate by the so-called low-K_m (K_m = 400 μM) mitochondrial aldehyde dehydrogenase-2 (ALDH2). The second pathway involves a two-enzyme system that converts glutathione-conjugated formaldehyde (S-hydroxymethylglutathione [HMGSH]) to the intermediate S-formylglutathione, which is subsequently metabolized to formate and GSH by S-formylglutathione hydrolase.

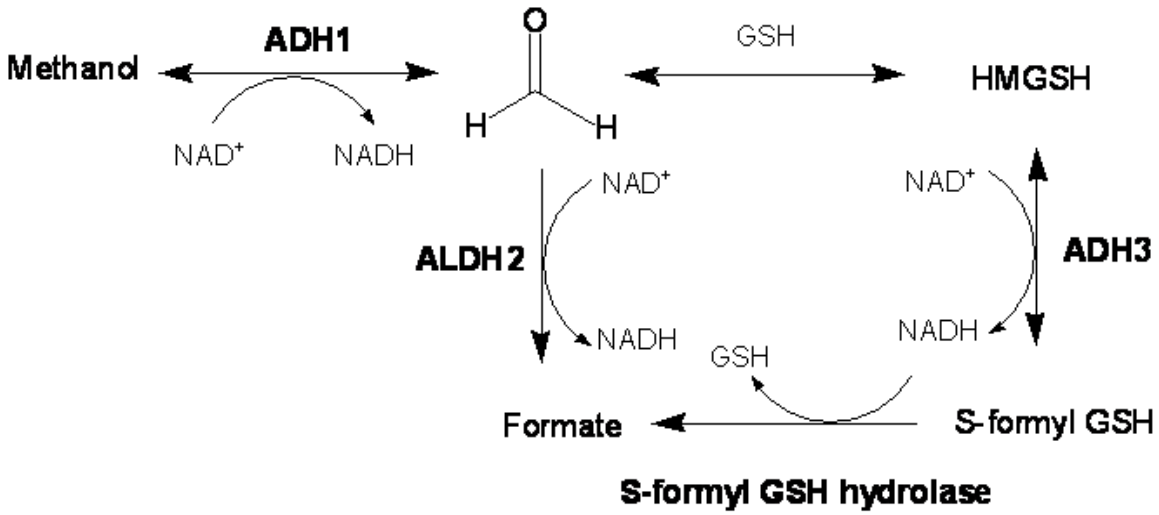

Figure 3-3. Formaldehyde clearance by ALDH2 (GSH-independent) and ADH3 (GSH-dependent).

The K_m value for ALDH2 and free formaldehyde is about 400 µM (Teng et al., 2001), whereas the K_m value for HMGSH and ADH3 is 6.5 µM (Uotila and Koivusalo, 1974a, b). The ADH-mediated reactions are reversible in the presence of excess reduced nicotinamide adenine dinucleotide (NADH).
Source: Adapted from Teng et al. (2001).

Though ADH3 is rate limiting in this second pathway, the affinity of HMGSH for ADH3 (K_m = 6.5 µM) is about 100-fold higher than that of free formaldehyde for ALDH2. In addition to the kinetic properties, this member of the ADH gene family (Høøg et al., 2003, 2001; Liu et al., 2001; Jornvall et al., 2000; Estonius et al., 1996) appears to be ubiquitously expressed in organ tissues (Molotkov et al., 2002; Ang et al., 1996a, b), exhibits cytoplasmic and nuclear localization (Fernandez et al., 2003), and is the most abundant ADH family member in the liver and brain (Galter et al., 2003).

In vitro studies have examined the clearance of formaldehyde in several human and rat tissues (see Table 3-1). Examination of formaldehyde metabolism in the rat nasal and olfactory mucosa indicates nearly identical pharmacokinetics in the rat liver on a per mg of cell lysate basis (Casanova-Schmitz et al., 1984b). Similar results have been obtained in the absence of GSH, where other ALDH family members oxidize formaldehyde, albeit with significantly lower affinity (i.e., higher K_m). Hedberg et al. (2000) demonstrated that human buccal tissue lysate kinetics are in close agreement with those reported for purified human liver ADH3 (Uotila and Koivusalo, 1974a). Additionally, micro-array analysis indicates that these cells express far more ADH3 and S-formylglutathione hydrolase than ALDH1 or ALDH2 (Hedberg et al., 2001a). The

results of Ovrebo et al. (2002) are not easily compared with the other studies in Table 3-1 because these studies were in intact cell cultures. However, it is apparent that the pharmacokinetic values in these human cells are comparable to intact rat liver cells.

Table 3-1. Formaldehyde kinetics in human and rat tissue samples

Source	K_m (µM)	V_{max} (nmol/mg protein × min)	Reference
Purified human liver ADH3	6.5	2.77 ± 0.12	Uotila and Koivusalo (1974a, b)
Rat olfactory mucosa (+ GSH)	2.6 ± 0.5	1.77 ± 0.12	Casanova-Schmitz et al. (1984b)
Rat olfactory mucosa (− GSH)	647 ± 43	4.39 ± 0.14	Casanova-Schmitz et al. (1984b)
Rat respiratory mucosa (+ GSH)	2.6 ± 2.6	0.90 ± 0.24	Casanova-Schmitz et al. (1984b)
Rat respiratory mucosa (− GSH)	481 ± 88	4.07 ± 0.35	Casanova-Schmitz et al. (1984b)
Rat liver (+ GSH)	5.0 ± 1.9	2.0 ± 0.3	Casanova-Schmitz et al. (1984b)
Human bronchial explants[a]	5,100	3.3	Ovrebo et al. (2002)
Human bronchial epithelial[a]	1,400	6.1	Ovrebo et al. (2002)
Rat hepatocytes[a]	1,250	4.2	Ovrebo et al. (2002)
Human buccal tissue (+ GSH)	11 ± 2	2.9 ± 0.6	Hedberg et al. (2000)
Human buccal tissue (− GSH)	360 ± 90	1.2 ± 0.7	Hedberg et al. (2000)
Human keratinocytes	n.d.[b]	14.5 ± 1.8	Hedberg et al. (2000)
Human fibroblasts	n.d.	17.9 ± 1.4	Hedberg et al. (2000)

[a]These studies were carried out in intact cells by measuring the formation of formate. This likely explains the nearly 1,000-fold increase in apparent K_m, since much of the formaldehyde was likely to be bound extracellularly. The remaining studies used either purified enzyme or cell lysates (as indicated) and measured the formation of NADH.
[b]n.d. = not determined.

The data in Table 3-2 along with data indicating the ubiquity of ADH3, indicate that many human tissues and cells, particularly in the respiratory tract, appear to exhibit significant capacity to metabolize formaldehyde. Molecular biology techniques have demonstrated the importance of ADH3 in formaldehyde clearance. For example, ADH-knockout studies have shown that the median lethal dose (LD_{50}) values for formaldehyde in wild type, $ADH1^{-/-}$, $ADH3^{-/-}$, and $ADH4^{-/-}$ mice strains were 0.200, 0.175, 0.135, and 0.190 g/kg, respectively (Deltour et al., 1999). Although the statistical significance was not reported, the data indicate that deletion of ADH3 increases the sensitivity of mice to formaldehyde.

Table 3-2. Allelic frequencies of ADH3 in human populations

Population, *n*	Allele frequencies (%)						
	AA$_{-197/-196}$	GG$_{-197/-196}$	A$_{-79}$	G$_{-79}$	T$_{+9}$	C$_{+9}$
Chinese, 83	22	78	100	–	–	100
Spanish, 95	41	59	62	38	–	100
Swedish, 96	47	53	67	33	1.5	98.5

Source: Adapted from Hedberg et al. (2001b).

The pharmacokinetics of formate are complex. Formate can undergo adenosine triphosphate (ATP)-dependent addition to tetrahydrofolate (THF), which can carry either one or two one-carbon groups. Formate can conjugate with THF to form N^{10}-formyl-THF and its isomer N^5-formyl-THF, both of which can be converted to N^5,N^{10}-methenyl-THF and subsequently to other derivatives that are ultimately incorporated into DNA and proteins via biosynthetic pathways (see Figure 3-4).

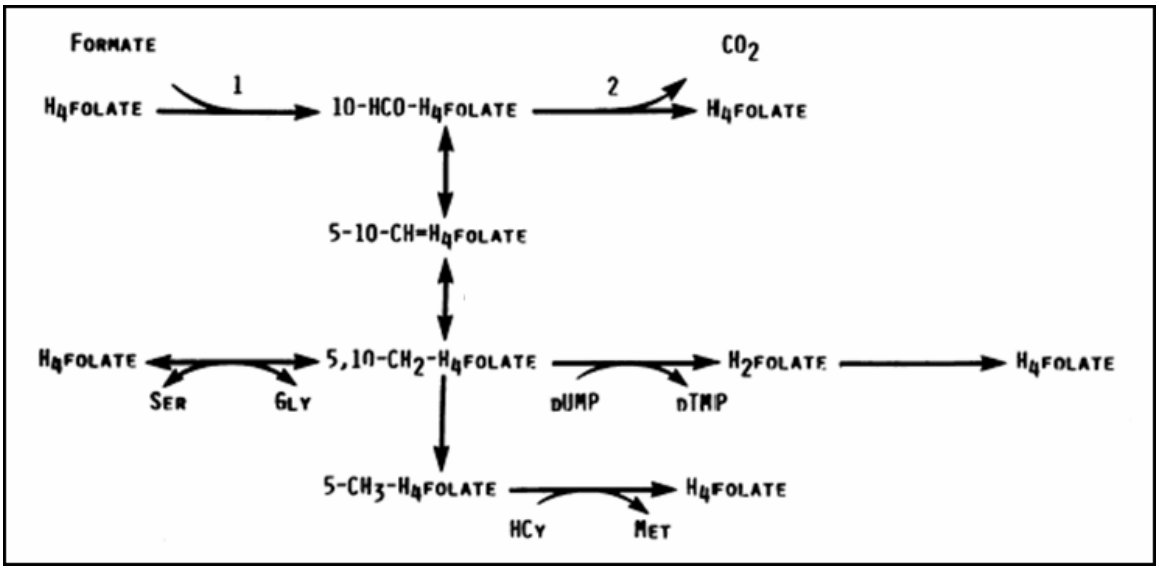

Figure 3-4. Metabolism of formate.

Note: 1, formyl-THF synthetase; 2, formyl-THF dehydrogenase.

Source: Adapted from Black et al. (1985).

Elevated levels of formate in urine have been detected following inhalation of methanol or formate under certain conditions (Liesivuori and Savolainen, 1987), although the interpretation of this finding is unclear. There is also evidence that formate generates CO_2^- radicals and can be metabolized to CO_2 via catalase and via the oxidation of N^{10}-formyl-THF (Dikalova et al., 2001, and references therein). The significance of formate in formaldehyde toxicity is unclear. Black et al. (1985) reported that hepatic tetrahydrofolate levels in monkeys are 60% of those in rats and that primates are far less efficient in clearing formate than are rats and dogs. Studies in rats involving [^{14}C]-formate suggest that about 80% is exhaled as $^{14}CO_2$, 2−7% is excreted in the urine, and about 10% undergoes metabolic incorporation (Hanzlik et al., 2005, and references therein). Mice deficient in formyl-THF dehydrogenase exhibit no change in LD_{50} (via I.P. dose) for methanol or in oxidation of high doses of formate (Cook et al., 2001). It has been suggested that rodents efficiently clear formate via folate-dependent pathways, peroxidation by catalase, or an unknown third pathway. Conversely, primates do not appear to exhibit such capacity and are more sensitive to metabolic acidosis following methanol poisoning (Cook et al., 2001).

3.4.2. Formaldehyde Exposure and Perturbation of Metabolic Pathways

The enzyme ADH3 has received renewed attention in recent years because of new functions that have been attributed to it. ADH3 is central to the metabolism of formaldehyde; however, exposure to formaldehyde in turn alters the activity of ADH3 (in multiple dose-dependent ways), thereby leading to perturbation of critical metabolic pathways. These are briefly mentioned below (refer to cited papers for details).

1. Exposure to formaldehyde increases cell replication. These proliferating epithelial and inflammatory cells are rich in both the messenger ribonucleic acid (mRNA) and protein of ADH3 (Nilsson et al., 2004; Hedberg et al., 2000). Studies in the rodent lung suggest that increases in ADH3 in such cells dramatically alter the biology of other important ADH3 substrates that are involved in protein modification and cell signaling (Que et al., 2005).
2. ADH3 also participates in the oxidation of retinol and long-chain primary alcohols, as well as the reduction of S-nitrosoglutathione (GSNO) (Staab et al., 2009; Thompson et al., 2009; Hedberg et al., 2003; Høøg et al., 2003; Molotkov et al., 2002; Liu et al., 2001; Jornvall et al., 2000; Jensen et al., 1998). The activity of ADH3 toward some of these substrates has been shown to be significantly increased in the presence of formaldehyde. Staab et al. (2009) showed that (in cultured cells) GSNO can accelerate ADH3-mediated formaldehyde oxidation and, likewise, that formaldehyde increases ADH3-mediated GSNO reduction nearly 25-fold. The following effects may be noted with regard to the relevance of such perturbations.

a. GSNO is an endogenous bronchodilator and reservoir of nitric oxide (NO) activity (Jensen et al., 1998). Details on the ADH3-mediated reduction of GSNO are shown in Thompson and Grafstrom (2008).

b. ADH3 is implicated in playing a central role in regulating bronchiole tone and allergen-induced hyperresponsiveness (Gerard, 2005; Que et al., 2005).

c. As concluded by California Environmental Protection Agency (CalEPA) (2008), "the dysregulation of NO by formaldehyde [in this manner] helps to explain the variety and variability in the toxic manifestations following formaldehyde inhalation."

3.4.3. Evidence for Susceptibility in Formaldehyde Metabolism

Teng et al. (2001) provided evidence that inhibition of ADH1, ALDH2, and ADH3 has significant impact on formaldehyde toxicity. The authors speculated that deficiencies in any of these enzymes would confer an increased susceptibility to formaldehyde toxicity (Teng et al., 2001). Polymorphism in ALDH2 has been shown to have implications in human risk assessment, specifically with regard to acetaldehyde metabolism (Ginsberg et al., 2002). It is worth noting, however, that Teng et al. (2001) only demonstrated the importance of ALDH2 in rat hepatocytes with formaldehyde concentrations of 2.5 mM and greater. Since this concentration is fivefold greater than the 0.5 mM K_m for free formaldehyde, ALDH2 involvement is not unexpected at such high concentrations. Teng et al. (2001) also demonstrated the importance of ADH1 in driving the reverse reaction (i.e., formaldehyde to methanol) by coadministration of NADH-generators. This would have the effect of prolonging the life of formaldehyde by continuous recycling. This is not surprising, given that many ADH reactions are reversible. However, levels of nicotinamide adenine dinucleotide (NAD^+) are normally much higher than NADH.

To date, two studies have reported polymorphisms in ADH3, using the new nomenclature.[5] ADH3 transcription appears to be regulated by specificity protein (Sp1), with a minimal promoter located at positions −34 to +61. The reported polymorphisms in ADH3 involve four base-pair substitutions in the promoter region and no polymorphisms in the coding region (Hedberg et al., 2001b). The three polymorphisms include −197/−196 (GG→AA), −79 (G→A), and +9 (C→T). The genotype frequencies are shown in Table 3-2. Of these alleles, the +9 (C→T) polymorphism (in the putative Sp1 minimal promoter region) reduced transcriptional

[5] Other epidemiologic studies investigating links between ADH3 and oral cancer use the older nomenclature and thus refer to Class I ADH (i.e., ADH1) enzymes.

activity twofold in in vitro reporter gene experiments. According to Hedberg et al. (2001b), no studies have demonstrated differences in ADH3 enzyme activity in humans. More recently, single nucleotide polymorphisms in ADH3 have been reported to be associated with childhood risk of asthma, although the functional relevance of these polymorphisms has not been published (Wu et al., 2007).

Alterations in THF pathways may also have an impact on formaldehyde toxicity. These could result from polymorphisms in various enzymes or differences in folate intake and absorption. Species differences in tetrahydrofolate levels (Black et al., 1985) are thought to play a role in the differential responses to methanol across species. Cook et al. (2001) speculate that rats have redundant pathways for formate clearance that may be absent or less efficient in primates.

3.5. ENDOGENOUS SOURCES OF FORMALDEHYDE

Endogenous formaldehyde is produced through normal cellular metabolism through enzymatic or nonenzymatic reactions, and also as a detoxification product of xenobiotics during cellular metabolism.

3.5.1.1. *Normal Cellular Metabolism (Enzymatic)*

Formaldehyde is produced during normal metabolism of methanol, amino acids (e.g., glycine, serine, and methionine), choline, dimethylglycine, and methylamine and through the folate-dependent endogenous one-carbon pool, etc.

a) One of the endogenous sources for formaldehyde production is methanol, formed during normal cellular metabolism. However, this fraction may also be derived through consumption of fruits, vegetables and alcohol (Shelby et al., 2004; IPCS, 1997). In studies conducted with healthy humans whose diet was devoid of methanol-containing or methanol-generating foods (such as cereals containing aspartame, a precursor of methanol) and who abstained from alcohol consumption, the background blood levels of methanol range from 0.25−4.7 mg/L (reviewed in Shelby et al., 2004 [CERHR]). Methanol is metabolized to formaldehyde predominantly by hepatic alcohol dehydrogenase-1 (ADH1) in primates and by ADH1 and catalase (CAT) in rodents, ADH1 requiring nicotinamide adenine dinucleotide (NAD^+) as a cofactor.

b) Dimethylglycine (DMG), one of the byproducts of choline metabolism endogenously present in the body, is an indirect source of endogenous formaldehyde. Two specific dehydrogenases, (a) dimethylglycine dehydrogenase (DMGDH) which converts DMG to sarcosine (methylglycine) and (b) sarcosine dehydrogenase (SDH) which converts sarcosine to glycine, have been shown to noncovalently bind to the folate enzyme,

tetrahydrofolate (THF). Further, these dehydrogenases form "active formaldehyde" by removing the 1-carbon groups from THF (Binzak et al., 2000).

c) Another source of endogenous formaldehyde is methylamine (MA), an intermediary component of the metabolism of adrenaline, sarcosine, creatine, lecithin, and other dietary sources (Yu and Zuo, 1996). The enzyme semicarbozole-sensitive amine oxidase (SSAO), predominantly present in the plasma membrane of endothelial smooth muscle cells and in circulating blood, converts methylamine to formaldehyde, hydrogen peroxide and ammonia. The formaldehyde thus released has been shown to cause endothelial injury eventually leading to atherosclerosis (Kalasz, 2003). Yu et al. (1997) have shown that adrenaline, released in the body as a response to stress, is known to be deaminated by the enzyme monoamine oxidase, with further conversion of methylamine to formaldehyde by SSAO (Yu et al., 1997). Creatine is another precursor for methylamine which is metabolized by SSAO to form formaldehyde. It has been shown that short-term, high-dose dietary supplementation of creatine in healthy humans causes a significant increase in urinary methylamine and formaldehyde levels (Poortmans et al., 2005).

d) Endogenous formaldehyde is also a constituent of the one-carbon pool, a network of interrelated biochemical reactions that involve the transfer of one-carbon groups from one compound to another (usually the transfer of the hydroxymethyl group of serine to tetrahydrofolic acid).

Tyihak et al. (1998) have demonstrated that formaldehyde, but not the methyl radical or methyl cation, is involved in the enzymatic transmethylation and demethylation reactions, and suggested the presence of a formaldehyde cycle in cells for the production and removal of formaldehyde utilizing the transfer through methionine → S-adenosylmethionine → S-adenosyl-homocysteine → homocysteine (Tyihak et al., 1998). However, these studies did not clearly show whether the formaldehyde released in this cycle is in free or bound form.

Formaldehyde has been shown to be produced in normal and leukemic leukocytes from N^5-methyl-THF by enzymatic degradation (Thorndike and Beck, 1977). This is a two-step reaction involving (1) enzymatic conversion of the methyl-THF to formaldehyde followed by (2) nonenzymatic reaction of formaldehyde with an amine. Thorndike and Beck (1977) showed that leukocyte (granulocyte and lymphocyte) cell extracts from normal individuals and patients with chronic lymphocytic leukemia (CLL) or chronic myelocytic leukemia (CML) incubated with ^{14}C-methyl-THF and saturating amounts of tryptamine produced free formaldehyde which is detected as its corresponding carboline derivative formed with tryptamine. These results demonstrate the activity of the enzyme N^5, N^{10}-methylene THF reductase which oxidizes N^5-methyltetrahydrofolate to N^5, N^{10} methylene THF. The authors noted that the enzyme levels were in the order of normal granulocytes < normal lymphocytes < granulocytes from a CML individual < lymphocytes from a CLL individual (Thorndike and Beck, 1977), suggesting

increased activity of formaldehyde producing enzyme in leukemic cells compared to normal leukocytes. Overall, formaldehyde might be a byproduct as well as an intermediary product in several of these reactions.

3.5.1.2. *Normal Metabolism (Nonenzymatic)*

i) Formaldehyde can also be formed nonenzymatically by the spontaneous reaction of methanol with hydroxyl radicals, wherein cellular hydrogen peroxide is the precursor for hydroxyl radicals generated through Fenton reaction (Cederbaum and Qureshi, 1982).

ii) Another mechanism of nonenzymatic production of formaldehyde is through lipid peroxidation of polyunsaturated fatty acids (PUFA) (Shibamoto, 2006; Slater, 1984). In this mechanism, reactive oxygen species (ROS) generated during oxidative stress abstract a hydrogen atom from a methylene group of polyunsaturated fatty acids (PUFA) in cell membranes causing autooxidation of lipids with the eventual production of free radicals (e.g., peroxy radical). It is known that a certain level of oxidative stress and lipid peroxidation does occur in normal individuals, and these cellular metabolic processes are likely to contribute to endogenous formaldehyde production.

3.5.1.3. *Exogenous Sources of Formaldehyde Production*

Microsomal cytochrome P450 enzymes catalyze oxidative demethylation of N-, O- and S-methyl groups of xenobiotic compounds whereby formaldehyde is produced as a primary product, which is subsequently incorporated into the one-carbon pool by reacting with tetrahydrofolic acid or is oxidized to formate (Dahl and Hadley, 1983; Heck et al., 1982). Also, some special peroxidases, such as peroxide-dependent horseradish peroxidase enzymatically catalyze xenobiotics to generate formaldehyde in the body. In particular, an ethyl peroxide-dependent horseradish peroxidase has been shown to act on *N*,*N*-dimethylaniline and produce equimolar amounts of *N*-methylaniline and formaldehyde (Kedderis and Hollenberg, 1983).

The tobacco-specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), is another source of formaldehyde. It has been shown that formaldehyde is also produced during the methyl hydroxylation of NNK by rat liver microsomes (Castonguay et al., 1991). Also recent studies have demonstrated the formation of formaldehyde-DNA adducts in NNK-treated rats using a highly sensitive liquid chromatography-electrospray ionization-tandem mass spectrometry with selected reaction monitoring (Wang et al., 2007), suggesting formation of formaldehyde from nitrosamines. Cigarette smoke is also a source of exogenously produced methylamine which is converted to formaldehyde by SSAO (Yu, 1998).

3.5.1.4. *Metabolic Products of Formaldehyde Metabolism (e.g., Formic Acid)*

Formate is converted to carbon dioxide (CO_2) in rodents predominantly by a folate-dependent enzyme pathway (Dikalova et al., 2001). Formate is also oxidized to CO_2 and water by a minor pathway involving catalase located in rat liver peroxisomes (Waydhas et al., 1978; Oshino et al., 1973). In the folate-dependent pathway, tetrahydrofolate (THF)-mediated oxidation of formate and the transfer of one-carbon compounds between different derivatives of THF has been described.

Endogenous levels of formate also will be affected by dietary intake of methanol-producing or methanol-containing diets since methanol is initially converted to formaldehyde and eventually metabolized to formate. It has been shown in several studies in human subjects who were restricted on consuming methanol producing diets, aspartame or alcohol, that the endogenous blood concentrations of formate ranged from 3.8 to 19.1 mg/L (Shelby et al., 2004 [CERHR]). The biological half life of formic acid is 77−90 minutes (Owen et al., 1990b). The levels of formate in the urine of unexposed individuals range from 11.7 to 18 mg/L (Boeniger, 1987). One source of formic acid intake is through diet which ranges from 0.4 to 1.2 mg per day (Boeniger, 1987). The half life for plasma formate is ~30 minutes or longer (Boeniger, 1987).

3.5.1.5. *Levels of Endogenous Formaldehyde in Animal and Human Tissues*

Heck et al. (1982) estimated that endogenous levels of formaldehyde (free as well as bound) in rats ranged from 0.05 to 0.5 µmole/g (1.5−15 µg/g) of wet tissue as analyzed by the stable isotope dilution with GC-MS method (Heck et al., 1982). Although the levels of free formaldehyde cannot be measured due to their high reactivity and short half life, they were calculated by Heck et al. (1985) using an indirect method. They added a molar excess of GSH or THF to the test tube containing formaldehyde in aqueous solution enabling complete binding. When estimated, they observed that the amount of formaldehyde detected was equal to the total amount added to the reaction suggesting that the formaldehyde measured contained both free and bound forms. Further, they calculated the free formaldehyde concentration using the dissociation constant of the HMGSH adduct and cellular concentration of GSH. Human formaldehyde dehydrogenase has been shown to have a dissociation constant of 1.5 mM for the formaldehyde-GSH hemithioacetal adduct (Uotila and Koivusalo, 1974), while the folate enzyme product N^5,N^{10}-methylene-THF has a dissociation constant of 30 mM (Kallen and Jencks 1966a, b). This could be evaluated using the Michaels-Menton constant (K_m) of formaldehyde dehydrogenase for the GSH adduct (~4 µM at 25°C), whereby they calculated the free formaldehyde level to be around 3−7 µM or 1−2% of the total formaldehyde as measured by GC-MS in rat tissues (Heck 1982).

Cascieri and Clary (1992) estimated the total body content of formaldehyde in human body based on the following assumptions. For an individual with an average body wt of 70 kg and with body fluids accounting for 70% of body weight, total formaldehyde content is distributed in ~49 kg of body mass or 49 L of body fluids, owing to the water solubility and uniform distribution of formaldehyde in body fluids. It has been shown that the average blood concentration (mean ± S.E.) of formaldehyde in unexposed rats and humans was 2.24 ± 0.07 and 2.61 ± 0.14 μg/g of blood, respectively (Heck et al., 1985), and in unexposed rhesus monkeys it was 2.42 ± 0.09 μg/g of blood (Casanova et al., 1988), overall giving an average of approximately 2.5 ppm (2.5 mg/L) formaldehyde across the species. All these studies used pentafluorophenyl hydrazine derived formaldehyde using GC-MS analysis (see Table 3-3). Assuming these values, the body content of total formaldehyde is 122.5 mg (49 L × 2.5 mg/L) or 1.75 mg/kg body wt at any given time. Formaldehyde given intravenously to rhesus monkeys has been shown to have a half life of ~1.5 minutes in blood, wherein formaldehyde in blood was measured by the dimedone method (McMartin et al., 1979). Using this information Cascieri and Clary (1992) calculated that the human body generates approximately 40.83 mg/minute [(122.5 mg/2 × 1.5] of formaldehyde. Biotransformation of formaldehyde to carbon dioxide in the liver alone has been estimated at 22 mg/minute (Owen et al., 1990a).

Free formaldehyde is detected in body fluids and tissues using dimedone (Szarvas et al., 1986) or 2,4-dinitrohenyhydrazine (DNPH) or pentafluorophenyl hydrazine (PFPH) derivative (Heck et al., 1985) or as a fluorescent derivative (Luo et al., 2001) as trapping agent and detected by analytical techniques such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) and gas-chromatography mass spectrometry (GC-MS). Data from several studies is summarized in Table 3-3. Using ^{14}C-labeled dimedone, a chemical which condenses with free formaldehyde forming a product termed “formaldemethone” enabling radiometric detection, Szarvas et al. (1986) estimated the levels of endogenous formaldehyde in human blood plasma to be 0.4−0.6 μg/mL and in human urine to be 2.5−4 μg/mL (Szarvas et al., 1986).

Hileman (1984) reported that the endogenous levels of metabolically derived formaldehyde will be in the range of 3−12 ng/g of tissue (Hileman, 1984). So for an average 70 kg individual, the endogenous level of metabolically derived formaldehyde would be 210 μg to 840 μg (3−12 ng/g × 0.001 μg/ng × 1,000 g/kg × 70 kg).

Table 3-3. Endogenous formaldehyde levels in animal and human tissues and body fluids

Tissue	Method	Detected as	Formaldehyde levels	Reference

Not specified	Not specified	Not specified	0.003−0.012 ppm (3−12 ng/g)	Hileman 1984
Not specified	GC-MS with stable isotope dilution method	As PFPH-derivative	1.5−15 ppm (0.05−0.5 µmole/g)	Heck et al., 1982a
Blood	GC-MS with select ion monitoring	As PFPH-derivative	2.24 ± 0.07 ppm (2.24 ± 0.07 µg/g)	Heck et al., 1985
Blood	GC-MS with select ion monitoring	As PFPH-derivative	2.61 ± 0.14 ppm (2.61 ± 0.14 µg/g)	Heck et al., 1985
Plasma	Reverse phase HPLC-fluorescent detection	As product of ampicillin	1.65 ppm (1.65 µg/mL)	Luo et al., 2001
Heart perfusate	HPLC	As DNPH adduct	0.089−0.126 ppm (2.98−4.21 nmol/mL)	Shibamoto 2006
Blood	GC-MS with select ion monitoring	As PFPH-derivative	2.42 ± 0.09 ppm (2.42 ± 0.09 µg/g)	Casanova et al., 1988
Plasma	Radiometric method	As formalde-methone adduct	0.4−0.6 ppm (0.4−0.6 µg/mL)	Szarvas et al., 1986
Urine	Radiometric method	As formalde-methone adduct	2.5−4.0 ppm (2.5−4.0 µg/mL)	Szarvas et al., 1986

Values in the parenthesis, originally cited in the references, are converted to parts per million (ppm) as indicated. PFPH, pentafluorophenyl hydrazone derivative; DNPH, dinitrophenyl hydrazine; GC-MS, gas-chromatography mass spectrometry; HPLC, high performance liquid chromatography.

3.6. EXCRETION

The main product of metabolic clearance of formaldehyde is formate, which is further metabolized to CO_2 and water, incorporated into the one-carbon pool, or eliminated in the urine. There is also some evidence that formaldehyde is present in exhaled breath; however, it is unclear whether this originates from endogenous sources, or is simply a function of ambient formaldehyde dissolved in fluids lining POEs. The following sections describe first experiments in laboratory species and then available data in humans. Broadly, these studies address two important questions that may be of relevance for risk assessment. First, it may be of interest to know what levels of formaldehyde are exhaled for comparison with inhaled levels, and whether there is any relationship between external exposure and exhaled levels. Second, there are recent studies that have attempted to relate genetic polymorphisms and changes in gene transcription level to levels of putative urinary formaldehyde biomarkers.

3.6.1. Formaldehyde Excretion in Rodents

Heck et al. (1983) determined the relative contributions of various excretion pathways in F344 rats following inhalation exposure to formaldehyde. Table 3-4 indicates that the relative excretion pathways were independent of exposure concentration (at least between 0.63 and 15 ppm). Nearly 40% of inhaled [^{14}C]-formaldehyde appeared to be eliminated via expiration, probably as CO_2 (it should be recalled that nearly 100% of inhaled formaldehyde is absorbed). Within 70 hours of a 6-hour exposure to formaldehyde, about 17 and 5% were eliminated in the urine and feces, respectively. Nearly 40% of inhaled [^{14}C]-formaldehyde remained in the carcass, presumably due to metabolic incorporation.

Table 3-4. Percent distribution of airborne [^{14}C]-formaldehyde in F344 rats

Source	Concentration of formaldehyde (ppm)	
	0.63	13.1
	Distribution (%)[a]	
Expired air	39.4 ± 1.5	41.9 ± 0.8
Urine	17.6 ± 1.2	17.3 ± 0.6
Feces	4.2 ± 1.5	5.3 ± 1.3
Tissues and carcass	38.9 ± 1.2	35.2 ± 0.5

[a]Values are means ± standard deviations (n = 4).

Source: Heck et al. (1983).

Mashford and Jones (1982) examined elimination pathways of formaldehyde in rats exposed by I.P. injection. Urine and exhaled gases were collected from rats exposed to 4 or 40 mg/kg [^{14}C]-formaldehyde. At 48 hours postinjection, 82 and 78% of the radiolabel were exhaled as $^{14}CO_2$, whereas exhaled [^{14}C]-formaldehyde was not detected. Mashford and Jones (1982) also further identified the urinary metabolites. Five hours after injection of the higher dose, formate was determined to comprise 80% of the urinary metabolites. The authors were unable to detect cysteine derivatives observed in other studies (see below) in the urine of these rats prior to or after formaldehyde exposure. The authors stated that if formaldehyde were to be excreted in urine containing cysteine, then thiazolidine-4-carboxylate (TZCA) would likely be produced. They speculated that species differences in urinary compounds may produce formaldehyde conjugates (or artifacts).

Hemminki (1982) reacted formaldehyde and acetaldehyde with cysteine, N-acetylcysteine, and GSH and found that formaldehyde reacted most rapidly with cysteine to form TZCA. Similarly, acetaldehyde reacted preferentially with cysteine, albeit slower than formaldehyde, to form a thiazolidine derivative. However, when each aldehyde was administered I.P. (10% formaldehyde, 50% acetaldehyde), thioether concentrations (nmol/mol creatinine) significantly increased in the 24 and 48 hour urine of acetaldehyde-treated rats but not formaldehyde-treated rats. These data suggest that formaldehyde is not appreciably excreted in urine and thus cysteine conjugates are not likely to represent formaldehyde exposure.

Most recently, Shin et al. (2007) attempted to show that formaldehyde inhalation increased urinary TZCA levels in Sprague-Dawley rats. Treated rats were exposed to 3.1 and 38.1 ppm formaldehyde for 6 hours/day for 2 weeks, and urine was collected for 3 days. The TZCA level in four control rats was 0.07 ± 0.02 mg/L, whereas levels in the 3 and 38 ppm groups were 0.18 ± 0.045 and 1.01 ± 0.36, respectively. Notably, the concentrations in the four highest exposed animals (0.71, 0.70, 1.20, and 1.43 ppm) exhibited a nearly twofold range. However, these comparisons are confounded if the exposures have any influence on urine production and urine cysteine levels. The study does not provide any data that might allow one to examine this issue.

3.6.2. Formaldehyde Excretion in Exhaled Human Breath

Several human and animal studies have attempted to measure the concentration of formaldehyde in exhaled breath. However, study design and limitations of available analytical techniques have resulted in little data which provide a basis for determining levels of formaldehyde in exhaled breath either from normal metabolism (in humans), or when formaldehyde is administered (animal study). The two major limitations of studies of human breath include the potential for false positives for formaldehyde from the primary analytical technique for breath analysis and the need for concurrent room air controls.

A recent study has illustrated that the use of proton transfer reaction in SIFT-MS may result in false positive results for formaldehyde as the characteristic analytical product ion for formaldehyde is also produced from methanol and ethanol found in exhaled breath (Španěl and Smith, 2008). Proton transfer reaction mass spectrometry (PTR-MS) has been applied to measure trace compounds in exhaled breath including volatile organics and specifically formaldehyde. The basic method of PTR-MS is based on the transfer of protons from H_30^+ to gases in exhaled breath and the in-line monitoring of products where gases are tentatively identified by the mass to charge ratio (*m/z*) where an *m/z* of 31 is consistent with protonated formaldehyde (Hansel et al., 1995; Lindinger et al., 1998). It is important to note that reaction

products from methanol and ethanol may also produce fragments with an *m/z* ratio of 31 (Kusch et al., 2008). Selected ion flow tube mass spectrometry (SIFT-MS) is an application of PTR-MS developed for real-time analysis of trace gases in breath (Smith and Španěl, 2005; Španěl and Smith, 2007). As shown in Figure 3-5 up to 1% of the mass of ethanol and methanol in exhaled breath may be detected with a mass-to-charge ratio (m/z ratio) of 31—which may have been reported as formaldehyde in earlier publications (Kusch et al., 2008; Španěl and Smith, 2008). The authors have improved the SIFT-MS software used in exhaled breath analysis to adjust the reported formaldehyde levels by accounting for the contribution of methanol and ethanol to the characteristic analytical product ion for formaldehyde (m/z- = 31). No published articles were available on formaldehyde in exhaled breath which adjusted for methanol and ethanol levels in exhaled breath. Therefore, the available articles discussed below will be evaluated with respect to the potential for ethanol or methanol to influence the reported formaldehyde levels.

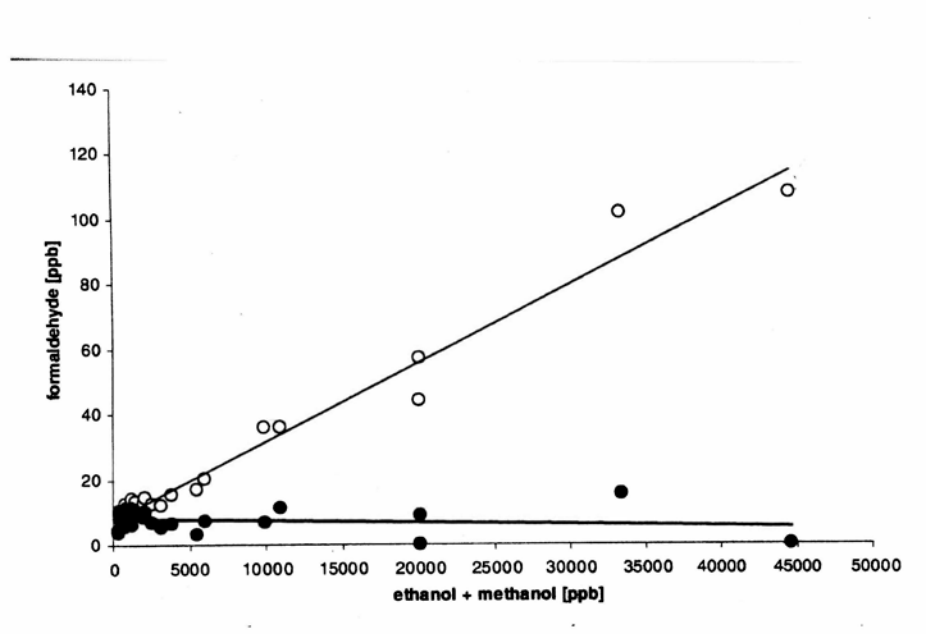

Figure 3-5. Detection of the characteristic analytical product ion for formaldehyde (m/z ratio of 31) by proton transfer reaction mass spectrometry (PTR-MS) in gas samples spiked with only methanol and ethanol. Open circles show the reported formaldehyde without adjustment for the methanol and ethanol present (each of which produces a small fraction of the analytical product with an m/z ratio of 31). Closed circles represent the same data, corrected by the SIFT-MS software to control for methanol and ethanol.

Source: Španěl and Smith (2008).

Six articles were located which reported formaldehyde levels in exhaled breath, three of which provide level of methanol and ethanol in exhaled breath in the same individuals or study group and are further discussed below (Wang et al., 2008; Cap et al., 2008; and Moser et al., 2005). Although Wehinger et al., (2007) report a compound tentatively identified as formaldehyde correlated with a diagnosis of lung caner, the PTR-MS was not controlled for any contribution of ethanol and methanol, and the levels of these compounds were not provided for comparison so it is not further discussed here. Turner et al. (2008) measured levels of volatile compounds including formaldehyde in exhaled breath five healthy males. The subjects fasted overnight, and measurements were taken before and after ingesting 75 g of glucose. The source of the inhaled air was laboratory air which contained an unreported concentration of formaldehyde. Formaldehyde was not detected in the exhaled breath of any subjects (5 ppb limit of detection) ethanol and methanol levels were not reported.

In a study designed to compare volatile organics in exhaled breath of smokers and nonsmokers, compounds tentatively identified as formaldehyde and methanol were not different between the populations (Kushch et al., 2008). The authors acknowledge that the reported formaldehyde (m/z = 31) might also represent fragments of reaction products from methanol and ethanol. Reported formaldehyde levels were approximately 5% of the methanol (e.g., mean of 9.9 ppb versus 208 ppb respectively).

Wang et al. (2008) measured the concentrations volatile organics, including formaldehyde, in the exhaled breath through the nose or mouth, and oral cavity during breath holding of three healthy male laboratory workers. Measurements were taken in each individual over a period of a month, 20 workdays. Formaldehyde levels (4−7 ppb) were lower than the inspired laboratory air (9.6 ppb) (see Table 3-5). Formaldehyde in the mouth during breath holding, did not differ from the exhaled air (nose or mouth). The SIFT-MS analysis did not adjust for any contribution of ethanol or methanol to the tentatively identified formaldehyde levels. Although only means are reported, a comparison of results in Table 3-5 does indicate that 1% of the reported ethanol and methanol may have contributed significantly to the reported formaldehyde levels.

Cáp et al. (2008) evaluated relationships between volatile organic compounds measured in exhaled breath and exhaled breath condensate. Exhaled breath condensate consists of aerosolized particles of airway lining fluid evolved from the airway wall by turbulent airflow that serve as seeds for substantial water vapor condensation, which then serves to trap water soluble volatile gases. This study also attempted to ascertain whether the source of each compound was endogenous or exogenous. According to the published article and electronic

communication with Dr. Patrik Španěl, a coauthor for this study, the limit of quantification was 3 ppb or better.

Table 3-5. Measurements of exhaled formaldehyde concentrations in the mouth and nose, and in the oral cavity after breath holding in three healthy male laboratory workers. The median levels are estimated as the geometric mean with the associated standard deviation (σ.)

Subject		Methanol (median ppb/σ)	Ethanol (median ppb/σ)	Formaldehyde (median ppb/σ)
A	Mouth	178/1.2	236/1.6	5/2.3
	Nose	167/1.2	28/1.3	7/2.1
	Oral cavity	149/1.2	412/1.4	5/2.3
B	Mouth	300/1.4	64/1.6	7/2.3
	Nose	396/1.4	27/1.4	5/2.1
	Oral cavity	358/1.4	93/1.4	6/1.9
C	Mouth	228/1.5	153/1.5	4/2.5
	Nose	229/1.5	26/1.4	6/1.9
	Oral cavity	162/1.7	163/1.4	6/1.9
Laboratory air		44 ± 9	101 ± 52	9.6 ± 1.5

Notes: The limit of quantification for formaldehyde was not reported.
Source: Wang et al. (2008).

However, the SIFT-MS protocol used in this study did not adjust for any contribution of ethanol or methanol to reported formaldehyde levels. Unadjusted reported formaldehyde levels in the direct exhaled breath of 34 subjects (25 to 62 years; 11 males; 2 smokers) varied from 0 to 12 ppb with a mean of 2 ppb and a median of 1 ppb (see Table 3-6). Measurements of formaldehyde in exhaled breath condensate ranged from 0 to 12 ppb with a mean of 2 ppb and a median of 0 ppb. All but one measurement was below the average ambient room air concentration of 9.6 ± 1.5 ppb. Although comparisons on the individual level could not be made from the data as reported, the range of ethanol and methanol levels in exhaled breath indicate that 1% of the reported ethanol and methanol may have contributed significantly to the reported formaldehyde levels in exhaled breath (see Table 3-6). It is unclear if the reported formaldehyde may represent in part inhaled formaldehyde, reduced by absorption in the upper respiratory tract, or is an artifact of the reported methanol and ethanol levels.

Table 3-6. Formaldehyde, methanol and ethanol levels reported in the exhaled breath of 34 subjects (25 to 62 years; 11 males; 2 smokers)

Chemical	Minimum (ppb)	Maximum (ppb)	Mean (ppb)	Median (ppb)
Methanol	102	2319	297	189
Ethanol	27	10262	447	82
1% of the reported levels of both ethanol and methanol	1.3	125	7.3	2.6
Formaldehyde (tentatively identified with a m/z ratio n = 31)	0	12	2	1

Source: Cáp et al. (2008).

Moser et al. (2005) measured levels of 179 volatile organic compounds (VOCs) in the exhaled breath of 344 individuals. This study was not designed to ascertain whether exhaled formaldehyde is of endogenous origin, but rather to demonstrate that proton transfer reaction-mass spectrometry can be used as a new method for rapid screening of large collectives for risk factors (e.g., smoking behavior), potential disease biomarkers, and ambient air characterization. The study was conducted at a health fair. The test subjects had a mean age of 61.6 years; 63% were males and 14% were smokers. Samples of room air were collected and evaluated in parallel with exhaled breath samples. The authors note that formaldehyde was detected in room air, but did not report the levels; rather they stated that the background concentrations were negligible. Of the 179 volatile organic compounds measured, data were reported for 14, including formaldehyde and formic acid. The report by Moser et al. (2005) does not provide the limit of detection for any of the compounds measured or details of the analytical method. Moser et al. (2005) do note that significant differences in exhaled breath composition could be found between smokers and nonsmokers for 32 of the 179 chemicals measured, but the 32 chemicals were not named and no substantiating data were provided.

The formaldehyde levels in exhaled breath spanned from 1.2 to 72.7 ppb with a median of 4.3 ppb and 75th percentile of 6.3 ppb (see Table 3-7) (Moser et al., 2005). The reported levels of formaldehyde (m/z ratio = 31) we not adjusted for any potential contribution from methanol or ethanol in exhaled breath. The levels of methanol and ethanol in exhaled breath were reported by Moser et al. (2005). Although the summary statistics do not allow comparison

of individual results, it is possible that reaction fragments from methanol and ethanol may have contributed to the reported formaldehyde levels (see Table 3-7).

Table 3-7. Apparent formaldehyde levels (ppb) in exhaled breath of individuals attending a health fair, adjusted for methanol and ethanol levels which contribute to the detection of the protonated species with a mass to charge ratio of 31 reported as formaldehyde (*m/z* = 31)

Chemical	Minimum	25th percentile	Median	75th percentile	97.5th percentile	Maximum
Methanol	13.367	106.227	161.179	243.185	643.614	1536.499
Ethanol	11.583	23.1	34.664	64.24	549.24	9779.768
1% of the reported levels of both ethanol and methanol	0.25	1.29	1.96	3.07	11.93	113.16
Mass of *m/z* = 31 reported as formaldehyde	1.23	3.1	4.26	6.33	39.8	72.7

Source: Moser et al. (2005).

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The range of reported formaldehyde is much greater in this study of the general population (attendees at a health fair) than that observed in healthy volunteers discussed above (Wang et al., 2008; Cap et al., 2008; Turner et al., 2008; Kushch et al., 2008). Moser et al. (2005) do not discuss potential causes for this wide range in values, and there was no distinction of the data by sex, age, or health. However, reported formaldehyde in exhaled breath (unadjusted) has been correlated to lung cancer diagnosis with a median of 7.0 ppb and upper 95th CI greater than 30 ppb (Wehinger et al., 2007). Although it is unknown if these results represent only formaldehyde, or are in part an artifact of increased ethanol and methanol in exhaled breath, the higher levels reported by Moser et al. (2005) may reflect volatile levels in unhealthy individuals who attended the public health fair.

Selected ion flow tube mass spectrometry (SIFT-MS), with the recent improvements by Španěl and Smith (2008) to account for the fragments of methanol and ethanol reaction products, have the ability to detect formaldehyde in exhaled breath. However, to date, no data has been published which makes this adjustment for reporting formaldehyde levels. Therefore all of the above reports of formaldehyde in exhaled breath should be carefully interpreted as the mass reported as formaldehyde—is only tentatively identified as formaldehyde. A careful review of the data where methanol and ethanol levels are also provided, indicate that levels of formaldehyde (tentatively identified as m/z = 31) may reflect a significant contribution from

reaction products of methanol and ethanol. In summary, there are insufficient data at this time to confidently establish a concentration of formaldehyde in exhaled breath that can be attributed to endogenous sources. Additional research is needed to further clarify.

3.6.3. Formaldehyde Excretion in Human Urine

Gottschling et al. (1984) examined urinary formic acid in 35 veterinary students. Personal monitoring badges were worn and returned after class, and urine samples were taken prior to class and within 2 hours after the class. Mean exposure levels were about 100 ppb. Baseline averages of urinary formic acid (as a sodium salt) were 12.47 mg/L and ranged from 2.43 to 28.38 mg/L among subjects. Post exposure formate levels were slightly elevated but were not statistically significant. Moreover, formate levels decreased in several individuals relative to pre-exposure levels. The authors concluded that variability in urinary formate may mask any changes and that monitoring formate within 2 hours of exposure is not informative. It is worth noting, however, that interpretation of this finding is confounded due to the fact that diet was not controlled and because no markers for urinary normalization were employed (Boeniger, 1987).

Boeniger (1987) reviewed previously published data on formate in urine (some of which were in German). In one occupational study, workers were exposed to an average formaldehyde exposure of 1.28 mg/m^3 over a 6-hour work shift. This implies an average intake of 6 mg;[6] Boeniger reported a range of 2.5 to 13 mg. However, the original study reported that post-shift formate levels were 152 mg/L, whereas the levels were only 24 mg/L 6 days later (no exposure). Considering that only a small percentage of inhaled formaldehyde would be excreted in urine, it is unclear how (or whether) formaldehyde exposure, with the highest total dose of 13 mg, could be responsible for the observed increase.

In the previously described study by Shin et al. (2007), human urine samples were shown to contain TZCA, although variability was not reported. A subsequent study reported that urine TZCA levels were higher in individuals living in newer apartments (0.18 ± 0.121 mg/g creatinine) as compared to older apartments (0.097 ± 0.040 mg/g creatinine) (Li et al., 2007).[7] The authors cited this as evidence that TZCA is a urinary marker for formaldehyde exposure, even though TZCA levels were not correlated to measured (or estimated) formaldehyde exposures. The individuals also differed significantly in age (21.5 vs. 28.6, $p = 0.053$) and

[6] 1.28 mg/m^3/1,000 L/m^3 × 13.8 L/minute × 60 minutes/hour × 6 hours.
[7] This study is described in greater detail in Chapter 5.

differed in smoking percentage (10 vs. 27%). Clearly these two studies do not establish a relationship between human formaldehyde exposure and urine TZCA levels.

3.7. MODELING THE TOXICOKINETICS OF FORMALDEHYDE AND DPX

3.7.1. Motivation

Airway geometry is expected to be an important determinant of inhaled formaldehyde dosimetry in the respiratory tract and its differences across species. The uptake of formaldehyde in the upper respiratory tract is highly nonhomogeneous and spatially localized and exhibits strong species differences. Species differences in kinetic factors have been argued to be the key determinants of species-specific lesion distributions for formaldehyde and other reactive inhaled gases. Section 3.7.2 details the benefits to the quantitative risk assessment of modeling these dosimetric differences in the upper respiratory tract. While frank effects were seen only in the upper respiratory tract in rodents, mild lesions were also present in the major bronchiolar region of the rhesus monkey. Therefore, with regard to extrapolation of cancer risk from animal bioassays to humans, it appears that the upper and lower human respiratory tract should both be considered potentially at risk of developing formaldehyde-induced squamous cell carcinoma. Therefore, formaldehyde dose to the lower human respiratory tract also needs to be quantified in order to develop a dose-response relationship that considers the entire respiratory tract.

This assessment uses internal dose metrics computed by using fluid dynamic models to compute regional formaldehyde uptake in the F344 rat and human nasal passages and in the human lower respiratory tract. The assessment also uses estimates of DPX levels in the nasal lining predicted by physiologically-based pharmacokinetic models which use the fluid dynamic model derived estimates of formaldehyde flux to the tissue as input. These computational models enable the derivation of more accurate human equivalent concentrations from the animal bioassays than would be obtained by averaging over the respiratory surface area. The following sections provide the motivation for these calculations, and discuss the strengths and uncertainties associated with the data and the models and their relevance to the hypothesized mode of action are discussed in some length.

3.7.2. Species Differences in Anatomy: Consequences for Gas Transport and Risk

As discussed earlier, formaldehyde is highly reactive and water soluble (categorized as a category 1 gas), thus its absorption in the mucus layer and tissue lining of the upper respiratory tract is known to be significant. The regional inhaled dose of formaldehyde to the respiratory tract of a given species depends on the amount of formaldehyde delivered by inhaled air, the absorption characteristics of the nasal lining, and reactions in the tissue. The amount delivered

by inhaled air is a function of the major airflow patterns, air-phase diffusion, and absorption at the airway-epithelial tissue interface. The dose of formaldehyde to the epithelial tissue, which is different from the amount delivered, depends on the amount absorbed at the airway-tissue interface, water solubility, mucus-to-tissue phase diffusion, and chemical reactions, such as hydrolysis, protein binding, and metabolism. It has been argued strongly that species differences in these kinetic factors are determinants of species-specific lesion distributions for formaldehyde and other inhaled gases (Moulin et al., 2002; Bogdanffy et al., 1999; Ibanes et al., 1996; Monticello et al., 1996; Monticello and Morgan, 1994; Morgan et al., 1991).

Because of the convoluted nature of the airways in the upper respiratory tract, the absorption of such gases in the upper respiratory tract is highly nonhomogeneous. There are large differences across species in the anatomy of the upper respiratory tract (see Figure 3-6) and in airflow patterns (see Figure 3-7). Therefore, as shown in the simulations in Figure 3-8, it may be expected that the uptake patterns, and thus risk due to inhaled formaldehyde, will also show strong species dependence. Morgan et al. (1991) concluded that airflow-driven dosimetry plays a critical role in determining the site specificity of various formaldehyde-induced responses, including tumors, in the nose of the F344 rat. The convoluted geometry of the airway passages in the upper respiratory tract, as seen from the cross sections of the nose in Figure 3-6, renders an idealized representation of fluid flow and uptake profiles almost impossible. For these reasons, Kimbell et al. (1998, 1993), Kepler et al. (1998), and Subramaniam et al. (1998) developed anatomically realistic finite-element representations of the noses of humans, F344 rats, and rhesus monkeys. These representations were subsequently used in physical and computational models (see Figure 3-6). This assessment utilizes dosimetry derived from these representations.

An accurate calculation of species differences in formaldehyde dosimetry in the upper respiratory tract is important to the extrapolation problem for another reason. The upper respiratory tract in rats is an extremely efficient scrubber of reactive gases (97% uptake) (Morgan et al., 1986), thereby protecting the lower respiratory tract from gaseous penetration. On the other hand, there is considerably more fractional penetration of formaldehyde into the lower respiratory tract of the rhesus monkey than in the rat (see Figure 3-8). Therefore, an accurate determination of scrubbing in the upper respiratory tract is important to delineate species differences in dosimetry in both the upper and lower respiratory tract. Thus, in the case of the rhesus monkey, the model by Kepler et al. (1998) included the trachea. It is important to note that the models mentioned above represent nasal passages reconstructed from a single individual from each species (Kimbell et al., 2001a, b; Conolly et al., 2000; CIIT, 1999; Subramaniam et al., 1998). This is discussed later in the context of intraspecies variability.

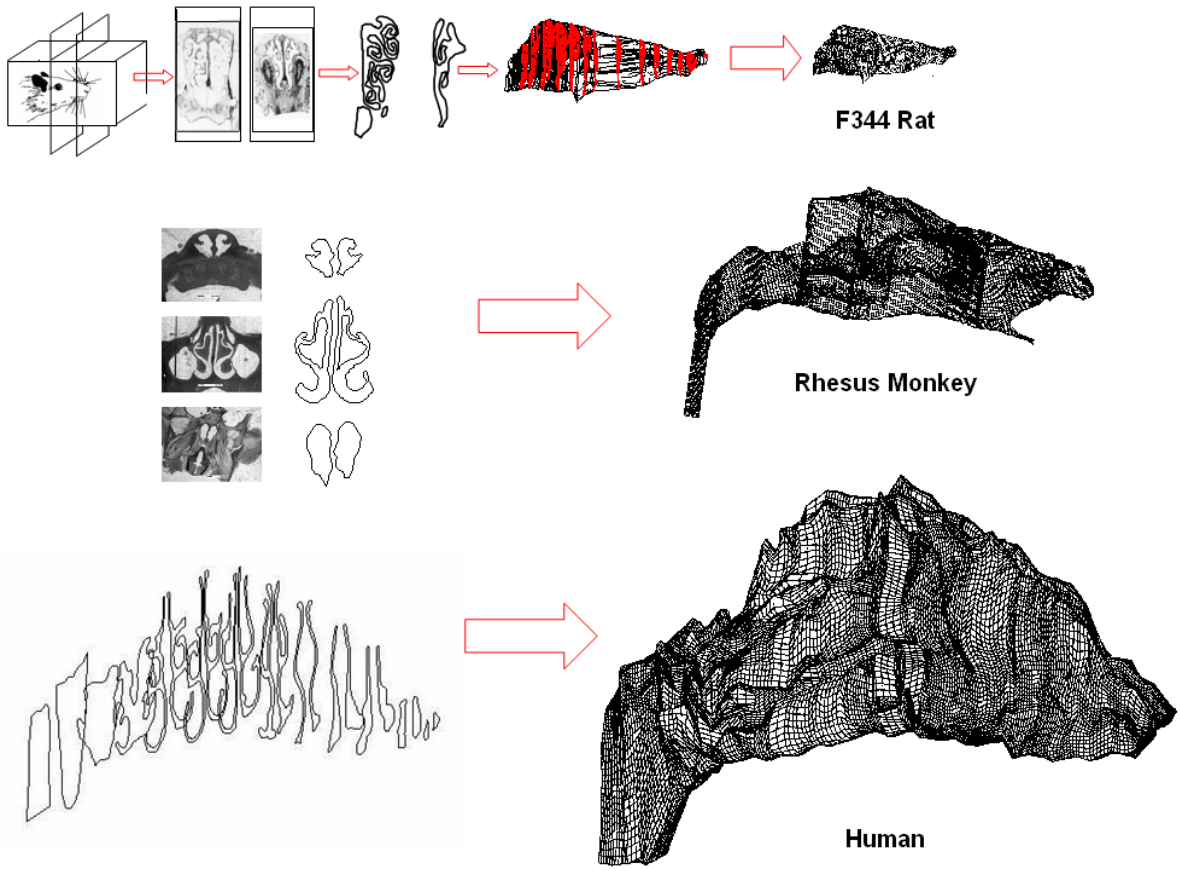

Figure 3-6. Reconstructed nasal passages of F344 rat, rhesus monkey, and human.

Note: Nostril is to the right, and the nasopharynx is to the left. Right side shows the finite element mesh. Left-hand side shows tracings of airways obtained from cross sections of fixed heads (F344 rat and rhesus monkey) and magnetic resonance image sectional scans (humans). Aligned cross sections were connected to form a three-dimensional reconstruction and finite-element computational mesh. Source: Adapted from Kimbell et al. (2001a). Additional images provided courtesy of Dr. J.S. Kimbell, CIIT Hamner Institutes.

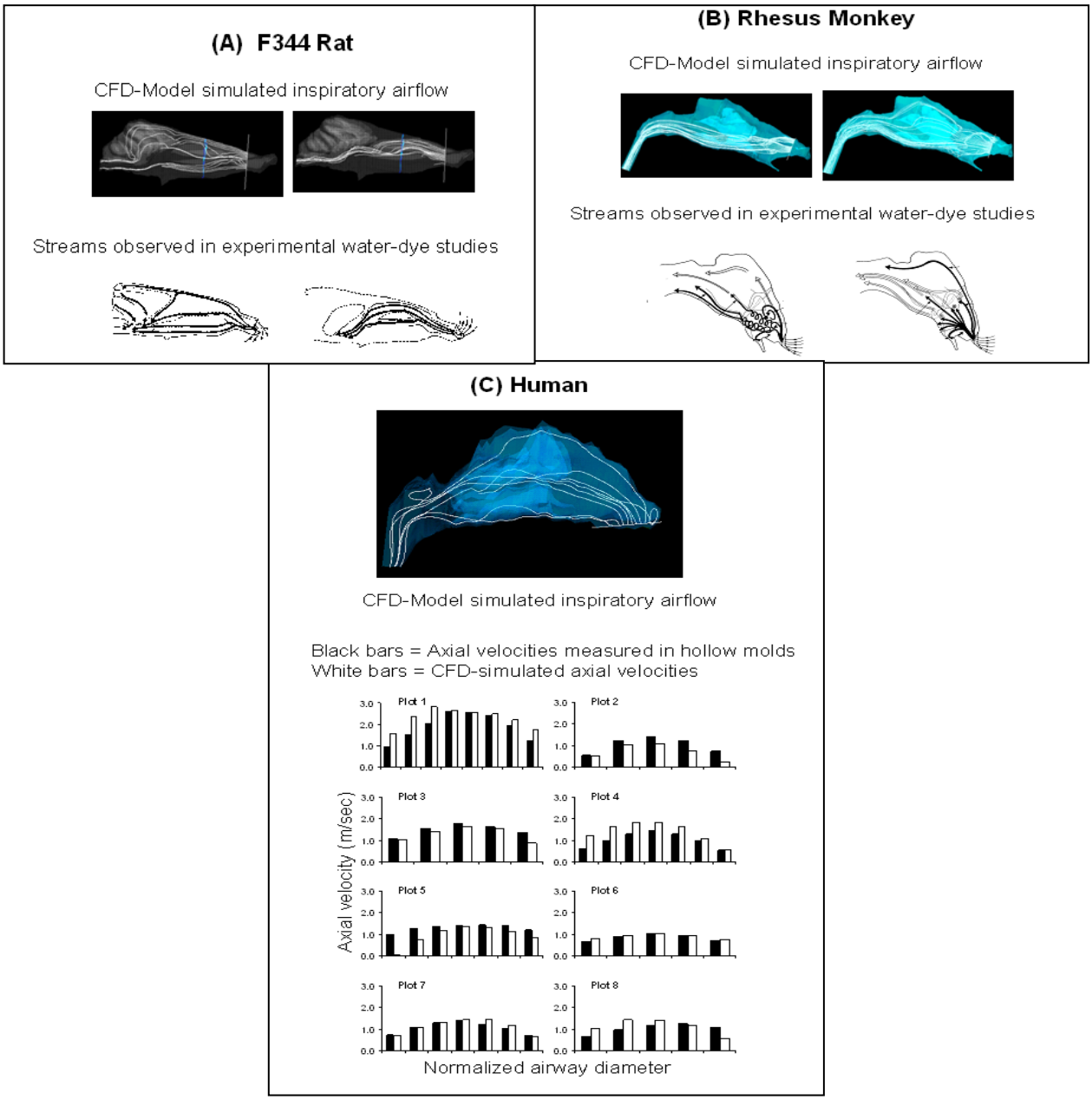

Figure 3-7. Illustration of interspecies differences in airflow and verification of CFD simulations with water-dye studies.

Note: Panels A and B show the simulated airflow pattern versus water-dye streams observed experimentally in casts of the nasal passages of rats and monkeys, respectively. Panel C shows the simulated inspiration airflow pattern, and the histogram depicts the simulated axial velocities (white bars) vs. experimental measurements made in hollow molds of the human nasal passages. Dye stream plots were compiled for the rat and monkey over the physiological range of inspiration flow rates. Modeled flow rates in humans were 15 L/minute. Source: Adapted from Kimbell et al. (2001a).

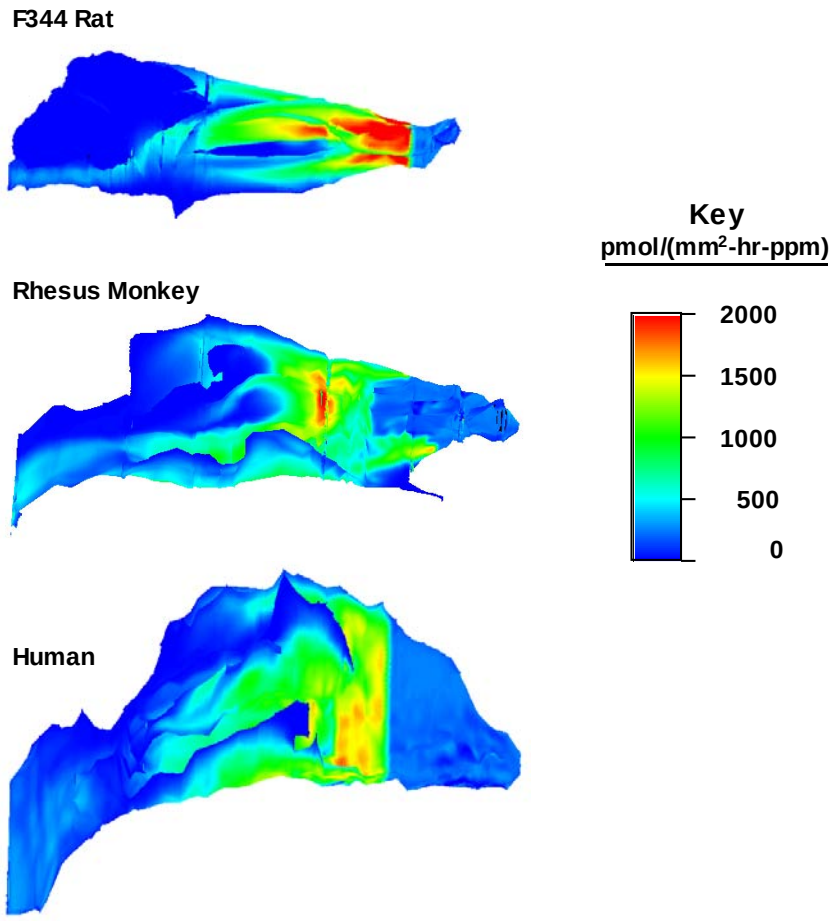

Figure 3-8. Lateral view of nasal wall mass flux of inhaled formaldehyde simulated in the F344 rat, rhesus monkey, and human.

Note: Nostrils are to the right. Simulations were exercised in each species at steady-state inspiration flow rates of 0.576 L/minute in the rat, 4.8 L/minute in the monkey, and 15 L/minute in the human. Flux was contoured over the range from 0−2,000 pmol/(mm^2-hour-ppm) in each species.

Source: Kimbell et al. (2001a).

The highly localized nature of uptake patterns shown in Figure 3-8 means that averaging uptake over the entire nasal surface area would dilute the regional dose over areas where response was observed and that an extrapolation based on such averaging would clearly not be accurate.

Another factor to consider in the extrapolation is that monkeys and humans are oronasal breathers while rats are obligate nose-only breathers. Thus, for humans and monkeys, oronasal or oral breathing implies a significantly higher uptake in the lower respiratory tract. It is known

that a significant fraction of the human population breathes normally through the mouth. Finally, activity profiles are also determinants of extraction efficiency (see Figure 3-9) and of breathing route (Niinimaa et al., 1981). Given the fact that formaldehyde-induced lesions were observed as far down the respiratory tract as the first bifurcation of the lungs in exposed monkeys, the entire human respiratory tract should be considered when extrapolating data from rats. Thus, for the human, Overton et al. (2001) attached an idealized single-path model of the lower respiratory tract to a model of the upper respiratory tract.

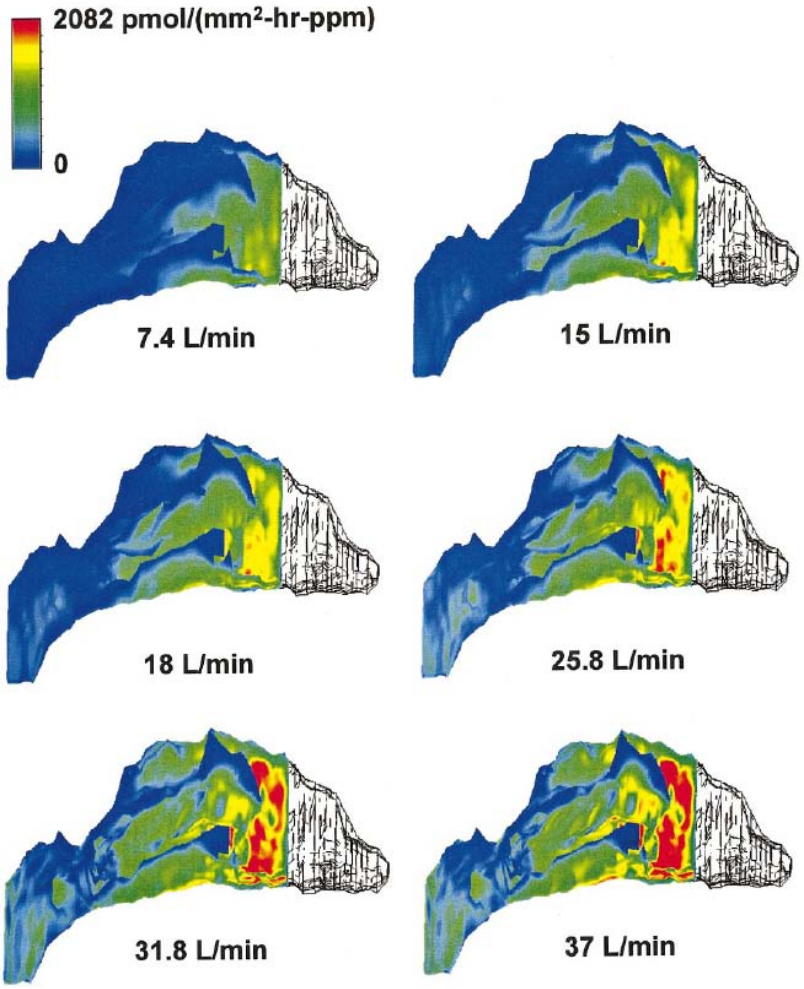

Figure 3-9. CFD simulations of formaldehyde flux to human nasal lining at different inspiratory flow rates.
Note: Right lateral view. Uptake is shown for the nonsquamous portion of the epithelium. The front portion of the nose (vestibule) is lined with keratinized squamous epithelium and is expected to absorb relatively much less formaldehyde.
Source: Kimbell et al. (2001b).

3.7.3. Modeling Formaldehyde Uptake in Nasal Passages

Computational models for air flow and formaldehyde uptake in the F344 rat, rhesus monkey, human nose, and human lung were developed by several scientists (Kimbell et al., 1998, 1993; Kepler et al., 1998; Subramaniam et al., 1998; Kimbell et al., 2001a, b). The F344 strain of the rat was chosen since it was assumed to be anatomically representative of its species and because it is widely used experimentally, most notably in bioassays sponsored by the National Toxicology Program. The approximate locations of squamous, mucus-coated, and nonmucus epithelial cells were mapped onto the reconstructed nasal geometry of the computer models. Taken together, these regions of nonmucus and mucus-coated cells comprise the entire surface area of the nasal passages (see original papers and CIIT [1999] for further details on reconstruction and morphometry). Types of nasal epithelium overlaid onto the geometry of the models were assumed to be similar in characteristics across all three species (rat, monkey, and human) except for thickness, surface area, and location. Species-specific mucosal thickness, surface area, and location were estimated from the literature or by direct measurements (Conolly et al., 2000; CIIT, 1999). The nasal passages of all three species were assumed to have a continuous mucus coating over all surfaces except specific areas in the nasal vestibule. As discussed at the beginning of this chapter, formaldehyde hydrolyzes in water and reacts readily with a number of components of nasal mucus. Absorption rates of inhaled formaldehyde by the nasal lining were therefore assumed to depend on where the epithelial lining is coated by mucus and where it is not.

To calculate an airflow rate that would be comparable among species, the amount of inspired air (tidal volume, V_T) was divided by the estimated time involved in inhalation (half the time a breath takes, or (1/2)(1/[breathing frequency, f]). Thus, an inspiratory flow rate was calculated to be $2V_Tf$, or twice the minute volume. Predicted flux values represent an average of one nasal cycle. Minute volumes were allometrically scaled to 0.288 L/minute for a 315 g rat from data given by Mauderly (1986). Simulations were therefore carried out at 0.576 L/minute for the rat.

The fluid dynamics modeling in the respiratory tract comprises two steps: modeling the airflow through the lumen (solution of Navier-Stokes equations) and modeling formaldehyde uptake by the respiratory tract lining (solution of convective-diffusion equations for a given airflow field). Details of these simulations, including boundary conditions for air flow and mass transfer, are provided in Kimbell et al. (2001a, b; 1998, 1993) and Subramaniam et al. (1998). Formaldehyde absorption at the airway-to-epithelial tissue interface was assumed to be proportional to the air-phase formaldehyde concentration adjacent to the nasal lining layer in

monkeys and humans (see the original paper [Kimbell et al., 2001a, b] for a more detailed elaboration of the calculations for these coefficients).

Because formaldehyde is highly water soluble and reactive, Kimbell at al. (2001a) assumed that absorption occurred only during inspiration. Thus, for each breath, flux into nasal passage walls (rate of mass transport in the direction perpendicular to the nasal wall per mm^2 of the wall surface) was assumed to be zero during exhalation, with no backpressure to uptake built up in the tissues. Overton et al. (2001) estimated the error due to this assumption to be small, roughly an underestimate of 3% in comparison to cyclic breathing. Also, this assumption is the same as that used in default methods for reference concentration determination and has been used in other PBPK model applications to describe nasal uptake (Andersen and Jarabek, 2001).

3.7.3.1. *Flux Bins*

A novel contribution of the CIIT biologically motivated dose-response model is that cell division rates and DPX concentrations are driven by the local concentration of formaldehyde. These were determined by partitioning the nasal surface by flux, resulting in 20 "flux bins." Each bin was comprised of elements (not necessarily contiguous) of the nasal surface that receive a particular interval of formaldehyde flux per ppm of exposure concentration (Kimbell et al., 2001a, b). The spatial coordinates of elements comprising a particular flux bin were fixed for all exposure concentrations, with formaldehyde flux in a bin scaling linearly with exposure concentration (ppm). Thus, formaldehyde flux was expressed as pmol/(mm^2-hour-ppm).

3.7.3.2. *Flux Estimates*

Formaldehyde flux was estimated for the rat, monkey, and human over the entire nasal surface and over the portion of the nasal surface that was lined by nonsquamous epithelium. Formaldehyde flux was also estimated for the rat and monkey over the areas where cell proliferation measurements were made (Monticello et al., 1991, 1989) and over the anterior portion of the human nasal passages that is lined by nonsquamous epithelium. Figure 3-8 shows the mass flux of inhaled formaldehyde to the lateral wall of nasal passages in the F344 rat, rhesus monkey, and human (Kimbell et al., 2001a, b).

Maximum flux estimates for the entire upper respiratory tract were located in the mucus-coated squamous epithelium on the dorsal aspect of the dorsal medial meatus near the boundary between nonmucus and mucus-coated squamous epithelium in the rat, at the anterior or rostral margin of the middle turbinate in the monkey, and in the nonsquamous epithelium on the proximal portion of the mid-septum near the boundary between squamous and nonsquamous

epithelium in the human (see Kimbell et al. [2001a, b] for tabulations of comparative estimates of formaldehyde flux across the species).

The rat-to-monkey ratio of the highest site-specific fluxes in the two species was 0.98. In the rat, the incidence of formaldehyde-induced squamous cell carcinomas in chronically exposed animals was high in the anterior lateral meatus (Monticello et al., 1996). Flux predicted per ppm in this site and flux predicted near the anterior or proximal aspect of the inferior turbinate and adjacent lateral walls and septum in the human were similar, with a rat-to-human ratio of 0.84.

3.7.3.3. *Mass Balance Errors*

Overall uptake of formaldehyde was calculated as 100% × (mass entering nostril − mass exiting outlet)/(mass entering nostril). Mass balance errors for air, 100% × (mass of air entering nostril − mass exiting outlet)/(mass entering nostril), and inhaled formaldehyde, 100% × (mass entering nostril − mass absorbed by airway walls − mass exiting outlet)/(mass entering nostril), were calculated. Mass balance errors associated with simulated formaldehyde uptake from air into tissue ranged from less than 14% for the rat, monkey, and human at 7.4 and 15 L/minute to approximately 27% at the highest inspiratory flow rates of 31.8 and 37 L/minute (Kimbell et al., 2001b). Kimbell et al. (2001b) corrected the simulation results for these errors by evenly distributing the lost mass over the entire nasal surface.

3.7.4. Modeling Formaldehyde Uptake in the Lower Respiratory Tract

Lesions were observed in the lower respiratory tract of rhesus monkeys exposed to 6 ppm formaldehyde. Therefore it is appropriate to consider the human lower respiratory tract as potentially at risk for formaldehyde-induced cancer. Accordingly, fluid flow and formaldehyde uptake in the lower respiratory tract were also modeled for the human in the CIIT approach by using dosimetry estimates for the human lower respiratory tract.

The single-path idealization of the human lung anatomy captures the geometrical characteristics of the airways for a given lung depth, and of airflow through these airways, in an average, homogeneous sense. For particulates, this has provided a reasonable representation of the average deposition in a given generation of the lung airways for a normal human population. The one-dimensional model by Weibel (1963) is generally considered adequate unless the fluid dynamics at airway bifurcations need to be explicitly modeled, and such an idealization of the lung geometry has been successfully used in various models for the dosimetry of ozone and particulate and fibrous matter. Most likely, the lung geometries of the susceptible population, such as those with chronic obstructive pulmonary disease, would depart significantly from the geometry described in Weibel (1963). Unlike the accurate representation of the nasal anatomy

used in the CFD modeling, the lung geometry is idealized in the CIIT approach as a typical path Weibel geometry. The single-path model used to calculate formaldehyde uptake in the human respiratory tract (Overton et al., 2001; CIIT, 1999) applied a one-dimensional equation of mass transport to each generation of an adult human symmetric, bifurcating Weibel-type respiratory tract anatomical model, augmented by an upper respiratory tract. The detailed CFD modeling of the upper respiratory tract was made consistent with the upper respiratory tract in the single-path model by requiring that the one-dimensional version of the nasal passages have the same inspiratory air-flow rate and uptake during inspiration as the CFD simulations for four daily human activity levels. The reader is referred to Overton et al. (2001) for further details of the simulations. Results most relevant to this assessment are shown in Figure 3-10.

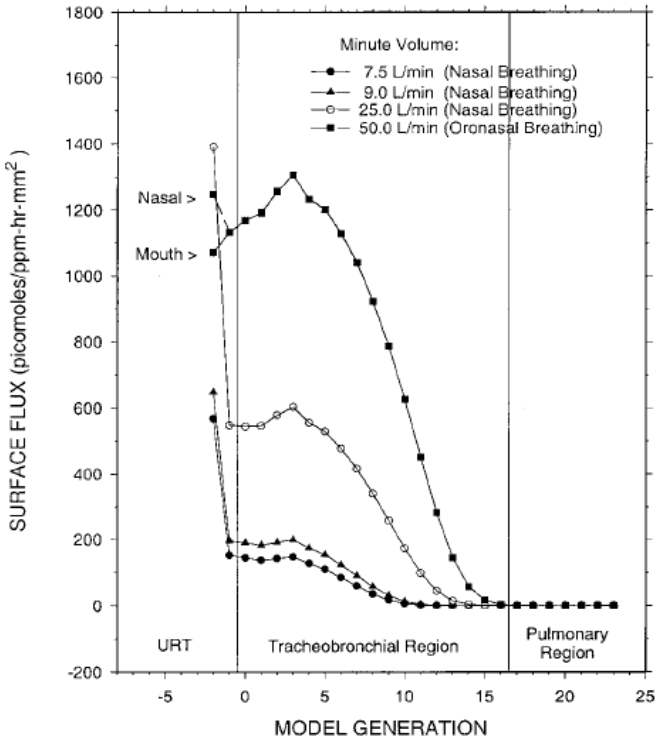

Figure 3-10. Single-path model simulations of surface flux per ppm of formaldehyde exposure concentration in an adult male human.

Source: Overton et al. (2001).

The primary predictions of the model, as shown in Figure 3-10, were that more than 95% of the inhaled formaldehyde would be retained and formaldehyde flux in the lower respiratory

tract would increase for several lung airway generations from that in the posterior-most segment of the nose and then decrease rapidly, resulting in almost zero flux to the alveolar sacs.

Overton et al. (2001) modeled uptake at higher inspiratory rates, including those at 50 L/minute of minute volume (well beyond levels where the oronasal switch occurs in the normal nasal breathing population). At these rates Figure 3-8 indicates that formaldehyde flux in the mouth cavity is comparable (but a bit less) to that occurring in the nasal passages. Overton et al. (2001) did not model uptake in the oral cavity at minute volumes less than 50 L/minute. This would be of interest because mouth breathers form a large segment of the population. Furthermore, at concentrations of formaldehyde where either odor or sensory irritation becomes a significant factor, humans are likely to switch to mouth breathing even at resting inspiration. At a minute volume of 50 L/minute, Overton et al. (2001) assumed, citing Niinimaa et al. (1981), that 0.55 of the inspired fraction is through the mouth. Therefore, based on the results in Figure 3-8, it is not unreasonable to assume that for mouth breathing conditions at resting or light exercise inspiratory rates, average flux across the human mouth lining would be comparable to the average flux across the nasal lining computed in Kimbell et al. (2001a, b).

3.7.5. Uncertainties in Formaldehyde Dosimetry Modeling

3.7.5.1. *Verification of Predicted Flow Profiles*

The simulated streamlines of steady-state inspiration airflow predicted by the CFD model agreed reasonably well with experimentally observed patterns of water-dye streams made in casts of the nasal passages for the rat and monkey as shown in panels A and B in Figure 3-7. The airflow velocity predicted by CFD model simulations of the human also agreed well with measurements taken in hollow molds of the human nasal passages (panel C, Figure 3-8) (Kepler et al., 1998; Subramaniam et al., 1998; Kimbell et al., 1997a, 1998, 1993). However, the accuracy and relevance of these comparisons are limited. The profiles were verified by video analysis of dye streak lines in the molds of rats and rhesus monkeys, although this method is reasonable for only the major airflow streams.

Plots of pressure drop vs. volumetric airflow rate predicted by the CFD simulations compared well with measurements made in rats in vivo (Gerde et al., 1991) and in acrylic casts of the rat nasal airways (Cheng et al., 1990) as shown in Figure 3-11. This latter comparison remains qualitative due to differences among the simulation and experiments as to where the outlet pressure was measured and because no tubing attachments or other experimental apparatus were included in the simulation geometry. The simulated pressure drop values were somewhat lower, possibly due to these differences.

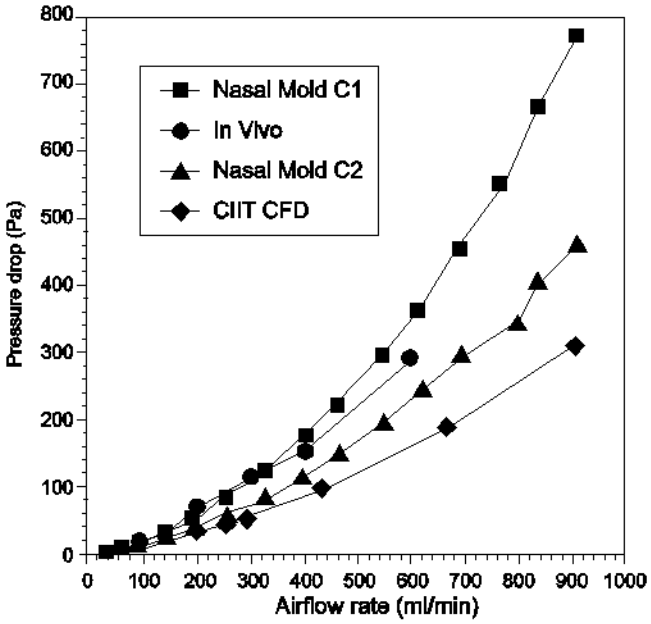

Figure 3-11. Pressure drop vs. volumetric airflow rate predicted by the CIIT CFD model compared with pressure drop measurements made in two hollow molds (C1 and C2) of the rat nasal passage (Cheng et al., 1990) or in rats in vivo (Gerde et al., 1991).

Source: Kimbell et al. (1997a).

Inspiratory airflow was assumed to be constant in time (steady state). Subramaniam et al. (1998) considered this to be a reasonable assumption during resting breathing conditions based on a value of 0.02 obtained for the Strouhal number. Unsteady effects are insignificant when this number is much less than one. However, this assumption may not be reasonable for light and heavy exercise breathing scenarios.

3.7.5.2. *Level of Confidence in Formaldehyde Uptake Simulations*

Unlike the airflow simulations, it was not possible to evaluate the formaldehyde uptake calculations directly. Since the mass transfer boundary conditions were set by fitting overall uptake to the average experimental data for various exposure concentrations, it was not possible to independently verify even the overall uptake values with empirical data. This assessment has relied on several indirect qualitative and quantitative lines of evidence listed below to provide general confidence in the uptake profile for the F344 rat nasal passages, as modeled in CIIT (1999), when gross averages are considered over certain regions of the nasal lining.

In an earlier simulation, where the nasal walls were set to be infinitely absorbing of formaldehyde, uptake of inhaled formaldehyde in the upper respiratory tract was predicted to be 90% in the rat for simulations corresponding to the resting minute volume in the F344 rat. This estimate compared reasonably well with the range of 91−98% observed by Morgan et al. (1986a).

Morgan et al. (1991) showed general qualitative correspondence between the main routes of flow and lesion distribution induced by formaldehyde in the rat nose. In their initial work with a CFD model that represented a highly reactive and soluble gas, Kimbell et al. (1998, 1993) described similarities in computed regional mass flux patterns and lesion distribution due to formaldehyde. When the results from this work in the coronal section immediately posterior to the vestibular region were considered, simulated flux levels over regions such as the medial aspect of the maxilloturbinate and the adjacent septum (where lesions were seen) were an order of magnitude higher than over other regions, such as the nasoturbinate (where lesions were not seen).[8]

The results of a PBPK model by Cohen-Hubal et al. (1997) provide a reasonable level of confidence in regional uptake simulations for the F344 rat when gross averages over nasal sites are carried out. Cohen-Hubal et al. (1997) linked the CFD dosimetry model for formaldehyde to a PBPK model for formaldehyde-DPX concentration in the F344 rat. This PBPK model was calibrated by optimizing the model to combined DPX data from all regions of the rat nose (high-tumor and low-tumor incidence regions) that were obtained in separate experiments by Casanova et al. (1991, 1989). These data were obtained at 0.3, 0.7, 2.0, 6.0, and 10 ppm for both regions. DPX data were also obtained at 15 ppm exposure from the high-tumor region; however these were not included for the calibration. Model prediction of DPX concentrations were then compared with data for the high-tumor region only and compared well with the experimental data, including the 15 ppm data for which the model had not been calibrated. This is shown in Figure 3-12. Such a verification, albeit indirect, is not available for the simulation of uptake patterns in the human.

The CFD simulations do not model reflex bradypnea, a protective reflex seen in rodents but not in humans. As discussed at length in Sections 3.2.3.1 and 4.2.1.1, it is reasonable to

[8] However, this 1993 CFD model differed somewhat from the subsequent model by Kimbell et al. (2001a) used in this assessment. In the 1993 model, the limiting mass-transfer resistance for the gas was assumed to be in the air phase; that is, the concentration of formaldehyde was set to zero at the airway lining. Furthermore, this same boundary condition was used on the nasal vestibule as well, while, in the more recent model, the vestibule was considered to be nonabsorbing. Unfortunately, Kimbell at al. (2001a) did not report on correspondences between flux patterns and lesion distribution.

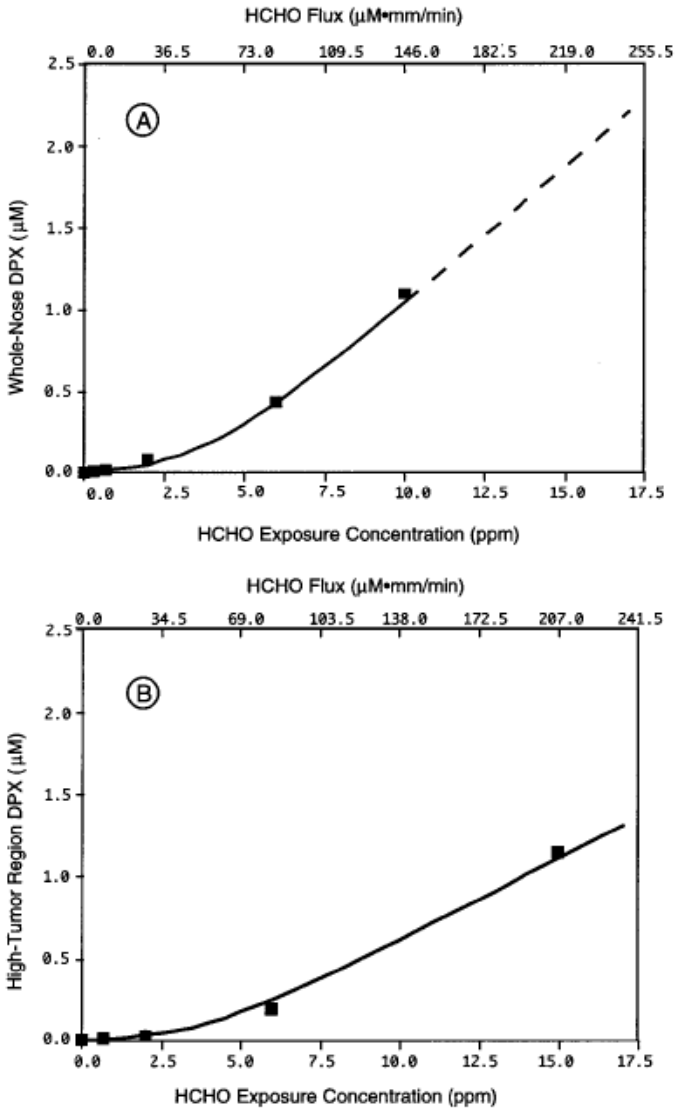

Figure 3-12. Formaldehyde-DPX dosimetry in the F344 rat.

Panel A: calibration of the PBPK model using data from high and low tumor incidence sites. Panel B: model prediction compared against data from high tumor incidence site. Dashed line in panel A shows the extrapolation outside the range of the calibrated data.

Source: Cohen-Hubal et al. (1997).

expect a range of 25% (Chang et al., 1983) to 45% (Barrow et al., 1983) decrease in minute volume in F344 rats at the exposure concentration of 15 ppm. Explicit omission of this effect in the modeling is, however, not likely to be a source of major uncertainty in the modeled results

for uptake of formaldehyde in the rat nose for the following reason. The CFD model for the F344 rat was calibrated to fit the overall experimental result for formaldehyde uptake in the F344 rat at 15 ppm exposure concentration. This was carried out by adjusting the mass transfer coefficient used as boundary condition on the absorbing portion of the nasal lining. Thus, the reflex bradypnea occurring in those experimental animals is phenomenologically factored into the value used for the boundary condition. Nonetheless, some error in the localized distribution of uptake patterns may be expected, even if the overall uptake is reproduced correctly. Furthermore, since the same value for the mass transfer coefficient was used in human simulations (as obtained from calibration of the rat model), there is additional uncertainty in the modeled human flux estimates. This issue was not addressed by Kimbell et al. (2001a, b), Conolly et al. (2004), or Schlosser et al. (2003), and we are unable to assess the extent of this error more accurately.

3.7.6. PBPK Modeling of DNA Protein Cross-Links (DPXs) Formed by Formaldehyde

3.7.6.1. *PBPK Models for DPXs*

As can be seen from the previous sections, measuring the distribution of the absorbed formaldehyde and identifying its form have proven difficult. Because of the high reactivity of formaldehyde, rapid metabolism of formaldehyde, and complexity of formate clearance, dose surrogates (or biomarkers) of exposure have been used to characterize the extent of absorption and distribution of formaldehyde. As with other soluble and reactive gases, typical PBPK models that predict steady-state blood concentrations are not useful for predicting formaldehyde dosimetry at this time. As noted previously, inhalation exposure to formaldehyde has not been shown to increase blood formaldehyde levels. Thus, most modeling efforts for formaldehyde have focused on disposition at the site of contact.

As discussed earlier, the concentration of DPXs formed by formaldehyde has been treated as a surrogate for the tissue dose of formaldehyde in earlier efforts by Casanova et al. (1991) and in EPA's efforts to update its health assessment of formaldehyde (Hernandez et al., 1994). These efforts used data from rats and rhesus monkeys (Casanova et al., 1991, 1989). Using DPXs in this manner allowed the incorporation of both clearance and metabolism of formaldehyde and the incorporation of the effect of saturation on detoxification of formaldehyde at higher doses. Calculation of the average DPX concentration from these data was seen as a surrogate for the area under the curve (AUC) of the reactive formaldehyde species in the epithelium. Based on these data, Casanova et al. (1991) developed a PBPK model for predicting DPXs in these species and for extrapolating to the human.

The Casanova et al. (1991) model consists of three anatomical compartments representing different parts of the upper respiratory tract of the rhesus monkey. The results indicated a 10-fold difference in DPX formation between rats and monkeys, due primarily to species differences in minute volume and differing quantities of DNA in the nasal mucosa. Casanova et al. (1991) then developed a monkey/rat scaling factor for these parameters by taking the ratio of nasal mucosa tissue between the two species, a determinant that was proportional to the total body weight differences between the two species. Using these scaling factors in their model, the authors' predictions in monkey (based on the rat data) were in close agreement with observed DPXs in monkey, particularly at higher formaldehyde concentrations. However, the model overpredicted DPX formation in the monkey at lower formaldehyde concentrations. Subsequent rat-human and monkey-human scaling results predicted much lower DPX formation in man. Again, the values obtained at lower concentrations may have been overpredicted, as was the case for the rat-monkey extrapolation.

Georgieva et al. (2003) developed a model for the uptake and disposition of formaldehyde in the rat nasal lining. This model was designed to predict the distribution of formaldehyde in the nasal mucosa. The model indicated that, at 6 ppm exposure, a steady-state elevation of 15−20 μM formaldehyde would be achieved within 30 seconds. Furthermore, this same elevation was predicted when the exposure was 6 ppm formaldehyde for 60 minutes. Given that human blood formaldehyde levels are predicted to be about 100 ± 15 μM (Heck et al., 1985) and assuming that blood formaldehyde concentration is roughly equivalent to the concentration predicted at the basement membrane of the epithelium, this model predicts roughly a 15−20% increase in blood formaldehyde. However, it should be noted that a 40-minute inhalation exposure of humans to 1.99 ppm formaldehyde did not lead to a measurable increase in blood formaldehyde (Heck et al., 1985).

Franks (2005) published a mathematical model for predicting the disposition of formaldehyde in the human nasal mucosa and blood. The calculated concentrations of formaldehyde in the mucus, the epithelium, and the blood attained steady-state profiles within a few seconds of exposure. The increase of the formaldehyde concentration in the blood was predicted to be insignificant compared with the existing pre-exposure levels in the body: an increase of 0.00044 mg/L in blood formaldehyde following exposure to 1.9 ppm formaldehyde for up to 8 hours. The model described formaldehyde concentration gradients across the mucus, epithelial, and submucosal compartments in the human nose. Transport of formaldehyde was governed by the following processes: diffusional (in the mucus); a combination of diffusional, two first order terms representing intrinsic reactivity of formaldehyde and binding to DNA, and Michaelis-Menten kinetics representing enzymatic metabolism (in the epithelial layer); a first-

order term representing nonenzymatic removal governed by the blood perfusion rate (in the submucosal compartment). The model used the values for the first order reaction rate constants and the Michaelis-Menten parameters (V_{max} and K_m) estimated by Conolly et al. (2000) in their model for extrapolating the rat and rhesus monkey data to the human. The modeling in Franks (2005) was not calibrated or validated against experimental data, but the predictions of negligible penetration of free formaldehyde to the blood are qualitatively in agreement with the conclusions in Heck et al. (1985).

Following the efforts by Casanova and coworkers, Cohen-Hubal et al. (1997), Conolly et al. (2000), and Georgieva et al. (2003) developed models that linked local formaldehyde flux from CFD models to DPX predictions. The focus here will be on the Conolly et al. (2000) effort for the following two reasons: it explicitly incorporates regional formaldehyde dosimetry in the nasal lining by using results from CFD modeling of airflow and gas uptake and it brings data across species (rat and rhesus monkey) to bear on model calibration, such a situation being relatively rare in chemical health risk assessments.

3.7.6.2. *A PBPK Model for DPXs in the F344 Rat and Rhesus Monkey that Uses Local Tissue Dose of Formaldehyde*

In earlier risk assessment efforts (Hernandez et al., 1994; Casanova et al., 1991; U.S. EPA, 1991b), the average DPX concentration was considered a surrogate tissue dose metric for the AUC of the reactive formaldehyde species. Conolly et al. (2003) assigned a more specific role for DPXs, treating local DPX concentration as a dose surrogate indicative of the intercellular concentration of formaldehyde, leading to formaldehyde-induced mutations. These authors indicated that it was not known whether DPXs directly induced mutations (Conolly et al., 2003; Merk and Speit, 1998). This is discussed in detail in the mode-of-action sections in this document. The Conolly et al. (2000) model for the disposition of inhaled formaldehyde gas and DPX in the rat and rhesus nasal lining is relatively simple in terms of model structure because it consists of a single well-mixed compartment for the nasal lining as follows:

1. Formaldehyde flux to a given region of the nasal lining is provided as input to the modeling and is obtained in turn as the result of a CFD model. This flux is defined as the amount of formaldehyde delivered to the nasal lining per unit time per unit area per ppm of concentration in the air in a direction transverse to the airflow. It is locally defined as a function of location in the nose and the inspiratory flow rate and is linear with exposure concentration.
2. The clearance of formaldehyde from the tissue is modeled as follows:

a. a saturable pathway representing enzymatic metabolism of formaldehyde, which is primarily by formaldehyde dehydrogenase (involving Michaelis-Menten parameters V_{max} and K_m)

b. a separate first-order pathway, which is assumed to represent the intrinsic reactivity of formaldehyde with tissue constituents (rate constant k_f)

c. first-order binding to DNA that leads to DPX formation (rate constant k_b)

3. The clearance or repair of this DPX is modeled as a first order process (rate constant k_{loss}).

DPX data. DPX concentrations were estimated from a study by Casanova et al. (1994) in which rats were exposed 6 hours/day, 5 days/week, plus 4 days for 11 weeks to filtered air (naive) or to 0.7, 2, 6, or 15 ppm (0.9, 2.5, 7.4, or 18 mg/m^3) formaldehyde (pre-exposed). On the 5th day of the 12th week, the rats were then exposed for 3 hours to 0, 0.7, 2, 6, or 15 ppm ^{14}C-labeled formaldehyde (with pre-exposed animals exposed to the same concentration as during the preceding 12 weeks and 4 days). The animals were sacrificed and DPX concentrations determined at two sites in the nasal mucosa. Conolly et al. (2000) used these naive rat data to develop a PBPK model that predicted the time-course of DPX concentrations as a function of formaldehyde flux at these sites.[9]

3.7.6.3. *Uncertainties in Modeling the Rat and Rhesus DPX Data*

3.6.6.3.1. ***Half-life of DPX repair.*** In the development of the PBPK model for DPXs, Conolly et al. (2000) assumed a value of 6.5×10^{-3} minute^{-1} for k_{loss}, the first-order rate constant for the clearance (repair) of DPXs, such that the DPXs predicted at the end of a 6-hour exposure to 15 ppm were reduced to exactly the detection limit for DPXs in 18 hours (the period between the end of 1 day's 6-hour exposure and the beginning of the next). This determination of rapid clearance was based on an observation by Casanova et al. (1994) that the DPX concentrations observed in the pre-exposed animals were not significantly higher than those in naïve animals (in which there was no significant DPX accumulation). However, in vitro data (Quievryn and Zhitkovich, 2000) indicate a much slower clearance, with an average k_{loss} of 9.24×10^{-4} minute^{-1}.

Subramaniam et al. (2007) examined the Casanova et al. (1994) data and argued that there was a significantly decreased (~ 40%) level of DPXs in high tumor regions of pre-exposed animals vs. naive animals at 6 and 15 ppm and that the weight of the tissues dissected from those

[9] Subramaniam et al. (2007) who also used the same data verified that they were on naïve rats; however, Conolly et

regions increased substantially, indicating a thickening of the tissues. After testing the outcome of changing the tissue thickness in the PBPK model for DPXs, it was apparent to these authors that such a change alone could not account for the dramatic reduction in DPX levels after pre-exposure, even with the higher value of k_{loss} used by Conolly et al. (2000). Therefore, in addition to the gross increase in tissue weight, these data indicated either an induction in the activity of enzymes that remove formaldehyde (aldehyde and formaldehyde dehydrogenase) or other changes in the biochemical properties of the highly exposed tissue that must have occurred. Given such a change, Subramaniam et al. (2007) concluded that the experimental results in Casanova et al. (1994) were consistent with the smaller experimental value of k_{loss} indicated by the Quievryn and Zhitkovich (2000) data. In particular, they argued that if V_{max} increased with exposure (in a tissue region- and dose-specific manner), then it was possible to explain the naïve vs. pre-exposed data of Casanova et al. (1994), with the value of k_{loss} effectively measured in vitro by Quievryn and Zhitkovich (2000). Furthermore, this value was measured directly, rather than obtained by indirect interpretation of measurements made at only two time points where significant changes in the tissue had occurred. Therefore, Subramaniam et al. (2007) considered the use of this lower value for k_{loss} to be more appropriate. The same lower value of k_{loss} was also used by Georgieva et al. (2003). Consequently, they reimplemented and reoptimized the Conolly et al. (2000) model with this modification and found that the fit so obtained to the acute DPX data was excellent. The reimplemented model will be used in this assessment, and more details can be found in Subramaniam et al. (2007).

It should be noted that this slower DPX repair rate was obtained in an in vitro study by using human cell lines that were transformed and immortalized. However, it appears that DPX repair in normal cells would be even slower. When nontransformed freshly purified human peripheral lymphocytes were used instead, the half-life for DPX repair was about 50% longer than in the cultured cells (Quievryn and Zhitkovich, 2000).

3.6.6.3.2. ***Statistical uncertainty in parameter estimates and extrapolation*.** Klein et al. (2010) developed methods for deriving statistical inferences of results from PBPK models, and used the structure of the Conolly et al. (2000) model for demonstrating their methods, specifically because of the sparse time-course information in the above DPX data. However, they used the value of k_{loss} deduced from Quievryn and Zhitkovich (2000) and fitted the model simultaneously to both the rat and rhesus monkey data, as opposed to the sequential fitting in Conolly et al. (2000). They found that the predicted DPX concentrations were extremely sensitive to V_{max} and

al. (2000) state that they used data on pre-exposed rats.

tissue thickness as was also concluded by Georgieva et al. (2003) and Cohen-Hubal et al. (1997). K_m was seen to be substantially different across species, a finding that was attributed plausibly to the involvement of more than one enzyme (Klein et al., 2010; Georgieva et al., 2003). Klein et al. (2010) concluded that the two efforts (Conolly et al. [2000] vs. Klein et al. [2010]) resulted in substantially different predictions outside the range of the observed data over which the models were calibrated.

The differences between these models occur in spite of the fact that both methods use all the available DPX data in both species and the same model structures. At the 0.1 ppm exposure concentration, in general these authors obtained three- to fourfold higher DPX concentrations averaged over a 24-hour period after exposure. Furthermore, the standard deviations in Klein et al. (2010) for V_{max} and K_m were an order of magnitude higher and that for k_f was 35-fold lower than the corresponding standard deviations reported in Conolly et al. (2000). The relatively larger standard deviation for k_f resulted in this parameter becoming negative in Conolly et al. (2000) at half the standard deviation below the maximum likelihood estimate (MLE) value. Note that, at a negative value of k_f, formaldehyde would be produced as opposed to being cleared through its intrinsic reactivity.

Klein et al. (2010) concluded that these "remarkable differences outside the range of the observed data suggest caution in the use of these models in a predictive sense for extrapolating to human exposures."

3.7.7. Uncertainty in Prediction of Human DPX Concentrations

Conolly et al. (2000) used both the rat and rhesus monkey data to predict human DPX concentrations and constructed a PBPK model for the rhesus monkey along similar lines as for the F344 rat. In the rhesus monkey model, they maintained the same values of k_b, k_{loss}, and k_f as in the rat model but optimized the values of V_{max} and K_m against the rhesus monkey data from Casanova et al. (1994). The rat and rhesus monkey parameters were then used to construct a human model (see Conolly et al. [2000] for a more detailed report of implementing the rhesus monkey model and the extrapolating to humans).

For the human, the model used the value of K_m obtained in the rhesus monkey model and the epithelial thickness averaged over three regions of the rhesus monkey nose. The maximum rate of metabolism, V_{max}, which was estimated independently for the rat and rhesus monkey by fitting to the DPX data available for these species, was then extrapolated to the human by assuming a power law scaling with body weight (BW) (i.e., $V_{max} = a \times BW^b$), and the coefficient "a" and exponent "b" were derived from the independently estimated values of $(V_{max})_{RAT}$ and

$(V_{max})_{MONKEY}$. Table 3-8 gives the values of V_{max} and K_m in the Conolly et al. (2000) extrapolation.

Table 3-8. Extrapolation of parameters for enzymatic metabolism to the human

Parameter	F344 rat	Rhesus monkey	Human
V_{max} (pmol/min-mm^3)	1,008.0	91.0	15.7
K_m (pmol/mm^3)	70.8	6.69	6.69

Source: Conolly et al. (2000).

The above scale-up procedure was an attempt to use both the rodent and primate DPX data. However, laws for allometric scaling across species, such as how enzymatic metabolic rates vary across organisms, are empirical regression relationships whose strength is that they are based on data from multiple species and usually multiple sources of data points. For example, West and Brown (2005) demonstrate that metabolic rates scale with mass$^{3/4}$ using data from organisms ranging over 27 orders of magnitude in mass (intracellular up to the largest organisms). In Conolly et al. (2000) the power-law relationship is derived using two data points (F344 rat and rhesus monkey for a single chemical) with log BW as x-axis and V_{max} on y-axis. Since such a regression does not have the power to delineate the curvature in the scaling function, the empirical strength of the allometric relationship derived in Conolly et al. (2000) is extremely weak for use in extrapolating from the rat to the human on the basis of body-weight.

The following observations point to the uncertainty in the values of the parameters V_{max} and K_m in the Conolly et al. (2000) models for predicting DPXs. First, K_m varies by an order of magnitude across the rat and monkey models but is then considered invariant between the monkey and human models (Conolly et al., 2000). Second, the values in Conolly et al. (2000) for V_{max}/K_m, the low-dose limit of the rate of enzymatic metabolism, is roughly similar between the rat and monkey but lower by a factor of six in the human.

Another factor that can substantially influence the above extrapolation of DPXs in the human is that Conolly et al. (2000) assumed the tissue to be a well-mixed compartment with regard to formaldehyde interaction with DNA and used the amount of formaldehyde bound to DNA per unit volume of tissue as the DPX dose metric. Considering formaldehyde's highly reactive nature, the concentrations of formaldehyde and DPX are likely to have a sharp gradient with distance into the nasal mucosa (Georgieva et al., 2003). Given the interspecies differences in tissue thickness, there is consequent uncertainty as to whether DPX per unit volume or DPX

per unit area of nasal lining is the more appropriate dose metric to be used in the extrapolation. In particular, it may be assumed that the cells at risk for tumor formation are only those in the epithelium and that measured DPX data (in monkeys and rats) are an average over the entire tissue thickness. Since the epithelial DPXs in monkeys (and presumably humans) would then be more greatly "diluted" by lower levels of DPX formation that occur deeper into the tissue than in rats, it could be predicted that the ratio of epithelial to measured DPXs in monkeys and humans would be much higher than the ratio in rats.

3.7.8. Modeling Interindividual Variability in the Nasal Dosimetry of Reactive and Soluble Gases

Garcia et al. (2009) used computational fluid dynamics to study human variability in the nasal dosimetry of reactive, water-soluble gases in 5 adults and 2 children, aged 7 and 8 years old. The sample size in this study is too small to consider the results representative of the population as a whole (as also recognized by the authors). Nonetheless, various comparisons with the characteristics of other study populations add to the strength of this study (see Appendix B). The authors considered two model categories of gases, corresponding to maximal and moderate absorption at the nasal lining. We focus here only on the "maximum uptake" simulations in Garcia et al. (2009). In this case, the gas was considered so highly reactive and soluble that it was reasonable to assume an infinitely fast reaction of the absorbed gas with compounds in the airway lining. Although such a gas could be reasonably considered as a proxy for formaldehyde, these results cannot be fully utilized to inform quantitative estimates of formaldehyde dosimetry (and it does not appear to have been the intent of the authors either). This is because the same boundary condition corresponding to maximal uptake was applied on the vestibular lining of the nose as well as on the respiratory and transitional epithelial lining on the rest of the nose. This is not appropriate for formaldehyde as the lining on the nasal vestibule is made of keratinized epithelium which is considerably less absorbing than the rest of the nose (Kimbell et al., 2001b).

The Garcia et al. (2009) study and the results of their analyses have been further described and evaluated in Appendix B. Overall uptake efficiency, average flux (rate of gas absorbed per unit surface area of the nasal lining) and maximum flux levels over the entire nasal lining did not vary substantially between adults (1.6-fold difference in average flux and much less in maximum flux), and the mean values of these quantities were comparable between adults and children. These results are also in agreement with conclusions reached by Ginsberg et al. (2005) that overall extrathoracic absorption of highly and moderately reactive and soluble gases (corresponding to Category 1 and 2 reactive gases as per the scheme in EPA [1994]) is similar in

adults and children. On the other hand, Figure 6A of the paper (reproduced as Figure B-1 in Appendix B), provides a different perspective on variations between the adults in flux values at specific points on the nasal walls. The plot indicates that local flux of formaldehyde may vary among individuals by a factor of 3 to 5 at various distances along the septal axis of the nose; such an evaluation of inter-individual variability in the spatial distribution of formaldehyde flux over the nasal lining is important for a highly reactive and soluble gas whose regional absorption is highly nonhomogeneously distributed (see text surrounding Figure 3-8).

End of Volume I

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TOXICOLOGICAL REVIEW OF FORMALDEHYDE - INHALATION ASSESSMENT

(CAS No. 50-00-0)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

VOLUME II of IV

Hazard Characterization

June 2, 2010

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4. HAZARD CHARACTERIZATION

4.1. HUMAN STUDIES

This chapter discusses epidemiologic studies of site-specific cancers and other adverse health effects that may be caused by exposure to formaldehyde. The primary focus is on the literature describing inhalation exposure and its potential carcinogenic and noncarcinogenic health risks. In addition, oral, dermal, and ocular exposures to formaldehyde are discussed.

The noncancer health effects section is organized by endpoint, beginning with sensory irritation (SI) and followed by pulmonary function, asthma, respiratory tract pathology, immunologic responses, neurological and behavioral responses, and, finally, developmental and reproductive outcomes.

The carcinogenicity section is divided into two parts, respiratory tract and nonrespiratory tract cancers. The first part discusses site-specific cancers that are chiefly located in the respiratory tract where direct contact with formaldehyde occurs: nasopharyngeal cancers (NPCs), nasal and paranasal cancers, other respiratory tract cancers, and lung cancers. The second part on nonrespiratory tract cancer discusses those cancers at other sites with more distant exposure to formaldehyde than respiratory epithelium—mainly, lymphohematopoietic (LHP) cancer, brain and central nervous system (CNS) cancer, pancreatic cancer, and cancer at other sites.

4.1.1. Noncancer Health Effects

4.1.1.1. ***Sensory Irritation (Eye, Nose, Throat Irritation)***

As a reactive gas, formaldehyde is a sensory irritant. Sensory irritation of the eyes and respiratory tract by formaldehyde has been observed consistently in clinical and epidemiologic studies in residential and occupational populations. Binding to sensory nerves at the portal of entry (POE) results in direct sensory responses (e.g., detection of odor and tissue irritation) as well as reflex responses to the sensory irritation and neurogenic sensitization. Reflex responses result from CNS stimulation by the afferent sensory signals and include lacrimation, coughing, sneezing, and bronchial constriction (BC). An additional reflex seen in rodents is reflex bradypnea (RB) (also known as reflex apnea [RA]). Formaldehyde-induced sensory irritation may be evident after acute exposures at average concentrations of 730 ppb (Kriebel et al., 1993), as well as in chronically exposed individuals at lower concentrations (100-300 ppb) (Ritchie and Lehnen, 1987). Formaldehyde-induced neurogenic sensitization and atopy may result in lifelong health effects from short-term or transient exposures. For this discussion, sensory irritation will include both direct sensory response to formaldehyde exposure and reflex responses (lacrimation, coughing, sneezing, RB, and BC, and sensitization.

Eye, nose, and throat irritation in response to formaldehyde inhalation exposure is well documented (Doty et al., 2004). Broadly, studies examining these endpoints are either controlled chamber studies with a defined population (e.g., healthy volunteers or sensitive individuals), worker/student studies, or population (e.g., residential) studies. Chamber studies, by design, are acute studies, although some researchers have investigated repeated exposures. Occupational, student, and residential exposures are generally longer duration, although there is variability in exposure and duration among subjects. Endpoints include both local effects and reflex effects of sensory irritation. The endpoints for assessing irritation include self-reporting of adverse symptoms (e.g., pain, burning, itching) as well as objective measures of irritation (e.g., eye-blink counts, lacrimation) (Doty et al., 2004). The following review focuses on eye, nose, and throat irritation but studies have documented other types of irritation, including dermal irritation eczema and dermatitis.

4.1.1.1.1. *Epidemiologic literature.*

A wide variety of epidemiologic studies have assessed the potential effects of exposure to formaldehyde on endpoints, indicating sensory irritation of the eye, nose, and throat. These studies generally include three different types of exposure populations: (1) Residents and visitors exposed to formaldehyde in homes and mobile buildings, where formaldehyde is present from various sources, including building components, furniture and home furnishings, heating and cooking combustion as well as active and passive smoking; (2) various occupational exposures from industrial processes related to wood products, furniture making, and formaldehyde-based resins; and (3) anatomy students who are exposed under well-defined conditions during academic courses where they are examining formaldehyde-preserved cadavers.

4.1.1.1.1.1. ***Residential epidemiology.***

Among the residential epidemiology studies of formaldehyde effects on sensory irritation, one of the strongest studies based on study design, execution, analysis, and sample size was the observational study undertaken by Ritchie and Lehnen (1987). In this cross-sectional study of nearly 2,000 Minnesota residents living in 397 mobile and 494 conventional homes, personal data and formaldehyde samples were collected from residents that had responded to an offer by the state health department to test homes for formaldehyde. Technicians administered a symptom questionnaire to participating residents at the time of formaldehyde sample collection. Residents were asked to close doors and windows of their homes for 12 hours before testing was conducted, and a standardized collection protocol was used for both sample collection and analysis. Measurements of formaldehyde exposure were taken from two rooms of the home,

usually the bedroom and living room, and the samples were kept refrigerated until analysis. Respondents were not aware of the results of the formaldehyde analyses in their homes at the time they responded to the symptom questionnaire. The results from Ritchie and Lehnen (1987) provide a clear dose-response relationship in the percentage of residential occupants reporting eye, nose, and throat irritation. Specifically, eye irritation responses increased from 1−2% in homes with formaldehyde concentrations lower than 0.1 ppm to 12−32% in homes with formaldehyde concentrations ranging from 0.1 to 0.3 ppm and 86−93% of residents reporting eye irritation at $\geq$ 0.3 ppm. These effects were found in the same concentration range for people living in either mobile (n = 851) or conventional (n = 1,156) homes. Similar percentages were found for nose/throat irritation. The authors reported that they controlled for smoking, age and sex in logistic regression models. Thus, smoking is not likely to be a confounder of the observed relation between formaldehyde and irritation. The percent reporting irritation increased with increasing formaldehyde concentration category within all strata for smoking (active, passive and nonsmokers) among residents in both mobile homes and conventional homes. While the participants in this study were self-selected and not a random residential sample, a clear concentration response was observed, and, even if participants sought testing because they suspected that they were being exposed to formaldehyde in their homes, they could not know the measured concentration of formaldehyde when reporting their irritation symptoms, so recall bias cannot explain the concentration response.

The results of an adverse association of sensory irritation with formaldehyde reported by Ritchie and Lehnen (1987) are corroborated by Hanrahan et al. (1984) who conducted a cross-sectional survey by using a random sample of mobile homes from mobile home parks in Wisconsin. Sixty-one teenage and adult residents participated. Health questionnaires were self-administered by each occupant. Respondents were blinded to the results of their home formaldehyde vapor measurements, which were sampled from two rooms in the homes following instruction to close windows, refrain from smoking, and turn off gas appliances for 30 minutes prior to air sampling. Logistic regression analyses were used to ascertain potential symptom risk ratio dependency on each respondent's age, smoking status, gender, and formaldehyde concentration measures in the home. Formaldehyde concentrations ranged from 0.1 ppm to 0.8 ppm with a median concentration of 0.16 ppm. Across this concentration range, a clear and statistically significant concentration-response relationship was reported in graphical form, controlling for age, gender, and smoking status. At 0.1 ppm, the regression model showed less than 5% predicted prevalence of burning eyes. At 0.2 ppm, the midpoint of the exposure category in Ritchie and Lehnen (1987) that was reported to be the lowest adverse effect level for eye irritation with 12−32% reporting eye irritation, the regression model of Hanrahan et al.

(1984) showed approximately 17.5% predicted prevalence of burning eyes. The prevalence of burning eyes rose linearly to approximately 65% prevalence at 0.5 ppm, with some diminishment in the rate of rise up to approximately 80% prevalence at 0.8 ppm. While only 65 out of 208 randomly selected homes volunteered to complete the health questionnaires, the investigators were able to complete home formaldehyde vapor measurements on all the homes and reported nearly an identical distribution of formaldehyde concentrations in participating and nonparticipating homes. Demographic characteristics of some of the nonrespondents were available and were reported as nearly identical to those of participants. There was no indication of selection bias. Confounding is unlikely to explain such a strong concentration response.

These findings of associations of sensory irritation with residential exposures to formaldehyde are further supported by studies that did not examine concentration response but nonetheless assessed the association of formaldehyde with sensory irritation. Similar findings to those of Ritchie and Lehnen (1987) and Hanrahan et al. (1984) have been reported in other residential studies of increased symptoms in association with formaldehyde exposure (Liu et al., 1991; Thun et al., 1982; Dally et al., 1981). Dally et al. (1981) collected data in 100 "complaint structures" (65% mobile homes, 27% conventional homes, 2% travel trailers, 2% office buildings, etc). Of these, 60% were from home owners contacting the health department and 30% from physician referrals. Twenty percent of the buildings had concentrations below the limit of detection (0.1 ppm), 20% had levels at or above 0.81 ppm, and overall the concentrations ranged from below detection to above 3 ppm with an overall median of 0.35 ppm. The median levels were 0.47 and 0.10 ppm for mobile and conventional homes, respectively. No other contaminants were measured. Eye, nose, and throat irritation were reported in a high percentage of occupants (eye irritation 68%, burning eyes 60%, runny nose 60%, dry or sore throat 57%, cough 51%), but these were not reported as a function of dose or home type. Thus, there was no control group to which rates of irritation could be compared. However, symptoms reportedly stopped in 89% of occupants when they left the "complaint structure." The most recent residential study was performed on over 1,000 mobile homes with 1,394 participants (Liu et al., 1991). Home formaldehyde concentration ranged from below 0.01 to 0.46 ppm. Analyses used logistic regression to control for potential confounders. Eye irritation was positively associated with formaldehyde with a clear concentration response demonstrated with cumulative exposure. During the summer and winter months, formaldehyde exposure was associated with burning eyes. In the winter months, formaldehyde exposure was associated with sore throat. There was no association of formaldehyde exposure with cough or running nose during either season. Liu et al. (1991) also report a synergistic effect on irritation by formaldehyde exposure and chronic disease prevalence. Thun et al. (1982) reported increased symptoms of itchy skin and "wheezing

and difficulty breathing" in residents in 395 homes insulated with urea-formaldehyde foam relative to nearby homes without urea foam formaldehyde insulation (UFFI); however, there were no measurements of formaldehyde concentration taken in this study.

While not strictly a residential epidemiology study, Olsen and Dossing (1982) studied occupational exposures within mobile and nonmobile daycare centers. They reported the mean concentration in mobile and nonmobile day care centers were 350 (200−450) ppb and 65 (40−90) ppb, respectively. Adverse eye, nose, and throat irritation were significantly elevated in the workers (n = 70) in the mobile units as compared with those in nonmobile units (n = 34). The authors also state that a high percentage of workers in the mobile day cares reported that the symptoms disappeared after working hours; however, the authors did not report any such percentages among those working in nonmobile units.

4.1.1.1.1.2. *Occupational epidemiology.*

Horvath et al. (1988) compared irritation symptoms between 109 workers at a particleboard manufacturing plant and 264 workers at food plants as a control group. The mean 8-hour time-weighted average (TWA) formaldehyde concentrations between these two groups were 0.69 ppm (range 0.17−2.93) and 0.05 ppm (range 0.03−0.12), respectively. Eye, nose, and throat irritation were more common among the former group (prevalence of symptoms during a work shift: throat sore or burning—test 22.0%, controls 3.9%; cough—test 34.9%, controls 18.9%; burning of nose—test 28.4%, controls 2.0%; stuffy nose—test 33.9%, controls 14.2%; itching of nose—test 21.1%, controls 7.9%; eyes burning or watering—test 39.5%, controls 9.1%; eyes itching—test 19.3%, controls 7.1%).

Similar results were reported for frequency of eye and nasal discomfort in a group of workers involved in the manufacture of formaldehyde resins. These workers were exposed to a mean concentration of 0.40 mg/m^3. Alexandersson and Hedenstierna (1988) reported that the frequency of eye, nose, and throat irritation was significantly greater in 38 workers exposed to formaldehyde and solvents in lacquers (average employment duration 7.8 years) as compared with 18 controls (nonexposed individuals working at the same factory). The frequency of eye irritation was 65.8% among those exposed and 16.7% among controls. No controls reported nose/throat irritation, but about 40% of those exposed did.

A Swedish study conducted at a chemical plant found that nasal and eye discomfort were reported by 64 and 24%, respectively, of workers (n = 70) exposed to formaldehyde (range 0.05−0.50 mg/m^3 with a mean of 0.26 mg/m^3) versus 25 and 6%, respectively, of the control group made up of clerks from the local government (n = 36). In addition, the majority of workers exposed to formaldehyde reported that their symptoms were relieved during weekends

and vacations (Holmström and Wilhelmsson, 1988). Another study by the same authors (Wilhelmsson and Holmström, 1992) reported similar results. In this study irritation prevalent among 66 workers from a formaldehyde-producing plant was compared with that seen among 36 community clerks. The workers were exposed to 0.26 mg/m^3 of formaldehyde (range 0.05−0.6 mg/m^3). The clerks were exposed to an average of 0.09 mg/m^3. Nasal and eye discomfort were reported at rates of 53 and 24%, respectively, among the workers. Among the community clerks, 3 and 6%, respectively, reported discomfort.

Holness and Nethercott (1989) reported significant increases in eye irritation (42 vs. 21%) and nose irritation (44 versus 16%) among 84 funeral service workers as compared with 38 controls (students and individuals from a service organization). The former group had been actively embalming for approximately 10 years and had nearly twice the pack-years smoked as the controls. The exposure concentration in both groups was 0.36 and 0.02 ppm, respectively.

4.1.1.1.1.3. ***Epidemiology on laboratory students.***

Several studies have monitored sensory irritation in medical/physical therapy students exposed to formaldehyde during anatomy courses. These studies have particular advantages: the student population generally has no former occupational exposure, and, oftentimes, preclass survey data serve as the control, providing a better basis for assessing the effects of formaldehyde exposure.

In a study of 24 formaldehyde-exposed anatomy students (personal breathing zone samples 0.73 ppm, range 0.49−0.93), the prevalence of eye irritation before the start of a cadaver dissection class was 16%, while after the class, the prevalence was 59%. The increase in prevalence of eye irritation was most pronounced (43%), but increases were also observed in the prevalence of irritation of the nose (21%) and throat (15%) (Kriebel et al., 1993). The authors also reported a tendency for this increase in intensity between the beginning and end of class to diminish over the 10-week course, especially for eye irritation. However, although the intensity of the irritation diminished, eye irritation was still present among the students after 10 weeks of intermittent exposure. The report of increase in post- versus preclass irritation symptoms in this study was no greater for asthmatic students (n = 5) compared with nonasthmatic students.

Takahashi et al. (2007) showed that 143 medical students reported various symptoms (including eye and throat irritation) and that the percentage of students reporting symptoms increased between the beginning and end of the course 2 months later. After the first day of class, approximately 35% of students reported eye soreness and about 15% reported throat irritation. After the course ended, these rates were close to 70% for eye soreness and slightly above 40% for throat irritation. The reported average room formaldehyde concentration was

2.12 ppm (range 1.7−2.4), while the gas samplers worn on the students' chests averaged 2.4 ppm (range 1.8−3.8). Another study of students in an anatomy laboratory class in Japan (Takigawa et al., 2005) measured formaldehyde concentrations and irritation symptoms before and after the installation of a ventilation system. This system reduced the median personal formaldehyde exposure concentration from 2.7 to 0.72 ppm. Before installation of the ventilation system, the students complained about exacerbation of all the sensory irritation symptoms on average. The increase in 8 out of 25 symptoms was significantly reduced after installing general ventilation ($p < 0.05$). After installation of the ventilation system, a dose-dependent relationship with formaldehyde was seen for irritated eyes but not for itchy nose.

Akbar-Khanzadeh et al. (1994) detected mean personal area levels of formaldehyde at 1.24 ppm and a range of 0.1−2.94 ppm from personal air sampling devices. Almost 90% of the students in this study reported eye irritation, 74% reported nose irritation, and close to 30% reported throat irritation during or after exposure to formaldehyde during the laboratory period after having completed at least 6 weeks of laboratory sessions with formaldehyde exposure. In addition, Uba et al. (1989) demonstrated that symptoms of eye, nose, and throat irritation were correlated with formaldehyde exposure among medical students by comparing students' responses on a questionnaire completed after a lab with formaldehyde exposure to a questionnaire completed after a lab with no formaldehyde exposure. The authors compared questionnaires completed prior to students' first anatomy lab to a questionnaire completed 7 months later. Reports of cough were more frequent after the 7 months. These students were exposed to a mean level of 1.9 ppm (range 0.1−5.0) while dissecting (measured using portable infrared spectrophotometer), and a TWA from all laboratory activities ranged from below limits of detection to 0.93 (measured using personal sampling devices in the students' breathing zones).

4.1.1.1.2. *Acute studies: controlled chamber exposures.*

Results from controlled human studies demonstrate eye, nose, and throat irritation in association with formaldehyde exposure (Lang et al., 2008; Yang et al., 2001; Krakowiak et al., 1998; Kulle, 1993; Green et al., 1989, 1987; Kulle et al., 1987; Sauder et al., 1987, 1986; Schachter et al., 1987, 1986; Witek et al., 1987; Day et al., 1984; Bender et al., 1983; Anderson et al., 1983; Weber-Tschopp et al., 1977; Andersen, 1979; Schuck et al., 1966). A key advantage of chamber studies is the ability to monitor and closely control formaldehyde concentrations during exposure. However, chamber studies may also be limited by other aspects of the study design, including small number of participants, use of healthy volunteers, short exposure durations (a few minutes), and often studies were conducted with only one exposure group and at relatively high concentrations (>1 ppm). The lack of multiple exposure levels in many studies

limits the understanding of exposure-response relationships. Additionally, numerous reports that demonstrate multiple symptoms of eye, nose, and throat irritation at levels at or above 1 ppm did not explore lower levels of exposure and can only be used for primary hazard identification (Yang et al., 2001; Green et al., 1989, 1987; Sauder et al., 1987, 1986; Schachter et al., 1987, 1986; Witek et al., 1987; Day et al., 1984).

The National Aeronautical and Space Administration conducted experiments in closed-environment living, including environmental monitoring and air quality. James et al. (2002) quantified air pollutants, including formaldehyde, during 30, 60, and 90-day tests in a closed chamber study of a Lunar-Mars life support chamber. Unfortunately, the detection methods used during the 30-day test were not sensitive enough to detect formaldehyde at levels below 2 mg/m^3. Thus, badge samples were obtained in the 60-day and 90-day tests and provided greater detection sensitivity (to 0.02 mg/m^3). Measured values of formaldehyde increased over time. In the 60-day test, formaldehyde levels were well above accepted limits (data not shown). Health effects data are limited since there were only four crew members. One crew member reported eye and upper airway irritation at formaldehyde concentrations of 0.25 mg/m^3 (203 ppb) on day 15. It should also be noted that astronauts are exceptionally healthy individuals, and these data should be interpreted carefully when determining expected health effects in the general population. The experimenters determined that formaldehyde levels increased as temperature increased. Formaldehyde was also linked to murals lining the chamber and was subsequently removed before executing the 90-day study. Between days 0 and 60, formaldehyde levels remained between 0.02 and 0.04 mg/m^3, with one sharp peak that occurred at day three to 0.07 mg/m^3. Between days 60 and 90, formaldehyde concentrations increased to 0.07 to 0.09 mg/m^3. The increase was attributed to an incomplete oxidation of methanol in a catalytic bed rather than in excessive off-gassing of formaldehyde. No crew members reported any adverse effects in the 90-day study.

A few studies have been conducted that specifically address sensitive populations (asthmatics) and/or individuals during exercise, which can exacerbate asthma (further details of these studies are in Section 4.1.1.3, Effects on Asthmatics). In Sauder et al. (1986), 8-minute bicycle exercise was completed multiple times during the exposure period (3 hours). However, irritation symptoms were only reported after 2 hours of exposure and do not address whether changes occurred during the periods of exercise. Overall, reports of eye, nose, and throat irritation increased with exposure to formaldehyde (3 ppm) compared with reports of irritation with no exposure to formaldehyde. Green et al. (1987) report that eye, nose, and throat irritation symptoms were greater immediately after exercise during exposure to 3 ppm formaldehyde. Additionally, the response levels were similar between asthmatic (n = 16) and nonasthmatic

(n = 22) subjects. Similar effects of exercise on certain symptoms, such as throat irritation, were reported in 15 asthmatic subjects exposed to 2 ppm formaldehyde at rest and after exercise (Witek et al., 1987).

Kulle (1993) and Kulle et al. (1987) enrolled 19 healthy volunteers and exposed them to a range of formaldehyde concentrations. At 2 ppm, 53% reported mild or moderate eye irritation (32% mild, 21% moderate). At 3 ppm, 100% of subjects exposed at this level (n = 9) reported irritation. The reported increase in irritation was shown to correspond with increasing formaldehyde concentration in a linear fashion. Mild nose/throat irritation was present among 37% of those exposed to 2 ppm of formaldehyde. Odor detection was very similar to the distribution seen for eye irritation. Nineteen subjects performed light to moderate exercise while exposed to 2 ppm; there was no increase in report of eye irritation, but nose/throat irritation did increase. The data were reanalyzed (Kulle, 1993), and thresholds for irritation were found to be 0.5−1 ppm for eye irritation and 1 ppm for nose/throat irritation.

Yang et al. (2001) reported that eight individuals exposed to varying levels of formaldehyde (1.65, 2.99, and 4.31 ppm) had mild to moderate eye irritation during the 5-minute exposures. The increase in irritation was detected at 30 seconds with exposure to 1.65 ppm of formaldehyde. The highest severity ratings at this concentration occurred between 60 and 90 seconds. Frequency of eye blinking was also measured. The peak in blinking rate occurred after about 1 minute of exposure and then decreased almost back to a normal rate after 5 minutes of exposure. Higher formaldehyde concentrations were associated with increased frequency of blinking compared with the 1.65 ppm exposure.

Other studies have examined responses across multiple exposure levels. For example, Weber-Tschopp et al. (1977) used two different methods of studying irritation resulting from formaldehyde exposure. For one, they exposed subjects (n = 33) to an increasing level of formaldehyde (maximum exposure was 3.2 ppm). This design precluded evaluation of distinct effects at different exposure levels. The researchers addressed this by examining another group of subjects (n = 48) that were exposed to 0, 1, 2, 3, or 4 ppm five times for 90 seconds. Levels of nasal and throat irritation for this discontinuous exposure were slightly higher than the irritation levels reported among those with continuous exposure. However, this was reversed for eye irritation; those with continuous exposure reported higher levels of irritation than those with discrete exposures. An objective measure, eye-blinking rate, was measured for those with continuous exposure and was found to have a statistically significant increase at 1.7 ppm.

Bender et al. (1983) conducted a study that enrolled individuals who “responded” to formaldehyde at 1.3 and 2.2 ppm and did not report irritation to the clean air control. They found that, among these subjects, exposure to 1 ppm of formaldehyde (n = 27) resulted in the

reporting of eye irritation with a median response time of 78 seconds. Reports of irritation were given as less than slightly irritating for formaldehyde concentrations of 0.3−0.9 ppm.

Assessment of sensory irritation for pain and discomfort often relies on self-reporting, using symptom questionnaires and severity ratings (e.g., mild, moderate, severe). In the case of formaldehyde, subjective ratings of eye irritation correlate positively with eye-blinking frequency (Lang et al., 2008). Lang et al. (2008) saw an increase in eye blinking after 195 minutes of exposure to formaldehyde at 0.5 ppm with four peak exposures of 1 ppm. After this amount of time and formaldehyde exposure, there was also an increase in moderate eye redness. Weber-Tschopp et al. (1977) reported that, among concentrations ranging from 0.03 to 3.2 ppm, eye-blinking frequency was increased at 1.7 ppm; similarly Yang et al. (2001) reported increased blinking at >1.5 ppm (the lowest concentration examined). There are studies that suggest that psychological factors (e.g., anxiety) can impact the perception of irritation—and perhaps more so at lower concentrations (Lang et al., 2008; Ihrig et al., 2006; Dalton, 2003). However, when Lang et al. (2008) controlled for mood prior to exposure, subjective symptoms of eye, nasal, and olfactory irritation were significantly related to exposure (0.5 ppm)

Schuck et al. (1966) performed a study that also examines self-reported eye irritation as well as blinking rate. Fourteen individuals were exposed to formaldehyde concentrations ranging from 0 to 1 ppm. Increased irritation was reported with increasing formaldehyde concentration. One subject, judged to be the least sensitive, was still able to detect formaldehyde levels as low as 0.01 ppm. In addition, the authors examined the blinking rate of participants, which they found was related to irritation intensity.

Andersen (1979) and Anderson and Mølhave (1983) reported on a controlled experiment in which 16 individuals were exposed to varying levels of formaldehyde for five hours and rated their level of discomfort over the exposure period. Discomfort occurred within 1 hour at formaldehyde exposure levels of 1 and 2 mg/m^3 (Andersen and Mølhave, 1983) After 2 hours, increasing discomfort was reported among the groups exposed to 0.3 and 0.5 mg/m^3. Subject reported that discomfort was mainly conjunctival irritation and dryness in the nose and throat. Subjects complained at all four concentrations of formaldehyde: 0.3, 0.5, 1.0, and 2.0 mg/m^3 and of 16 subjects, 3, 5, 15, and 15 subjects complained at each respective exposure concentration (Andersen and Mølhave, 1983).

Controlled chamber studies have also been conducted on various populations of previously exposed individuals to determine if formaldehyde exposure potentiates an individual's response to acute exposures. Schachter et al. (1987) reported on 15 laboratory workers "frequently exposed to formaldehyde" (no quantification of exposure is given; however, the workers report being exposed for 1 to 7 days per week from a range of 1 to 21 years). Tests

performed at the start of the study found that these individuals had pulmonary function similar to that seen in healthy individuals. The workers in this study reported subjective measures of eye, nose, and throat irritation after 40 minutes of exposure to 2 ppm of formaldehyde. However, the 2 ppm acute exposure in this study may be sufficiently high to induce significant irritation in most individuals. Krakowiak et al. (1998) reported that 10 asthmatics with occupational exposure to formaldehyde (via formaldehyde solutions or pure gaseous formaldehyde) exhibited similar symptom scores to healthy controls (never exposed to formaldehyde in the workplace) exposed to 0.4 ppm formaldehyde for 2 hours. The mean symptom scores and standard deviation (SD), which included information on sneezing, rhinorrhea, mucosal edema, and itching, were 4.6 ± 1.6 (mean ± SD) for asthmatics and 4.3 ± 1.2 for healthy subjects immediately after inhalation. These dropped to 1.8 ± 1.2 and 1.2 ± 1.3, respectively, 4 hours after the exposure. It is unclear if sensitive individuals may not be represented in either of these groups, as the workers were tolerating their exposures during the work shift "healthy worker" effect. However, residents (n = 9) exposed to formaldehyde in their homes, who complained about adverse effects from the material, but with no occupational exposure reported eye, nose, and throat irritation at a similar rate as controls (individuals in homes without formaldehyde or individuals in homes with formaldehyde but not reporting adverse effects [n = 9]) after a 90-minute exposure to 1 ppm (Day et al., 1984). The number of individuals reporting eye irritation, nasal congestion, and throat irritation were seven, three, and two among sensitive individuals and eight, four, and three among controls, respectively. These individuals may be considered a sensitive population since they had "previously complained of various nonrespiratory effects from the UFFI in their homes" (household concentrations unknown).

4.1.1.2. *Pulmonary Function*

Pulmonary function is assessed using spirometry which measures the volume and speed of air that is exhaled or inhaled. Multiple parameters can be measured during spirometric testing. Forced vital capacity (FVC) measures the volume of air that can be exhaled. This volume can be partitioned into the volume that is exhaled in the first second (FEV_1) or that which is exhaled during the middle of a breath between the 25^{th} and 75^{th} percentiles called the forced expiratory flow 25-75% (FEF_{25-75}) and is also called the maximal mid-expiratory flow (MMEF). Other common metrics of lung function are the ratio of FEV_1 to FVC (FEV_1/FVC) and the peak expiratory flow rate (PEF or PEFR). Spirometric results can be important diagnostic criteria for physician-diagnosed asthma. Changes in lung function are an important health endpoint with potentially long-term consequences. The observed consequences of early

life exposure to adverse levels of air pollutants include diminished lung function, increased susceptibility to acute respiratory illness and asthma (Bateson and Schwartz, 2008).

Absolute values for lung function parameters are likely to vary by gender, age, height, and smoking status and are best compared when normalized to the expected lung function based on these variables (Schoenberg et al., 1978). Therefore, these well-known predictors of lung function should be controlled for in an evaluation of spirometric data. Individual variation can also be addressed by each subject serving as his/her control with measurements taken before, during, and after exposure. Analysis of the percent change in various parameters in this context may have greater sensitivity to detect exposure-related changes in function. In addition to individual variation in baseline lung function, there is also individual variation in bronchial responsiveness. Reduced lung function parameters in response to methacholine challenge is a standard test for bronchial constriction, and this can be used to define responsive, sensitive, or susceptible individuals. Since formaldehyde-induced bronchial constriction is measured with these lung function tests, variability in bronchial responsiveness may impact interpretation of formaldehyde-induced changes. Depending upon the proportion of susceptible individuals in a study population, the group-mean change in lung function parameters may or may not reflect any effect of exposure. Studies that exclude sensitive or responsive individuals may not detect changes in lung function. Studies based on random population samples may include some sensitive individuals who respond to exposures with large changes in lung function parameters that may be difficult to detect if only the group mean change in lung function is examined. Experiments that report individual-level changes in lung function parameters or that focus on sensitive individuals can help address this question. An additional complication in the interpretation of pulmonary function experiments is that 'sensitivity' may be specific to timing of exposure in relation to the potential allergen and the individuals' atopic status.

Formaldehyde may itself be an allergen or it may potentiate the ability of other allergens to cause atopic switching or increase the sensitivity of atopic individuals. Thus formaldehyde exposure among nonatopic individuals could theoretically cause atopic switching in the presence or absence of allergens possibly resulting in a diagnosis of asthma. Formaldehyde could also cause an asthma attack or potentiate the influence of other stimuli on the risk of asthma attacks. Demonstration of a clear association of formaldehyde exposure, or the lack of an association, at one particular time prior to or following the onset of asthma does not necessarily imply that exposure to formaldehyde is causing or not causing adverse outcomes at other times.

Workers chronically exposed to formaldehyde have exhibited signs of reduced lung function consistent with bronchial constriction, inflammation, or chronic obstructive lung disease. Worker exposures that report cross-shift differences in spirometric values are consistent

with formaldehyde-induced sensory irritation. Additionally, concordance has been reported between subjective irritant response and measured changes in pulmonary function, further supporting the possibility that cross-shift and short-term evidence of bronchial constriction may be a reflexive response to sensory irritation.

In occupational studies of formaldehyde exposure, lung function deficits have been reported both in preshift versus postshift measurements and as a result of long-term exposures (Pourmahabadian et al., 2006; Herbert et al., 1994; Malaka and Kodama, 1990; Alexandersson and Hedenstierna, 1989; Alexandersson et al., 1982). Decreases in spirometric values, including peak expiratory flow, vital capacity, forced expiratory volume, forced vital capacity, and FEV/FVC have been reported. Studies of long-term exposure also report increased respiratory symptoms, such as cough, increased phlegm, asthma, chest tightness, and chest colds, in exposed workers (Pourmahabadian et al., 2006; Herbert et al., 1994; Malaka and Kodama, 1990; Alexandersson and Hedenstierna, 1989; Alexandersson et al., 1982). Similar findings have been reported for low-level residential formaldehyde exposure, including decreased PEF rates in children (Krzyzanowski et al., 1990).

4.1.1.2.1. ***Epidemiologic literature.***

The potential adverse effects of formaldehyde exposure on pulmonary function in humans can be examined on several time scales of interest. The epidemiologic literature supports the assessment of exposures among exposed anatomy medical students where all participants have well-defined and similar duration of exposure (i.e., a semester-long class) (Kriebel et al., 2001, 1993; Akbar-Khanzadeh and Mlynek, 1997; Akbar-Khanzadeh et al., 1994; Uba et al., 1989; Fleisher, 1987); among individuals living or working in buildings with formaldehyde exposure (Franklin et al., 2000; Krzyzanowski et al., 1990; Main and Hogan, 1983); and among workers (industrial, manufacturing, mortuary, hospital staff, etc.) (Ostojic et al., 2006; Herbert et al., 1994; Khamgaonkar and Fulare, 1991; Malaka and Kodama, 1990; Nunn et al., 1990; Alexandersson and Hedenstierna, 1989; Holness and Nethercott, 1989; Holmström and Wilhelmsson, 1988; Horvath et al., 1988; Kilburn et al., 1985; Alexandersson et al., 1982). These studies are summarized in Table 4-1.

Studies of anatomy students provided information about acute effects related to formaldehyde exposures experienced in the laboratory (Kriebel et al., 2001, 1993) as well as special insight into the intermediate stages of possible sensitization (Kriebel et al., 1993). Kriebel and colleagues (1993) examined the prelaboratory and postlaboratory PEF using Mini-Wright peak flowmeters in 24 students attending 3-hour anatomy classes to dissect cadavers once per week over 10 weeks. Formaldehyde concentrations collected in the breathing zone

Table 4-1. Epidemiologic studies of formaldehyde and pulmonary function

Reference	Study design	Exposure assessment	Results, effect estimate, statistical significance				
Uba et al., 1989	Panel study of symptoms and respiratory function among 103 medical students related to formaldehyde exposure during a 7 month anatomy class meeting twice a week for 4 hours (September 1984−April 1985). Pre- and postlab spirometric measures were taken before the class began, after the first 2 weeks, and after 7 months. Complete data was available for 96 students. Cross-shift change in pulmonary function analyzed using repeated measures ANOVA.	Personal sampling monitors (impingers) in the breathing zone measured time-weighted average formaldehyde exposure during the gross anatomy laboratory. A total of 32 samples were taken during 7 months. Short-term samples were taken (N = 16) for peak concentrations using a portable infrared spectrophotometer. formaldehyde ranged from below LOD (0.05 ppm) to 0.93 ppm for TWA and 0.1 to 5.0 ppm during dissection. Concentrations declined over the 7 months.		Prelab	Value (SD)		
			Test Day 1	FVC (L)	5.246 (1.025)		
				FEV_1 (L)	4.379 (0.846)		
				FEF_{25-75} (L/sec)	4.492 (1.216)		
				FEV_1/FVC	0.835		
			Test Day 2	FVC (L)	5.277 (1.027)		
				FEV_1 (L)	4.409 (0.824)		
				FEF_{25-75} (L/sec)	4.484 (1.151)		
				FEV_1/FVC	0.836		
			Test Day 3	FVC (L)	5.308 (1.027)		
				FEV_1 (L)	4.399 (0.823)		
				FEF_{25-75} (L/sec)	4.392 (1.198)		
				FEV_1/FVC	0.829		
				Cross-Lab Change	Mean change (%)		
			Test Day 1	FVC (L)	-0.012 (-0.23)		
				FEV_1 (L)	-0.031 (-0.71)		
				FEF_{25-75} (L/sec)	-0.079 (-1.76)		
				FEV_1/FVC	-0.004 (-0.48)		
			Test Day 2	FVC (L)	-0.042[a] (-0.80)		
				FEV_1 (L)	-0.046[b] (-1.04)		
				FEF_{25-75} (L/sec)	-0.089 (-1.99)		
				FEV_1/FVC	-0.003 (-0.36)		

Table 4-1. Epidemiologic studies of formaldehyde and pulmonary function (continued)

Reference	Study design	Exposure assessment	Results, effect estimate, statistical significance				
Uba et al., 1989 (continued)			Test Day 3	FVC (L)	-0.042[a] (-0.79)		
				FEV_1 (L)	-0.021[c] (-0.48)		
				FEF_{25-75} (L/sec)	0.003 (0.07)		
				FEV_1/FVC	0.002 (0.24)		
				[a]Day 2&3 vs Day 1, $p < 0.001$ [b]Day 2 vs Day 1, $p = 0.03$) [c]Day 3 vs Day 1 $p = 0.01$)			
Kriebel et al. (1993)	Panel study of symptoms and respiratory function related to formaldehyde exposure among 24 clinical anatomy students during a 10 week anatomy class meeting once a week for 3 hours. PEF measured by trained students pre- and postlab and 1-3 times during lab using Mini-Wright peak flowmeters. Mean prelab and cross-lab change in pulmonary function analyzed using random effects models.	Personal samples in the breathing zone formaldehyde sampling for 1−1.5 hours. Concentrations ranged from 0.49−0.93 ppm, 8 samples. No trend in concentrations over semester.		Prelab	Mean (SD) (N = 20)		
			Weeks 1-2	PEF (L/min)	538.9 (86.9)		
			Weeks 9-10[a]	PEF (L/min)	529.4 (88.4)		
			Weeks 24-25	PEF (L/min)	536.6 (86.2)		
			GLM of prelab decrement over 10 week course[b]				
				ß = -2.7 ± 1.1 L/min per week; $p = 0.01$			
			[a]End of course				
			[b]Model included asthma, asthma*week, eye symptoms, nose symptoms				
				Cross-lab change	Change (% of prelab)		
			Weeks 1-2	PEF (L/min)	-10.8 (-2.0)		
Kriebel et al.. (2001)	Panel study of symptoms and respiratory function and formaldehyde exposure among 51 gross anatomy students during a 12 week class meeting once per week for 2.5 hours. Pre- and postlab measurements obtained for at least one week for 38. Individual pre- and	Formaldehyde concentrations were monitored continuously in six homogenous sampling zones in the lab (LOD = 0.05 ppm). Work location every 12 minutes was recorded and 12 minute work-zone concentrations were calculated for each student.		PEF as fraction of baseline (before 1st lab)			
				ß (SE)	p value		
			Recent exposure	-1.05 (0.33)	0.002		
			Recent exposure *ln(wk)	0.69 (0.24)	0.004		
			Past exposure	-0.52 (0.30)	0.08		
			Cold on lab day	-1.67 (0.41)	0.001		

Table 4-1. Epidemiologic studies of formaldehyde and pulmonary function (continued)

Reference	Study design	Exposure assessment	Results, effect estimate, statistical significance				
Kriebel et al.. (2001) (continued)	postlab data analyzed together in generalized estimating equations.	Three exposure metrics were developed. Recent exposure: Mean concentration during 2.5 hour lab Cumulative exposure: PPM-minutes for all previous weeks Past average exposure: Cumulative exposure divided by the total number of minutes of exposure Geometric mean concentration 0.7 ppm (GSD:2.13 ppm). Peak 12 min concentration was 10.91 ppm. Average concentration 1.1 ppm (SD = 0.56 ppm). Formaldehyde concentrations decreased over the 12 week semester.					
Akbar-Khanzadeh et al. (1994)	Comparison of pulmonary function (spirometry) among 34 nonsmoking exposed medical students and instructors and 12 nonmedical unexposed students before and after their work in the gross anatomy laboratory or at predetermined times for the unexposed (approximately 3 hours) over five consecutive weeks.	TWA personal breathing zone samples were obtained for each exposed subject over 9 days and 1 unexposed subject over 6 days. The TWA exposure from personal sampling ranged from 0.07-2.94 ppm. More than 94% of the subjects were exposed to >0.3 ppm and 31.7% were exposed to an 8-hour time-weighted average of >0.5 ppm.		Study group (n = 34)	Referent (n = 12)		
				Percent cross-lab change (SD)			
			FVC	-1.4 (4.4)[a]	-0.3 (4.6)		
			FEV_1	-0.03 (3.4)	1.0 (4.0)		
			FEV_3	-1.2 (4.2)	1.3 (3.29)[c]		
			FEF_{25-75}	2.5 (8.7)	2.31 (2.71)		
			FEV_1/FVC	1.6 (3.8)[b]	0.6 (2.9)		
				[a]$p < 0.1$, [b]$p < 0.05$, [c]independent group, $p < 0.1$			

Table 4-1. Epidemiologic studies of formaldehyde and pulmonary function (continued)

Reference	Study design	Exposure assessment	Results, effect estimate, statistical significance				
Akbar-Khanzadeh et al. 1997	Comparison of pulmonary function (spirometry) among 50 exposed first-year medical students and 36 unexposed second-year physiotherapy students during a 3-hour anatomy lab. At least 2 exposed and 2 unexposed students, close in age to the exposed, were evaluated on each test day for the duration of the course. Lung function over one 3-hour lab session was evaluated for each participant. Prelab spirometric variables, expressed as a percentage of reference values accounting for height, weight, age, sex, and race. Cross-lab change analyzed within and between groups.	Personal (breathing zone) (n = 44) and area (n = 76) formaldehyde samples weer collected. Average formaldehyde concentrations measured in the breathing zone of the students for an average of 157 minutes was 1.88 (SD = 0.96) ppm with a range of 0.30-4.45 ppm.		Mean percent cross-lab change over 3 hours (SD)			
				Exposed (n = 50)	Referent (n = 36)		
			FVC	2.5 (5.4)[a]	4.6 (6.4)[b]		
			FEV_1	2.4 (5.1)[a]	6.2 (7.0)[bc]		
			FEV_3	2.7 (4.6)[b]	5.2 (6.5)[bd]		
			FEF_{25-75}	2.2 (9.4)	9.3 (11.9)[bc]		
			[a]$p < 0.01$, [b]$p < 0.001$, [c]independent group, $p < 0.01$, [d]independent group, $p < 0.1$				
Krzyzanowski et al. (1990)	Cross-sectional study of residential formaldehyde exposure. A stratified random sample of households of municipal employees was selected based on information about potential exposure (age of housing) and potential susceptibility obtained from an initial screening questionnaire. Households with children aged 5−15 years (613 adults and 298 children) were eligible for inclusion. Trained subjects	Residential exposures to formaldehyde were based on two one-week samples from each individual's kitchen, living area, and bedroom using passive sampling tubes. The average formaldehyde concentration was 26 ppb, with a maximum sample value of 140 ppb. The majority of subjects (83%) lived in homes with 2-week average concentrations below 40 ppb.	Random effects model, ages ≤ 15 (N = 208; 3021 observations)				
			Factor	ß (SD)			
			Formaldehyde (household mean)	-1.28 (0.46)[a]			
			Morning FA (vs bedtime)	-6.1 (3.0)[a]			
			Bedroom FA *morning	0.09 (0.15)			
			Bedroom FA squared*morning	0.0031 (0.0015)[a]			
Krzyzanowski et	measured peak expiratory flow		Morning*asthma	4.59 (9.60)			

Table 4-1. Epidemiologic studies of formaldehyde and pulmonary function (continued)

Reference	Study design	Exposure assessment	Results, effect estimate, statistical significance				
al. (1990) (continued)	rates (PEFRs) using mini-Wright peak flow meters four times daily, in the morning, at noon, in the early evening, and before bed, for 2 weeks. The largest of three tests was recorded for each test period. PEFR was analyzed using a random effects model adjusting for asthma status, smoking status, SES, NO_2 levels, episodes of acute respiratory illness, and time of day. Analysis performed separately for ages below and over 15 years of age.		Bedroom FA*morning*asthma	-1.45 (0.53)[a]			
			Bedroom FA sq*morning*asthma	0.031 (0.006)[a]			
			Constant	349.6 (13.2)			
			[a]$p < 0.05$				
			Random effects model, ages > 15 (N = 526; 8463 observations)				
			Formaldehyde (household mean)	0.09 (0.27)			
			Morning FA (vs bedtime)	-5.9 (1.1) [a]			
			Bedroom FA *morning	-0.07 (0.04)[b]			
			Morning*smoking	-7.4 (2.6)[a]			
			Bedroom FA*morning*smoking	0.59 (0.13)[a]			
			Bedroom FA sq *morning *smoking	-0.007 (0.001)[a]			
			Constant	491.7 (8.5)			
			[a]$p < 0.05$, [b]$0.05 < p < 0.10$				

Table 4-1. Epidemiologic studies of formaldehyde and pulmonary function (continued)

Reference	Study design	Exposure assessment	Results, effect estimate, statistical significance				
Franklin et al. (2000)	Cross-sectional study of residential exposure among 224 children (116 girls, 108 boys) with no current, or history of, upper or lower respiratory tract disease were included from responses to a respiratory health questionnaire and household inventory distributed through local primary schools. Clinical respiratory measures were obtained at the children's hospital. Exhaled nitric oxide and skin prick tests for 7 common allergens were measured.	Three to four-day passive samples were collected in the child's bedroom and the main living area of the house and formaldehyde levels were recorded as a time-weighted average.	FVC	No association	Data not presented		
			FEV_1	No association	Data not presented		
			Formaldehyde	eNO (ppb)	Range		
			≥50 ppb	15.5	10.5-22.9		
			<50 ppb	8.7[a]	7.9-9.6		
			[a]p = 0.002, adjusted for age, atopic status				
Main and Hogan (1983)	Cross-sectional comparison of 21 individuals working in two mobile trailers for 34 months (mean age 38 ± 9 years, 76% male, 19% nonsmokers) and 18 individuals who did not work in the trailers (mean age 30 ± 6, 50% male, 22% nonsmokers). Percent predicted FEV_1 and FVC stratified by smoking status (unadjusted group means compared using t tests).	Three 1-hour area samples using impingers were taken on 4 occasions (August, September, December, April) always on a Monday. At least 1 sample was taken from each office in both trailers. Concentrations ranged from 0.12 to 1.6 ppm.		Mean percent predicted			
				Exposed (*N* = 14)	Unexposed (*N* = 17)		
			FEV_1	98	99		
			FVC	94	97		
			FEF_{50}	93	90		
			FEF_{75}	69	70		
			%Δ FEF_{50}	55	43		

Table 4-1. Epidemiologic studies of formaldehyde and pulmonary function (continued)

Reference	Study design	Exposure assessment	Results, effect estimate, statistical significance				
Alexandersson et al. (1982)	Cross-sectional occupational study at a carpentry works. A total of 47 exposed workers employed at the plant for > 1 year were included (mean age 35 years, mean duration 5.9 years) and compared to 20 unexposed employees. Spirometric measurements were obtained Monday morning preshift and after work for exposed. Lung function was measured in the unexposed in the morning or the afternoon.	TWA formaldehyde concentration, measured using personal sampling in the working zone over a working day, was 0.36 ppm (0.04-1.25).		Preshift lung function (SD)			
				Exposed	Referent		
			FVC (L)	5.73 (0.14)	6.0 (0.2)		
			FEV_1 (L)	4.52 (0.12)[a]	4.86 (0.15)		
			FEV%	792 (1.0)	80.7 (1.32)		
			MMF (L/sec)	4.94 (0.2)	5.08 (0.31)		
			CV%	16.7 (1.07)	17.1 (1.5)		
			[a]Difference from reference value, $p = 0.08$				
				Cross-shift change			
			FVC (L)	-0.05			
			FEV_1 (L)	-0.17[a]			
			FEV%	-2.1[b]			
			MMF (L/sec)	-0.32[b]			
			CV%	3.4[a]			
			[a]$p < 0.001$, [b]$p < 0.05$				
Alexandersson and Hedenstierna, 1989	Prospective occupational study of cabinetry workers first reported by Alexandersson et al., 1982. Of 47 exposed workers and 20 unexposed workers examined in 1980, 34 exposed and 18 unexposed were examined again in 1984. Of the 34 originally exposed, 13 had been reassigned to other unexposed jobs. The exposed	Personal exposure monitored during 3-4 15-minute periods during the work day. The time-weighted formaldehyde concentration, measured using personal sampling in the working zone over a working day, was 0.42 ± 0.27 mg/m3 (0.34 ppm) in 1980 and 0.50 ± 0.12 mg/m3 (0.4 ppm) in 1984.		Annual change (1980-1984), Mean (SD)			
				Smokers ($N = 10$)	Nonsmokers ($N = 11$)		
			FVC (L)	-15 (24)	-10 (26)		
			FEV_1 (L)	-15 (21)	-31 (20)		
			FEV_1/FVC (%)	-0.1 (0.4)	-0.4 (0.2)[a]		
			FEV_{25-75}	-60 (69)	-212 (66)[a]		

Table 4-1. Epidemiologic studies of formaldehyde and pulmonary function (continued)

Reference	Study design	Exposure assessment	Results, effect estimate, statistical significance				
Alexandersson and Hedenstierna (1989) (continued)	and transferred workers had been exposed to formaldehyde an average of 11 years. Spirometric measures were compared with reference values for sex, age, height, and weight. The 5-year change was corrected for age-dependent change. Results were presented by smoking status.		CV%	-0.6 (0.3)	0.2 (0.4)		
			[a]$p < 0.001$				
				Cross-shift change (All $N = 21$)			
			FVC (L)	-0.03 (0.09)			
			FEV_1 (L)	-0.05 (0.09)			
			FEV_1/FVC (%)	-0.3 (0.18)[a]			
			FEV_{25-75}	-0.1 (0.18)			
			CV%	-0.8 (0.14)[a]			
			[a]$p < 0.05$				
Kilburn et al. (1985)	Occupational study of 45 fiberglass batt makers (out of 110); aged 21-55 years (40% Hispanic, 60% white) Another exposed group of 18 male histology technicians, aged 25-48 years (4 Hispanic, 2 Oriental, 12 white). Reference group was hospital employees, 20-62 years of age (35% Hispanic, 65% white). Spirometry measurements were taken before and after an 8-hour work shift.	Formaldehyde exposure categorized as high or low based on reported work assignments.	Batt Makers Lung function (% cutoff)	Percent with specified percent decrease or more relative to preshift value			
				Smokers ($N = 35$)	Nonsmokers ($N = 9$)		
			FVC (L) (5%)	8.6[a]	22.2		
			FEV_1 (L) (10%)	11.4 [a]	33.3		
			FEV_{25-75} (L/sec) (15%)	11.4 [a]	33.3		
			FEV_{75-85} (L/sec) (15%)	40.0 [a]	22.2		
				[a]$p < 0.01$			

Table 4-1. Epidemiologic studies of formaldehyde and pulmonary function (continued)

Reference	Study design	Exposure assessment	Results, effect estimate, statistical significance
Horvath et al., 1988	Occupational study of wood products workers compared pulmonary function between 109 exposed (workers at a particleboard and molded products operation, 68.6% of all exposed) and 254 unexposed (workers from nearby food processing facilities). The exposed workers had been employed an average of 10.3 years (1−20 years). Spirometry was conducted before and after the work shift. Lung function as percent of predicted normal was compared between exposed and unexposed (unpaired t-test).	8-hour TWA formaldehyde was measured using individual passive monitors on the day of the exam (LOD 0.1 ppm). Area levels were measured with an active sampling train (impingers). TWA formaldehyde averaged 0.69 ppm (0.17−2.93 ppm) and 0.05 ppm (0.03−0.12 ppm) in the exposed and unexposed industries, respectively.,	Preshift Lung Function: % Predicted (SD) Exposed / Referent FEV_1 (L): 103 (13) / 105 (13) FVC (L): 105 (12) / 107 (13) FEV_1/FVC: 96 (8) / 95 (8) PEFR (L/sec): 100 (23) / 103 (22) FEV_{25-75} (L/sec): 83 (22) / 85 (25) $p > 0.05$ Cross-shift change in lung function compared between exposed and referent. Statistically significant differences reported for FVC, FEV_1/FVC, FEV_{25-75}, FEF_{50} and FEF_{75} (Data not presented).
Holmström and Wilhelmsson (1988)	Cross-sectional occupational study of 70 individuals (87% male) from a chemical plant where formaldehyde and formaldehyde products were made. Exposure levels varied from 0.05−0.5 mg/m^3. A group of 100 furniture workers was exposed to formaldehyde and wood dust with mean concentrations of 0.25 mg/m^3. A comparison group of 36 persons (56% male) was mostly comprised of clerks for the local government in an office with mean formaldehyde concentrations of 0.09 mg/m^3.	Mean annual exposure to formaldehyde was estimated for each participant from the beginning of employment. Data on formaldehyde concentrations was available between 1979−1984 and from 1-2 hour personal sampling in breathing zone at different workstations in 1985. Dose-years were calculated for each worker.	FA exposed (N = 70) / FA-dust exposed (N = 98) / Referent (N = 36) FVC Observed: [a]4.979 / [a]4.929 / 4.539 Expected: 5.556 / 5.593 / 4.718 FEV% Observed: 80.8 / 78.3 / 81.4 Expected: 80.6 / 79.5 / 80.7 [a]paired t-test comparing observed to expected, $p < 0.001$
Holmström and	Mean duration of employment		

Table 4-1. Epidemiologic studies of formaldehyde and pulmonary function (continued)

Reference	Study design	Exposure assessment	Results, effect estimate, statistical significance				
Wilhelmsson (1988) (continued)	was 10.4 years for exposed and 11.4 for referent group. Spirometric measures were analyzed as percent of expected normal based on age, sex, smoking, height and weight.						
Holness and Nethercott (1989)	Cross-sectional study of funeral workers, including 67 currently active embalmers and 17 who were no longer active, were recruited through a list of funeral homes developed by the Metropolitan District Funeral Directors Association in Toronto, Canada (86.6% participation). An unexposed group (*N* = 38) was recruited from a large service organization and paid student volunteers. Information on symptoms, past and family medical history, and work practices was collected by questionnaire, and pulmonary function and skin tests were administered. Lung function tests were performed on 22 embalmers just prior to and following an embalming procedure, and on 13 controls 2-3 hours after for first test. Funeral workers had performed the embalming procedure for an average of 10 years. Lung	The average formaldehyde concentration from 2 area samples (impingers), measured during embalming procedures lasting from 30 to 180 minutes, was 0.36 ± 0.19 ppm (0.08−0.81 ppm). Unexposed participants were stated to be exposed to an average concentration of 0.02 ppm.	Lung function (% predicted) (SD)	Exposed (*N* = 84)	Unexposed (*N* = 38)		
			FVC	100.5 (12.3)	100.9 (11.5)		
			FEV_1	99.2 (12.9)	100.7 (12.9)		
			FEV_1/FVC	98.4 (7.9)	99.4 (8.7)		
			FEF_{50}	104.8 (29.7)	110.3 (34.5)		
			FEF_{75}	76.2 (32.9)	86.6 (36.0)		
				Active (*N* = 67)	Inactive (*N* = 17)		
			FVC	100.7 (12.2)	95.8 (12.0)[a]		
			FEV_1	100.8 (12.19)	93.1 (14.1)[b]		
			FEV_1/FVC	98.9 (7.8)	96.6 (8.0)		
			FEF_{50}	107.5 (28.7)	94.1 (32.3)		
			FEF_{75}	80.8 (33.1)	57.1 (24.7)		
				[a]p = 0.0385, [b]p = 0.0652			
			% Change in Lung Function during embalming	Exposed (*N* = 22)	Unexposed (*N* = 13)		
			FVC	+0.88 (2.95)	+1.13 (3.98)		
			FEV_1	-0.03 (2.4)	+1.45 (4.43)		
			FEF_{50}	-2.28 (13.43)	+1.23 (12.44)		
Holness and	function as percent predicted		FEF_{75}	-8.55 (15.09)	+1.93 (27.54)		

Table 4-1. Epidemiologic studies of formaldehyde and pulmonary function (continued)

Reference	Study design	Exposure assessment	Results, effect estimate, statistical significance				
Nethercott (1989) (continued)	was compared between exposed and unexposed using multiple regression correcting for age, height, and pack-years smoked.						
Nunn et al. (1990)	Prospective, occupational study of workers, aged 25 or older, at a chemical factory in Duxford, England, manufacturing urea formaldehyde resin. A total of 164 workers exposed to free formaldehyde in 1980 and a group of 129 workers from the bonded structures division at the same factory in 1980 were followed over a 6 year period from 1980-1985. Data on FEV_1 and FVC (highest of two readings within 5% of each other) were obtained from routine annual health screenings conducted by the same nurse throughout the study period. Follow-up was complete for 76% of the exposed and 74% of the unexposed workers. FEV_1 values ($FEV_1/height^3$), adjusted for height, were regressed on time of screening visit for each worker, adjusting for age in 1980, smoking status in 1980 and at final assessment, maximum and mean exposure assessment level and total duration of exposure.	Data on formaldehyde concentrations from area samples (1−6 hours sample collections) taken periodically between 1979 and 1985, and from personal samplers attached to representative exposed workers from 1985 to 1987 were used to categorize the workers' employment experience into low, medium and high formaldehyde groups corresponding to a 8-hour TWA of 0.1-5.0 ppm, 0.6-2.0 ppm, and >2 ppm, respectively. Exposure assessments prior to 1976 were based on subjective determinations and knowledge of process changes and industrial hygiene measures.	Decline in FEV_1 with age by smoking history (Mean slope, ml/year (95% CI)				
			Smoking status	Exposed	*N*	Unexposed	*N*
			Never	45 (28-62)	26	29 (7-51)	13
			Ex-smoker	33 (20-46)	34	40 (26-54)	31
			Current	46 (33-59)	57	46 (32-61)	36
			Total	42 (34-51)	117	41 (32-50)	80
Malaka and	Cross-sectional occupational	Exposed and unexposed		Mean Baseline Spirometric Values			

Table 4-1. Epidemiologic studies of formaldehyde and pulmonary function (continued)

Reference	Study design	Exposure assessment	Results, effect estimate, statistical significance
Kodama, 1990	study at a plywood company comparing a group of formaldehyde exposed with plant workers not exposed to formaldehyde, matched for age, ethnicity, and smoking status. Exposed workers (N = 100) were randomly selected with stratification by smoking status and years of occupation (< 5 and ≥5 years). The unexposed group (N = 100) worked in areas where formaldehyde was not used (range 0.003−0.07 ppm) and had no previous or current exposure to formaldehyde based on occupational histories. A total of 93 exposed and 93 unexposed participants completed the protocol (93% participation). Baseline and cross-shift spirometric measurements were taken. Lung function (percentage of expected function) was analyzed using analysis of covariance adjusting for sampled dust levels and stepwise regression. Unexposed workers had been employed slightly longer than those in the exposed group (6.7 ± 2.3 versus 6.2 ± 2.4 years, $p < 0.05$).	workers were identified using area formaldehyde measurements and personal monitoring. Formaldehyde levels ranged between 0.22 and 3.48 ppm (average 1.13 ppm). Exposure was evaluated using a cumulative measure using area concentrations and duration in current job (mean 6.29 ppm-year, SD 2.72).	(adjusted for dust) (SD) Exposed / Referent FEV_1/FVC (%): 84.7 (6.5) / 86.9 (4.9)[a] FEV_1 (L): 2.78 (0.41) / 2.82 (0.30)[a] FVC (L): 3.28 (0.44) / 3.37 (0.36) $FEF_{25-75\%}$ (L/sec): 3.04 (0.76) / 3.44 (0.78)[a] [a]$p < 0.001$ Multiple regression model of pulmonary function[a] ß (per ppm-yr FA) FEV_1/FVC (%): -0.347[b] FEV_1 (L): -0.015[b] FVC (L): NS $FEF_{25-75\%}$ (L/sec): -0.043[b] [a]adjusted for age, height, weight, cigarettes/day, and dust [b]$p < 0.05$
Khamagaonkar	Cross-sectional occupational	Multiple thirty minute air	Mean lung function

Table 4-1. Epidemiologic studies of formaldehyde and pulmonary function (continued)

Reference	Study design	Exposure assessment	Results, effect estimate, statistical significance				
and Fulare, 1991	study of 74 individuals working in anatomy and histopathology departments at 3 colleges in India and exposed to formaldehyde. A comparison group was matched by age and sex (N = 74) (individuals not working in laboratories with formaldehyde). Persons with a history of lung disease before their present occupation were excluded. Lung function tests were performed on a subset of 37 exposed and 37 comparison individuals on a Monday morning after days of no exposure.	samples were collected in the breathing zone in both the exposed (N = 43) and unexposed (N = 18) areas for formaldehyde analysis. Mean formaldehyde concentrations were 1.00 ppm (range 0.036−2.27) and 0.102 ppm (range 0−0.52) among the exposed and referent groups, respectively.		Exposed (N = 37)	Referent (N = 37)		
		FVC (L)	2.18	2.63[a]		
		MMEFR (L/sec)	1.55	2.71[b]		
		FEV_1 (%)	60.68	78.74[a]		
			[a]$p < 0.01$, [b]$p < 0.05$			
Herbert et al. (1994)	Cross-sectional occupational study comparing 99 oriented strand board workers (exposed to formaldehyde) (98% participation rate) with 165 oil/gas field plant workers (not exposed to formaldehyde) from the same geographic area (82% participation rate). Duration of employment was a mean of 5.1 and 10 years for OSB and oilfield workers, respectively. Spirometric testing (best of 5 satisfactory maneuvers) was conducted at start of work shift and after 6 hours. Lung function was analyzed using	Time weighted average formaldehyde concentrations based on 21 hour continuous sampling in the breathing zone at 5 work sites on 2 separate days ranged between 0.07 and 0.27 ppm.		Preshift lung function (Mean)			
			OSB	Oilfield		
		FEV_1 (ml)	4.203	4.223		
		FVC (ml)	5.364	5.257		
		FEV_1/FVC (%)	78.6	80.3[a]		
			[a]p = 0.028			
			Cross-shift difference in lung function			
		FEV_1 (ml)	39[b]			
		FVC (ml)	47 [b]			
		FEV_1/FVC (%)	0.1			
			[b]$p < 0.05$			
Herbert et al.	ANCOVA controlling for age,						

Table 4-1. Epidemiologic studies of formaldehyde and pulmonary function (continued)

Reference	Study design	Exposure assessment	Results, effect estimate, statistical significance				
(1994) (continued)	height, and smoking.						
Pourmahabadian et al. (2006)	Cross-sectional study of formaldehyde exposure among staff working in different departments in 7 large hospitals of Tehran University of Medical Sciences in Iran. Pre- and postshift lung function testing was conducted for 124 workers from the pathology labs (38), surgery (65), endoscopy (21) departments and 56 administrative affairs staff during February 2002 to February 2003.	Formaldehyde concentrations were highest in the pathology lab (8-hour average [SD]: 0.96 [0.74] ppm) compared to the surgery rooms (0.25 [0.18] ppm) and endoscopy departments (0.25 [0.22] ppm). Highest one-hour spot samples recorded concentrations of 6.5 ppm, 2.7 ppm and 0.6 ppm in pathology, surgery and endoscopy, respectively. Formaldehyde levels in administrative offices were not reported.		Preshift lung function, Mean (SD)			
				Exposed	Referent		
			VC (L)	3.34 (1.07)	3.92 (1.26)[a]		
			FVC (L)	3.46 (1.04)	4.03 (1.23)[a]		
			FEV_1 (L)	2.45 (1.02)	2.90 (1.21)[b]		
			FEV_1/VC (%)	73.35 (21.53)	73.98 (21.84)		
			FEV_1/FVC (%)	70.81 (17.8)	71.96 (24.4)		
			FEF_{25-75} (L/sec)	2.79 (1.4)	3.38 (1.7)[b]		
				[a]$p < 0.002$, [b]$p < 0.01$			
				Cross-shift difference, Mean (SD)			
				Exposed	Referent		
			VC (L)	0.14 (0.15)	0.02 (0.04)[a]		
			FVC (L)	0.16 (0.03)	0.03 (0.03)[b]		
			FEV_1 (L)	0.15 (0.02)	0.00 (0.01)[b]		
			FEV_1/VC (%)	1.48 (6.67)	0.38 (0.16)		
			FEV_1/FVC (%)	1.11 (2.9)	0.54 (0.20)		
			FEF_{25-75} (L/sec)	0.13 (0.10)	0.03 (0.10)		
				[a]$p < 0.001$, [b]$p < 0.04$			

Table 4-1. Epidemiologic studies of formaldehyde and pulmonary function (continued)

Reference	Study design	Exposure assessment	Results, effect estimate, statistical significance				
Ostojić et al. (2006)	Cross-sectional occupational study of 16 male health-service professionals (8 medical doctors, 8 lab technicians) at a university in the pathoanatomic laboratory for at least 4 years with daily exposure to formaldehyde. Exposed were compared to 16 males matched by age ad stature. All were nonsmokers. The timing of the spirometric tests was not stated.	Assessment of formaldehyde exposure was not described.		Lung function (percent of expected, SD)			
				Exposed	Referent		
			FVC	111 (14)	106 (11)		
			FEV_1	112 (13)	102 (9)		
			FEV_1/VC	108 (6)	96 (6)		
			PEF	99 (19)	92 (13)		
			MEF_{50}	104 (22)	110 (20)		
			MEF_{25}	102 (22)	105 (25)		

ranged between 0.49−0.93 ppm with a geometric average concentration of 0.73 (SD 1.22) ppm. Students, trained in the use of the flowmeters, took measurements at the beginning and end of each session, and an additional 1 to 3 times during the sessions. The average of the 3 largest values in a set was used in the analyses. The strongest pulmonary response was observed for the average cross-laboratory decrement in PEF in the first 2 weeks of the study. Overall, the students exhibited a 2% decrement in PEF during the laboratory session in the first 2 weeks, while 5 students with any history of asthma showed a 7.3% decrement in PEF. The numbers in the two groups were too small to evaluate differences in response statistically. The Kriebel et al. (1993) study also shows how the acute effects of formaldehyde exposure were altered following several weeks of weekly episodic exposure. By the fifth week of class, the pre- and postlaboratory measurements of PEF were no longer reflecting a clearly demonstrated acute effect, but, following the seventh week of episodic exposure, both pre- and postlaboratory PEF continued to drop steadily until the class adjourned after 10 weeks. While the acute effects of formaldehyde exposure appeared to diminish after several weeks of exposure, the intermediate effect across 10 weeks was a 2.7 ± 1.1 L/minute per week drop in PEF that was statistically significant ($p < 0.01$) in a model adjusting for random person effects, asthma, interaction between time and asthma, and eye as well as nose symptoms of irritation. Prevalence of eye and nose symptoms was associated with decreased PEF ($p < 0.02$).

These findings were partially corroborated in a subsequent study, which used a similar study design and more thorough exposure assessment (Kriebel et al., 2001). This study, involving 38 students with data for at least one week, measured formaldehyde in 6 zones in the laboratory and linked students locations, recorded every 12 minutes, to the zone measurements to estimate a mean formaldehyde exposure over the entire 2.5 hour lab. The geometric mean formaldehyde concentration was 0.70 (GSD 2.13) ppm and the highest 12 minute exposure was 10.91 ppm. Generalized estimating equation models included an interaction term for recent exposure (mean formaldehyde concentration during the lab) and time (natural log weeks) which indicated an association with post lab PEF (percent of prelab PEF at first session) and recent exposure that attenuated over several weeks. Past average exposure (average exposure over all previous weeks) was associated with prelab PEF. The model indicated an approximate overall decrease in maximum PEF of 1% per ppm formaldehyde associated with formaldehyde levels during the laboratory in the early weeks of the course and an additional 0.7% (SE 0.3, $p = 0.02$) decrease per ppm associated with past average formaldehyde levels during the weeks previous to the current week. The addition of having a cold on the day of the lab reduced the coefficient for past average exposure somewhat (ß = -0.52 ±0.3, $p = 0.08$). Spirometry measurements (FEV_1 and FVC) before and after the course were not related to formaldehyde exposure. Class

attendance decreased markedly over the semester and it is not clear whether attendance could have been related to perceived tolerance of formaldehyde exposure in which case only the results based on the first weeks of the class can be construed to corroborate the earlier results by Kriebel et al. (1993).

A study of 103 medical students was performed over a period of 7 months in which the students were exposed to formaldehyde in the breathing zone at a time weighted average that ranged from 0.93 ppm to below the limit of detection (0.05 ppm) during anatomy laboratory sessions meeting twice a week (Uba et al., 1989). Mean concentrations during dissection were 1.9 ppm (0.1−5 ppm). Monthly average formaldehyde concentrations were highest during the first two months of the course (0.6 and 0.8 ppm) and declined over the next 5 months to 0.1 ppm. Twelve students were asthmatics. Pulmonary function tests were conducted according to criteria published by the American Thoracic Society before the start and at the end of the anatomy laboratory on three occasions, before the laboratory began, at 2 weeks and after 7 months. The largest of all acceptable spirometry values were used. Unlike the studies by Kriebel et al. (2001, 1993), these researchers did not find a prelab decrement in pulmonary function over the course of 7 months. However, mean change for pulmonary function before and after the exposure, expressed as percent of the prelab value, showed a similar pattern; at 2 weeks, all parameters were decreased and, except for FEV1/FVC, the changes were larger compared to the first test day when students were not exposed to formaldehyde (FVC -0.80 versus -0.23%, p value < 0.001; FEV1 -1.04 versus -0.71%, p value 0.03; FEF 25-75 -1.99 versus -1.76%, p value > 0.05; FEV1/FVC -0.36 versus -.48%, p value > 0.05). At 7 months, the changes were less marked, with measures showing decreases in function at the end of the laboratory session (measurements taken at the 7-month time point: FVC −0.79% (change greater than day 1, $p > 0.001$), FEV_1 −0.48% (change less than day 1, $p < 0.01$), $FEF_{25–75\%}$ 0.07%, FEV_1/FVC 0.24%). Symptoms of eye, nose and throat irritation, sneezing, rhinorrhea and chest tightness ($p = 0.05$) were more frequent during the exposure ($p < 0.01$). While formaldehyde concentrations appear to be comparable to those measured by Kriebel et al., Uba et al., used spirometry to assess lung function at 3 separate points in time while Kriebel et al. measured peak expiratory flow among subjects using multiple measures during each laboratory session providing greater statistical power to detect a response to formaldehyde. Also, the lung function parameters used by the two studies may have measured effects in different parts of the lung.

Similar studies among medical students have been performed. In one study, 34 nonsmoking exposed medical students and instructors and 12 nonmedical unexposed students completed pulmonary function tests according to criteria published by the American Thoracic Society before and after their work in the laboratory or at predetermined times for the unexposed

(approximately 3 hours) over five consecutive weeks (Akbar-Khanzadeh et al., 1994). The medical students had been exposed to formaldehyde for at least 6 weeks prior to the study. The time-weighted average exposure from personal sampling ranged from 0.07-2.94 ppm. More than 94% of the subjects were exposed to >0.3 ppm and 31.7% were exposed to an 8-hour time-weighted average of >0.5 ppm. The best of 4 spirometric measurements were used in the analysis. Comparing pre- and postexposures among the exposed students, on average FVC decreased by 1.4%, FEV_3 decreased by 1.2%, FEV_1/FVC increased by 1.6%, and $FVC_{25–75\%}$ increased 2.5%. The average percent changes in the unexposed group were −0.3, 1.30, 2.31, and 0.6%, respectively. The researchers also calculated correlation coefficients by examining the relationship between lung function and formaldehyde concentration, but no association was found. However, the pulmonary function changes were not evaluated with statistical models and baseline parameters were not adjusted for height, gender, or age.. Further, age and race composition varied between exposed and unexposed. Therefore, it is not clear that the results of this study are informative. Akbar-Khanzadeh and Mlynek (1997) performed another study with 50 exposed first-year medical students and 36 unexposed second-year physiotherapy students to evaluate pulmonary function changes during a 3-hour anatomy lab that met during the morning. Average formaldehyde concentrations measured in the breathing zone of the students was 1.88 (SD = 0.96) ppm with a range of 0.30-4.45 ppm. At least 2 exposed and 2 unexposed students, close in age to the exposed, were evaluated on each test day for the duration of the course. Lung function over one 3-hour lab session was evaluated for each participant. Spirometric measurements were conducted according to criteria published by the American Thoracic Society. Prelab spirometric variables, expressed as a percentage of reference values accounting for height, weight, age, sex, and race, were comparable between the two exposure groups. Although lung function (percent change from prelab value) increased in both groups during the first hour, the authors reported a larger increase among the unexposed students when compared with exposed students during the second two hours of exposure (FVC 3.0 versus 0.9%, FEV_1 4.1 versus 1.2%, FEV_3 3.3 versus 0.8%, forced expiratory flow during the middle of the FVC [$FEF_{25–75\%}$] 6.1 versus 0.7%). These differences between exposed and unexposed remained for FEV_1, FEV_3, and $FEF_{25–75\%}$ after 3 hours. Since lung function has been shown to have diurnal variation, increasing during the morning, the authors suggested that formaldehyde exposure may have inhibited a normal increase in respiratory function over the 3 hour period.

Finally, Fleisher (1987) gave self-administered questionnaires to 204 medical students one month after completing an anatomy laboratory course (formaldehyde concentrations <1 ppm) and again after a subsequent pathology/microbiology laboratory course (no formaldehyde exposure). Area samples taken in the anatomy labs on one day during the semester measured <1

ppm formaldehyde. Of the 38 students who completed the questionnaire after both courses, over 8% reported experiencing shortness of breath during the laboratory with formaldehyde exposure, but none of the students reported shortness of breath in the laboratory session with no exposure. No objective measurements of formaldehyde exposure were used and spirometric tests were not performed. A higher proportion of students reported other symptoms after the anatomy class compared to the pathology/microbiology class including eye and nose irritation, sneezing, headache, nausea, cough, throat irritation and sinus problems (p value ≤ 0.01).

Three studies have been performed that examine formaldehyde exposure from the buildings in which individuals live or work. Krzyzanowski et al. (1990) studied the health effects of formaldehyde exposure in a residential population in Arizona. A stratified random sample of households of municipal employees was selected based on information about potential exposure (age of housing) and potential susceptibility obtained from an initial screening questionnaire. Households with children aged 5−15 years (613 adults and 298 children) were eligible for inclusion in this cross-sectional study of home exposures. Residential exposures to formaldehyde were based on two one-week samples from each individual's kitchen, living area, and bedroom using passive sampling tubes. The average formaldehyde concentration was 26 ppb, with a maximum sample value of 140 ppb. The majority of subjects (83%) lived in homes with 2-week average concentrations below 40 ppb.

Subjects' peak expiratory flow rates (PEFRs) were determined four times daily, in the morning, at noon, in the early evening, and before bed, for 2 weeks. Subjects were trained to use the mini-Wright peak flow meters and the largest of three tests was recorded for each test period. PEFR data from the first 2 days of observations were excluded to account for a learning effect. A statistically significant linear relationship between increased household mean formaldehyde exposure and decreased PEFR was reported in children but not adults (ß = -1.28 ± 0.46 L/minute per ppb formaldehyde). A curvilinear (concave) relation was observed among asthmatic children for increasing formaldehyde levels between zero and 50 ppb and decreasing morning PEFR. Higher prevalence rates of physician-diagnosed asthma and chronic bronchitis were also shown at higher concentrations of formaldehyde (60−140 ppb), an effect that was exacerbated by environmental tobacco exposures. Among children with physician-diagnosed asthma, the observed effects of increased formaldehyde exposure on decreased PEFR were more pronounced ($p < 0.05$). All statistical models controlled for socioeconomic status, tobacco smoking (current active or environmental tobacco smoking), and nitrogen dioxide concentrations.

Among adult smokers, there was a statistically significant nonlinear relationship with decreasing morning PEFR at formaldehyde concentration > 40 ppb. In addition, the investigators also demonstrated statistically significant interaction between formaldehyde

exposures, smoking status, and prevalence of chronic cough among adults. That is, a formaldehyde concentration that caused decreased pulmonary function at residential levels also caused chronic cough among nonsmokers.

The formaldehyde monitors were prepared by the Lawrence Berkeley Laboratories and were considered to be precise and highly reliable. The 7-day passive formaldehyde monitors generally provide the lowest limit of formaldehyde detection. This study found decrements in lung function among children in a large, representative sample of the community associated with formaldehyde concentrations in their homes. The investigators specifically tested an a priori hypothesis and demonstrated to a high level of statistical significance that increased residential formaldehyde exposures were associated with decreased pulmonary function as measured by PEFR in children.

Franklin et al. (2000) studied children 6−13 years of age (median age = 9.5 years) and measured the levels of formaldehyde in their homes. A total of 224 children (116 girls, 108 boys) with no current, or history of, upper or lower respiratory tract disease were included from responses to a respiratory health questionnaire and household inventory distributed through local primary schools. The length of time the children had lived in their homes was not reported. Three to four-day passive samples were collected in the child's bedroom and the main living area of the house and formaldehyde levels were recorded as a time-weighted average. Clinical respiratory measures were obtained at the children's hospital. Exhaled nitric oxide was measured during a single-breath exhalation and the average of three plateaus varying by less than 10% was used for the NO concentration value. Spirometry was conducted on all children according to ATS guidelines and skin prick tests were measured for 7 common allergens. There was no association between FVC or FEV1 and the indoor concentrations of formaldehyde, although there were signs of lower airway inflammation as measured by levels of exhaled nitric oxide (NO) in children exposed to average formaldehyde levels ≥0.05 ppm compared to <0.05 ppm (Franklin et al., 2000). The multiple regression models controlled for age and atopic status. Other housing factors evaluated in bivariate analyses, were not associated with eNOS or spirometry measures, and therefore were not included in the regression models ($p > 0.1$); however, the effect of environmental tobacco smoke was not evaluated. The measurement of lung volume at one point in time by Franklin et al. may have been less sensitive to detect an early change in small airways than the measurement of flow rate used by Krzyzanowski et al.

Main and Hogan (1983) reported on a group of individuals ($n = 21$) working in two mobile trailers for 34 months and exposed to levels of formaldehyde ranging from 0.12 to 1.6 ppm (mean age 38 ± 9 years, 76% male, 19% nonsmokers). The unexposed population was comprised of individuals who did not work in the trailers ($n = 18$; mean age 30 ± 6, 50% male,

22% nonsmokers). There were no differences between the exposed and unexposed groups' percent predicted FEV_1 or FVC regardless of smoking status (unadjusted group means compared using t tests). Although pulmonary function measures were analyzed stratified by smoking status in this small study, exposure to environmental tobacco smoke was more common among the unexposed (44%) compared to the exposed group (29%). Since ETS is associated with lung function, this parameter may have acted as a confounder.

Several studies allowed for the examination of potential chronic effects of formaldehyde exposure. These included an occupational study at a plywood company by Malaka and Kodama (1990) that reported preshift pulmonary function as a percentage of expected function among the formaldehyde exposed compared with plant workers not exposed to formaldehyde, matched for age, ethnicity, and smoking status. Exposed and unexposed workers were identified using area formaldehyde measurements. Exposed workers (N = 100) were randomly selected with stratification by smoking status and years of occupation (< 5 and ≥5 years). Formaldehyde levels ranged between 0.22 and 3.48 ppm. The unexposed group (N = 100) worked in areas where formaldehyde was not used (range 0.003−0.07 ppm) and had no previous or current exposure to formaldehyde based on occupational histories. Among the 93 exposed and 93 unexposed participants who completed the protocol (93% participation), an average formaldehyde exposure of 1.13 ppm from area samples was associated with statistically significant decrements in FEV_1, FEV_1/FVC, and $FEF_{25-75\%}$ using analysis of covariance adjusting for sampled dust levels. The strongest response was for $FEF_{25-75\%}$, which showed a 12% drop in observed function compared with expected function in the unexposed. Workers in the unexposed group had been employed slightly longer than those in the exposed group (6.7 ± 2.3 versus 6.2 ± 2.4 years, $p < 0.05$). When the exposed group was categorized into groups of low and high exposure based on a cumulative measure calculated from area concentrations and length of employment in the current job, a decrease was reported for one measure, FEV1/FVC (percent of expected 86.9 ± 4.9, 85.3 ± 6.4, and 84.4 ± 6.5 among unexposed, low and high formaldehyde groups, respectively (statistical tests not reported). In multiple regression models adjusting for age, height, weight, cigarettes/day, and dust, formaldehyde as a continuous variable was a significant predictor for FEV_1, FEV_1/FVC, and $FEF_{25-75\%}$. Each unit increase in formaldehyde (ppm-years) was associated with a decrease of 0.015 liters, 0.347%, and 0.043 l/s in FEV_1, FEV_1/FVC, and $FEF_{25-75\%}$, respectively. Changes in spirometric measures across the shift were not associated with formaldehyde exposure in a subgroup of 55 exposed and 50 unexposed participants. A higher prevalence of cough, phlegm, chronic bronchitis, asthma, occupational asthma, shortness of breath and chest colds were reported by the exposed group ($p < 0.04$). This was a carefully

conducted occupational study with a high participation rate, relatively thorough exposure estimates, appropriate reporting of methods, and control for potential confounders.

A cross-sectional study comparing 99 oriented strand board workers (exposed to formaldehyde) (98% participation rate) with 165 oil/gas field plant workers (not exposed to formaldehyde) from the same geographic area (82% participation rate) demonstrated a difference in pulmonary function between the two groups (Herbert et al., 1994). Time weighted average formaldehyde concentrations based on 21 hour continuous sampling in the breathing zone at 5 work sites on 2 separate days ranged between 0.07 and 0.27 ppm. The groups were similar in regard to measured FVC and FEV_1 (controlled for age, height, and smoking), but the workers exposed to formaldehyde had lower FEV_1/FVC (78.6% and 80.3% for oriented strand board and oil workers, respectively ($p = 0.028$)). In addition, those exposed to formaldehyde showed a decrease in FVC and FEV_1 after their shift, with an average pre- and postshift difference of 47 mL ($p = 0.022$) and 39 mL ($p = 0.044$) for FVC and FEV_1, respectively (however change could not be compared with the controls of this study because no postshift measurements were taken). Strand board workers were more likely to report respiratory symptoms in analyses controlling for age and smoking status including cough, phlegm, shortness of breath, wheeze, and chest tightness. Odds ratios were statistically significantly elevated.

Another occupational study of wood products workers found no differences in preshift percent predicted pulmonary function between 109 exposed (workers at a particleboard and molded products operation, 68.6% of all exposed) and 254 unexposed (workers from nearby food processing facilities) (Horvath et al., 1988). Regression models controlled for height, sex, age, and smoking. Formaldehyde measured using individual monitors averaged 0.69 ppm (0.17−2.93 ppm) and 0.05 ppm (0.03−0.12 ppm) in the exposed and unexposed industries, respectively., The exposed workers had been employed an average of 10.3 years (1−20 years). Although there was no difference in preshift measurements, the authors found a cross-shift decline in $FEV_{1,}$ FEV_1/FVC, $FEF_{25–75\%}$, $FEF_{25\%–}$ and $FEF_{75\%}$ among exposed workers and in FVC and FEV_1 among the unexposed group (paired t-test, $p < 0.05$). Thus, while acute declines in large airway function over the shift were observed in both groups, the exposed group also demonstrated declines in small airway function. The authors also evaluated cross-shift changes between the groups and reported a significant difference for FVC, FEV_1/FVC, $FEF_{25–75\%}$, $FEF_{25\%–}$ and $FEF_{75\%}$ (data were not presented). When the investigators assigned all unexposed workers a formaldehyde exposure value of 0.05 ppm and evaluated formaldehyde associations among exposed and unexposed combined, a correlation was detected in pre-and postshift pulmonary function changes and formaldehyde levels, though no specific details on regression

analyses were provided. Symptom prevalence related to cough, chest tightness and eye, nose and throat irritation during the work shift was higher among the exposed workers ($p < 0.01$).

No association between formaldehyde and lung function was observed among funeral workers and an unexposed control group (Holness and Nethercott, 1989). Funeral workers, including 67 currently active embalmers and 17 who were no longer active, were recruited through a list of funeral homes developed by the Metropolitan District Funeral Directors Association in Toronto, Canada (86.6% participation). An unexposed group ($N = 38$) was recruited from a large service organization and paid student volunteers. Information on symptoms, past and family medical history, and work practices was collected by questionnaire, and pulmonary function and skin tests were administered. The average formaldehyde concentration, measured during embalming procedures lasting from 30 to 180 minutes, was 0.36 ± 0.19 ppm (0.08−0.81 ppm). Funeral workers had performed the embalming procedure for an average of 10 years. Unexposed participants were stated to be exposed to an average concentration of 0.02 ppm. The authors reported no difference in percent predicted pulmonary function of the two groups at baseline, although 10% of the funeral workers compared to 3% of the unexposed group had an FVC or FEV1 value less than 80% of predicted. Exposed and unexposed were of similar age, height and years worked, although embalmers had higher weights, a higher proportion of current smokers, and a higher number of pack-years smoked. The 17 funeral workers who were no longer active had lower FVC (95.8 ± 12.0 versus 101.7 ± 12.2, $p = 0.04$) and FEV1 (93.1 ± 14.1 versus 100.8 ± 12.1, $p = 0.07$) compared to the 67 active embalmers, when corrected for age, height, and pack-years smoked. The authors reported that active workers were younger and had worked less time in the industry. After exposure, there was no change in lung function for the exposed or unexposed when comparing lung function tests done immediately before and after an embalming procedure (for controls the repeat measures were taken approximately 2−3 hours after the first measure) (changes in percentage predicted FVC and FEV_1 were 0.88 ± 2.95 and −0.03 ± 2.40 for exposed and 1.13 ± 3.98 and 1.45 ± 4.43 for unexposed). Further analysis showed no association between formaldehyde levels and changes in lung function during the embalming procedure. Although this study did not find lung function decrements among embalmers compared to an unexposed group, decrements in FVC and FEV_1 among those who had stopped embalming suggests a possible response among more sensitive individuals. Sample sizes in the exposure group were small, and the null association with formaldehyde exposure may have been influenced by differences between a healthy occupational population and the service organization volunteers.

A cross-sectional study of formaldehyde exposure among staff working in different departments in 7 large hospitals of Tehran University of Medical Sciences in Iran reported

differences in spirometric measures related to formaldehyde (Pourmahabadian et al., 2006). Lung function testing was conducted for 124 workers from the pathology labs (38), surgery (65), endoscopy (21) departments and 56 administrative affairs staff during February 2002 to February 2003. Formaldehyde concentrations were highest in the pathology lab (8-hour average [SD]: 0.96 [0.74] ppm) compared to the surgery rooms (0.25 [0.18] ppm) and endoscopy departments (0.25 [0.22] ppm). Highest one-hour spot samples recorded concentrations of 6.5 ppm, 2.7 ppm and 0.6 ppm in pathology, surgery and endoscopy, respectively. Formaldehyde levels in administrative offices were not reported. Pulmonary function measured before the work shift was lower in the exposed group compared to the administrative staff. Average VC (liters, SD), FVC, FEV_1, and FEF_{25-75} were 3.34 (1.07), 3.46 (1.04), 2.45 (1.02) and 2.79 (1.4) in the exposed groups and 3.92 (1.26), 4.03 (1.23), 1.21 (2.59) and 1.7 (2.5) among the unexposed group ($p < 0.01$). FEV_1/FC and FEV_1/FVC were not significantly different between exposed and unexposed. In addition, the change in pulmonary function across the shift was significantly greater among the exposed workers compared to the unexposed ($p < 0.04$). Over 80% of the exposed staff reported eye irritation and the prevalence of nose and throat irritation ranged between 57 and 81%. The differences between the exposure groups is difficult to interpret because the pulmonary function measures were not adjusted for age, gender, and height while distribution by age, sex and smoking status varied between the groups.

A study performed in India (Khamgaonkar and Fulare, 1991) examined 74 individuals working in anatomy and histopathology departments at 3 colleges in India and exposed to formaldehyde (mean 1.00 ppm, range 0.036−2.27). A comparison group matched by age and sex ($N = 74$) (individuals not working in laboratories with formaldehyde) was exposed to an average of 0.102 ppm formaldehyde (range 0−0.52). Persons with a history of lung disease before their present occupation were excluded. Multiple thirty minute air samples were collected in the breathing zone in both the exposed ($N = 43$) and unexposed ($N = 18$) areas for formaldehyde analysis. Lung function tests were performed on a subset of 37 exposed and 37 comparison individuals on a Monday morning after days of no exposure in order to examine chronic effects. The FVC, FEV_1%, and maximum mid-expiratory flow rate of the exposed group, respectively, were 17.12 ($p < 0.01$), 22.94 ($p < 0.01$), and 42.81% ($p < 0.05$) lower compared with the unexposed. Mean height was comparable in the two groups. The exposed group also had a higher prevalence of symptoms including productive cough, breathlessness and tightness of chest ($p < 0.01$). However, while the pool of exposed and unexposed were matched on age and gender, there was no mention by the investigators of normalizing the pulmonary function metrics by gender and height, which would have made for more appropriate comparisons. Kilburn et al. (1985) also demonstrated reduced pulmonary function (lower percent predicted FVC, FEV_1, and

$FEF_{25-75\%}$) among 45 male fiberglass bat makers exposed to formaldehyde when compared with 26 hospital employees, including respiratory therapists, gardeners, and attendants, without formaldehyde exposure. However, formaldehyde levels in work areas were not measured and group differences were not analyzed statistically in this small study.

Two occupational studies found no association between formaldehyde exposure and deficits in pulmonary function (Ostojic et al., 2006; Holmström and Wilhelmsson, 1988). Ostojic et al. (2006) examined nonsmoking male health service professionals working in pathoanatomic laboratories with 8 hours of formaldehyde exposure per day at an unspecified concentration for at least 4 years (n = 16). The source of the comparison group, comprised of sixteen age- and stature-matched nonsmoking male controls, was not described. There was no difference in mean percent predicted FVC or FEV_1 between exposed and unexposed. The researchers also examined values for diffusing lung capacity for carbon monoxide and membrane diffusion capacity, which were similar between the exposed and control groups. However, blood volume of pulmonary capillaries was found to be higher in the exposed group. Holmström and Wilhelmsson (1988) recruited individuals from a chemical plant where formaldehyde and formaldehyde products were made (n = 70). Exposure levels varied from 0.05−0.5 mg/m^3. A comparison group, mostly comprised of clerks for the local government worked in an office with mean formaldehyde concentrations of 0.09 mg/m^3 (n = 36). FEV% was not different from the predicted value among either the exposed or comparison groups. Mean FVC was lower than expected among the exposed group (expected values were based on age, sex, smoking habits, height, and weight) but not among the comparison group. Spirometric measures between the exposure groups were not statistically compared. The comparison group was older (39.9 versus 36.9 years of age) and contained more women (44% versus 13%). The investigators measured changes in pulmonary function for those employed more than 5 years and reported no signs of increasing restrictivity after 5 years. Cumulative exposure to formaldehyde was estimated using mean annual formaldehyde concentrations based on sampling conducted between 1979 and 1985 summed over the number of years employed. There was no correlation between pulmonary function and cumulative dose of formaldehyde (Holmström and Wilhelmsson, 1988). The studies by Ostojic et al. and Holmström and Wilhelmsson are less informative because the adequacy of the comparison group cannot be evaluated, sample sizes were small, and analytic methods were not adequate. There have been two studies that have reported on the longitudinal follow-up of workers exposed to formaldehyde (Nunn et al., 1990; Alexandersson and Hedenstierna, 1989). The Alexandersson and Hedenstierna (1989) investigation examined Monday morning preshift lung function and the acute effects of exposure across shift at a carpentry works in central Sweden in 1980 and then again in 1984 (Alexandersson et al., 1982).

The time-weighted formaldehyde concentration, measured using personal sampling in the working zone over a working day, was 0.42 ± 0.27 mg/m^3 (0.34 ppm) in 1980 and 0.50 ± 0.12 mg/m^3 (0.4 ppm) in 1984. Of 47 exposed workers and 20 unexposed workers examined in 1980, 34 exposed and 18 unexposed were examined again in 1984. Of the 34 originally exposed, 13 had been reassigned to other unexposed jobs. The exposed and transferred workers had been exposed to formaldehyde an average of 11 years. Cross-sectionally, a decreased preshift FEV1 relative to reference values (according to sex, age, height) was suggested among exposed workers in 1980 ($p < 0.1$). In 1984, preshift FEV1, FVC, and $FEF_{25–75\%}$ were significantly different from predicted among the 21 currently exposed workers. Statistically significant annual decreases in FEV_1/FVC and $FEF_{25–75\%}$ were noted over the intervening 5 years in nonsmokers after correction for normal aging and reference lung function spirometry values. The decrease in $FEF_{25–75\%}$ was 0.212 ± 0.066 L/second (mean ± SD) for each year of exposure and was significant ($p < 0.01$). The decrease in FEV1/FVC was -0.4 ± 0.2 ($p < 0.01$).

For comparison with the 12% drop in the same pulmonary metric reported by Malaka and Kodama (1990) over an estimated 6.5 years, the extrapolated percentage decrease in $FEF_{25–75\%}$ was computed for the Alexandersson and Hedenstierna (1989) study by using the reported yearly decrement applied to the preshift values at the time of the initial study period. From the predicted value of 4.26 L/second, a decrease of 0.168 L/second for each year of exposure regardless of smoking status was calculated. For 6.5 years of exposure, this would result in a 24% drop in $FEF_{25–75\%}$. Significant across shift decreases in FEV1/FVC and $FEF_{25–75\%}$ were reported in 1980 and cross-shift decreases in FEV1, FVC%, and MMF were report in 1984, particularly for nonsmokers.

The study by Nunn et al. (1990) assessed the decrease in FEV_1 among workers, aged 25 or older, at a chemical factory in Duxford, England manufacturing urea formaldehyde resin followed over a 6 year period from 1980. A total of 164 workers exposed to free formaldehyde in 1980 and a group of 129 workers from the bonded structures division at the same factory in 1980 were included. Data on formaldehyde concentrations from area samples (1−6 hours sample collections) taken periodically between 1979 and 1985, and from personal samplers attached to representative exposed workers from 1985 to 1987 were used to categorize the workers' employment experience into low, medium and high formaldehyde groups. Exposure assessments prior to 1976 were based on subjective determinations and knowledge of process changes and industrial hygiene measures. FEV1 and FVC measurements (highest of two readings within 5% of each other) were obtained during annual health screenings by the same nurse throughout the study. Follow-up was complete for 76% of the exposed and 74% of the

unexposed workers. FEV1 values, adjusted for height, for each worker were regressed on time of screening visit. The mean decrease in FEV_1 was 42 mL/year among workers exposed to formaldehyde and 41 mL/year for workers who were not exposed to formaldehyde, however a larger decline appeared to be evident among 36 nonsmoking exposed (45 ml/year, 95% CI: 28−62) compared to 13 nonsmoking unexposed (29 ml/year, 95% CI: 7-51). Other covariates stated to be analyzed in relation to FEV1 decline were FEV1 level, age in 1980, smoking state in 1980 and final visit, maximum and mean exposure assessment level (1−5), and duration of exposure but analytic details were not presented. The exposed were on average older and had worked longer in the factory than the unexposed. It is difficult to interpret the findings of this study because, although a small subset (N = 20) among the unexposed group were assessed and not found to be exposed to other potential irritants above recommended limits, this may not have been the case in the past. This group was exposed to asbestos, carbon and glass fibers, silicaceous fillers, acid anhydrides, aliphatic amines, phenol formaldehyde (not free formaldehyde) and urea formaldehyde (not free formaldehyde).

To summarize, the epidemiologic literature contains studies of formaldehyde exposure and both acute and chronic effects on pulmonary function. Several studies with adequate analytical methods and reporting have assessed acute exposures among naïvely exposed anatomy graduate students (Kriebel et al., 1993; 2001), anatomy graduate students with several weeks of episodic exposure (Kriebel et al., 1993; Uba et al., 1989), and postshift versus preshift worker pulmonary function among those with regular occupational exposure (Malaka and Kodama, 1990; Herbert et al., 1994; Alexandersson et al., 1982; Alexandersson and Hedenstierna, 1989; Holness and Nethercott, 1989; Horvath et al., 1988). Depending on whether the exposures are naïve or not, the epidemiologic studies that assessed the pulmonary effects after acute exposures to formaldehyde are assessing different biological responses, namely, the acute effect alone or the acute effect(s) in people who may have already been sensitized to different and unknown degrees. Generally, these studies found that intermittent laboratory or occupational shift exposures to high formaldehyde concentrations (time weighted averages between 0.3−3 ppm) were associated with deficits in lung function across the shift or laboratory session and, in some studies, over time (Kriebel et al., 1993; 2001; Uba et al., 1989; Herbert et al., 1994; Horvath et al., 1988; Alexandersson et al., 1982; Alexandersson and Hedenstierna, 1989. Pourmahadadian et al. (2006) reported cross-shift reductions in pulmonary function but results were not adjusted for age, height or gender. Acute exposure associated with eye, nose and throat irritation and other respiratory symptoms including chest tightness and cough also was reported by some studies (Kreibel et al., 1993; Uba et al., 1989; Fleisher, 1987; Horvath et al., 1988; Pourmahadadian et al., 2006).

Several adequately conducted and reported studies evaluated chronic effects of occupational (Malada and Kodama, 1990; Herbert et al., 1994; Horvath et al., 1988; Holness and Nethercott, 1989; Alexandersson et al., 1982; Alexandersson and Hedenstierna, 1989) or residential exposure to formaldehyde (Krzyzanowski et al., 1990; Franklin et al., 2000). Decreased preshift lung function relative to unexposed groups was reported in several different occupational groups including plywood, oriented strand board and carpentry (Malaka and Kodama, 1990; Herbert et al., 1994; Alexandersson et al., 1982; Alexandersson and Hedenstierna, 1989). The studies included appropriate comparison groups, formaldehyde measurements, adequate reporting of methods, and adjustment for potential confounding variables including age and smoking status (in some studies weight and ethnicity). All studies presented lung function values as percent of expected based on age, height, and sex or adjusted for these variables in the analysis. Some studies reported lower mean values for several preshift measures of lung function among formaldehyde exposed workers (university laboratories, fiberglass bat makers) suggestive of chronic decrements in pulmonary function but results were not adjusted for age, height or gender, or statistical analyses were not reported complicating their interpretation (Khamgaonkar and Fulare, 1991; Pourmahadadian et al. (2006); Kilburn et al., 1985). Studies of long-term exposure also reported increased respiratory symptoms such as cough, increased phlegm, asthma, chest tightness and chest colds in exposed workers (Malaka et al., 1990; Herbert et al., 1994; Pourmahabadian et al., 2006, Alexandersson et al., 1982; Alexandersson and Hedentierna 1989). Some of the studies reporting null findings suffered from small sample size, methodological, analytical or reporting deficiencies complicating the interpretation of the findings (Main and Hogan, 1983; Ostojic et al., 2006; Holmström and Wilhelmsson, 1988; Nunn et al., 1990). Also, occupationally exposed groups compared to nonoccupational referent groups may have exhibited a healthy worker effect masking any formaldehyde related lung function decrements (Holness and Nethercott, 1989).

The longitudinal study by Alexandersson and Hedenstierna (1989) provides support for the association of lung function decrements over time with occupational formaldehyde concentrations of 0.42−0.5 mg/m^3 (340−400 ppb) over 5 years. Kryzanowski et al. (1990), a well-designed and executed cross-sectional study of residential formaldehyde exposure in a large, representative sample, provides clear evidence of a linear relationship between increased formaldehyde exposure and decreased peak expiratory flow rate among children. The average formaldehyde concentration was 26 ppb, with a maximum sample value of 140 ppb. Decrements in PEFR associated with increasing formaldehyde concentrations were observed among adults beginning at an average concentration of 40 ppb. While Franklin et al. (2000) did not observe an association between a one-time measurement of FVC or FEV_1 among children aged 6−13 years

and indoor concentrations of formaldehyde, levels of exhaled nitric oxide (NO) were higher in children exposed to average formaldehyde levels ≥0.05 ppm compared to < 0.05 ppm. These findings indicate that formaldehyde may increase lower airway inflammation at concentrations associated with effects on pulmonary function. Malaka and Kodama (1990) also observed an exposure-response pattern for a cumulative formaldehyde measure and declines in FEV_1/FVC.

The pulmonary function measures associated with formaldehyde exposure are consistent with bronchial constriction, inflammation, or chronic obstructive lung disease. Decreases in spirometric values, including vital capacity (VC), forced expiratory volume (FEV), forced vital capacity (FVC) and FEV/FVC have been documented. Decreases in lung volume (FEV_1, FVC) indicate possible pulmonary obstruction (narrowing of the airways during exhalation) (Pellegrino et al., 2005). Early changes in small airways are observed as reductions in expiratory flow in the terminal portion of the spirogram (PEF, $FEF_{75\%}$, $MEF_{25\%-75\%}$). These changes may be observed even if FEV_1 is not affected.

Worker and laboratory exposures associated with cross-shift differences in spirometric values are consistent with formaldehyde-induced sensory irritation. Concordance has also been reported between subjective irritant response and measured changes in pulmonary function further supporting the possibility that cross-shift and short-term evidence of bronchial constriction may be a reflexive response to sensory irritation (Kriebel et al., 1993). Similar findings have been reported following low-level residential formaldehyde exposure including decreased PEFRs (Krzyzanowski et al., 1990).

4.1.1.2.2. *Acute studies: controlled chamber exposures.*

Pulmonary effects of acute formaldehyde exposure have been studied in both healthy volunteers and sensitive populations under controlled conditions. Controlled chamber studies have the advantage of measured controlled exposures, but other factors can limit the usefulness of the studies, especially when study populations are small and there is high variability in the measured parameters.

Anderson and Mølhave (1983) described a 5 hour controlled exposure study with 16 healthy students (5 female and 11 male). The students were an average of 23 years (20-33) and included 5 smokers. After a 2 hour exposure to clean air, subjects in groups of four, were exposed to four 5-hour formaldehyde exposures (0.3, 0.5, 1.0, and 2.0 mg/m^3) on 4 consecutive days in an order determined by a Latin square design. Measurements were taken prior to, after 2−3 hours, and 4-5 hours of exposure to formaldehyde. Only average standard deviations from the mean were presented for vital capacity (0.28), $FEF_{25-75\%}$, (0.28), and FEV1.0 (0.24). The authors concluded that no significant changes were measured in the airway resistance measures.

Day et al. (1984) exposed 18 volunteers to formaldehyde (1.0 ppm for 90 minutes) generated either from formalin in chambers or offgasing from urea formaldehyde foam insulation (1.2 ppm for 30 minutes) in individual hoods. Pulmonary function tests were conducted before formaldehyde exposures in room air (0.02 ppm formaldehyde), immediately after exposures, and 8 hours after the beginning of the exposure. No differences in absolute values for FVC, FEV1 or $FEF_{25-75\%}$ before and after formaldehyde exposures, analyzed using paired t-tests, were observed among 9 subjects who claimed to experience adverse effects to UFFI and 9 who had not. For example, FEV1 before, immediately after, and 6.5 hours after exposure was 3.31 ± 0.79, 3.32 ± 0.81, and 3.41 ± 0.77 L among those with complaints and 3.81 ± 1.0, 3.75 ± 1.04, and 3.71 ± 1.02 L among those with no complaints or not living in homes with UFFI. Mean change in FEV1 after methacholine challenge was -3.2% (-13−7.4%) and 0.04% (-6.0−5.0%) among those with prior complaints and those with no prior complaints, respectively. Since demographic and other characteristics of the subjects were not presented, and only absolute values were analyzed, the null findings in this small study cannot be adequately interpreted.

A study of healthy nonsmokers evaluated a dose-response relation using 2 groups (mean age 26.3 ± 4.7 years, 10 males and 9 females) exposed at rest and during exercise (Kulle et al., 1987). Subjects had no history of allergy, asthma, hay fever, or upper respiratory infection in the 6 weeks before the study began. One group of 10 subjects were exposed to formaldehyde concentrations of 0.0, 0.5, 1.0, and 2.0 ppm in random order and the other group was exposed to 0.0, 1.0, 2.0, and 3.0 ppm in random order. Both groups were also exposed to 2.0 ppm with exercise. Each session was separated by 1 week. Spirometric measurements were taken at the beginning and end of each exposure, at several points during the 3 hour session, and 24 hours post exposure. Airway resistance and thoracic gas volume were measured at the beginning and end of each exposure. Nonspecific airway reactivity, measured by methacholine challenge, was assessed at the end of the exposure and after 24 hours. No differences between dose levels and no trend with increasing dose was found in analyses using lung function values at each time point as a ratio of the value measured before exposure began. Lung function was not expressed as a percent of predicted according to age, sex or height and dose level means were presented by time point without standard deviations to characterize individual variability. In addition, no differences in bronchial reactivity were observed.

A study of sensory irritation and subjective symptoms among 21 healthy volunteers with 4 hour exposures to 0, 0.15, 0.3 and 0.5 ppm formaldehyde over 2 weeks did not observe differences in pulmonary function measured at baseline, before the first exposure and after the last exposure (Lang et al., 2007). The differences between an individual's postexposure and pre-exposure values were analyzed in relation to formaldehyde. The data were not presented.

Schachter et al. (1986) measured lung function among 15 subjects, aged 25.4 ± 4 years, exposed for 40 minutes to 0 or 2 ppm formaldehyde at rest or during exercise. Each exposure session was conducted on 4 separate days. Bronchial reactivity assessed by methacholine inhalation challenge was measured in 6 subjects. Subjects were healthy nonsmokers with no history of asthma. Lung function parameters, expressed as a percent change from the baseline measurement, showed slight improvements at the end of 40 minutes some of which were statistically significant. For example, FEV1 at 40 minutes was 1.65 ± 4.5 and 4.56 ± 5.3 liters for subjects exposed to 2 ppm formaldehyde at rest and during exercise, respectively. FEV1 after exposure to room air only was -1.14 ± 4.8 and 1.6 ±7.7, respectively. Maximum expiratory flow at 50% of expired vital capacity (MEF 50%) was 7.4 ± 5.0 and 8.8 ± 8.1 L/second at rest and during exercise. MEF50% after exposure to room air only was 2.74 ± 4.4 and 8.72 ± 12.6. Standard deviations indicate large variability in individual responses to exposure. Differences in responses related to formaldehyde versus room air exposures were not evaluated statistically.

In a subsequent report the research team described a study using the same exposure protocol examining lung function among 15 healthy laboratory workers frequently exposed to formaldehyde in their occupation (Schachter et al., 1987). The group of 5 men and 10 women ranged in age from 19 to 60 years and included 3 current smokers. The frequency of formaldehyde exposure varied considerably (between 1 and 7 days per week for 1 to 21 years. Chamber exposures of 2 ppm formaldehyde at rest or with exercise did not induce lung function changes (percent of baseline) during the 40 minute exposure and up to 30 minutes afterward. While individual responses were not presented, the standard deviations for the mean percent change from baseline were large.

Witek et al. (1986) performed the same 40 minute exposure protocol with 0 and 2 µg/L formaldehyde exposures at rest and during exercise among 15 healthy subjects and 15 subjects with asthma. Again, lung function parameters, as a percent change from the baseline measurement, showed slight improvements at the end of 40 minutes some of which were statistically significant. For example, FEV1 at 40 minutes was 1.65 ± 4.5 and 4.56 ± 5.3 liters for subjects exposed to 2 ppm formaldehyde at rest and during exercise, respectively. FEV1 after exposure to room air only was -0.41 ± 5.0 and 4.87 ± 8.3, respectively. Again, standard deviations indicated large variability in individual responses to exposure.

Other acute controlled studies including asthmatics also reported no changes in pulmonary function associated with formaldehyde exposure (Sheppard et al., 1984, Ezratty et al., 2007; Harving et al., 1990; Green et al., 1987; Sauder et al., 1987; Witek et al., 1987, 1986), including studies of individuals thought to have formaldehyde-induced bronchial asthma (Krakowiak et al., 1998). Specific airway resistance was not significantly increased among

7 volunteers (2 females, 5 males) aged 18 to 37 years old with physician-diagnosed asthma after exposure to 1 or 3 ppm formaldehyde for 10 minutes at rest or with moderate exercise (Sheppard et al., 1984). Subjects were exposed via mouthpiece. Two of the subjects experienced large increases (over 3 liters) in airway resistance after formaldehyde exposures of 1 and 3 ppm, however these two individuals also exhibited increased airway resistance after air-only exposure. Witek et al. (1986, 1987) evaluated lung function and bronchial reactivity among 15 nonsmoking subjects with asthma (18−35 years of age) using the same exposure protocol as that used with healthy subjects described in Witek et al. (1986). Similar to the response noted in healthy subjects, the mean percent change from baseline in FEV1 during formaldehyde exposure among asthmatics was increased. FEV1 values at 40 minutes were 4.59 ± 4.9 and 6.63 ± 11.2 liters for subjects exposed to 2 ppm formaldehyde at rest and during exercise, respectively. FEV1 after exposure to room air only was 2.85 ± 4.6 and 5.81 ± 8.0, respectively. Differences in responses related to formaldehyde versus room air exposures were not evaluated statistically. The threshold dose of methacholine required to produce a 20% decrease in FEV1 was determined immediately following an additional 40 minute exposure to formaldehyde among 12 subjects with asthma. Mean PD_{20}FEV1.0 after exposure was decreased (13.6 ± 20.5) compared to the response assessed at baseline (24.0 ± 15.7) (p = 0.12).

Harving et al. (1990) evaluated pulmonary function and bronchial reactivity among 15 nonsmoking volunteers with asthma (8 female and 7 male) exposed to filtered air (0.008 mg/m^3 formaldehyde), 0.12 mg/m^3 and 0.85 mg/m^3 formaldehyde for 90 minutes. The subjects, 15 to 36 years of age, were assigned at random to 1 of 3 groups and each group (5 subjects per group) was exposed to formaldehyde or filtered air in random order over a 3 week period. FEV1 as a percent of baseline was not significantly changed after a 90 minute exposure. Values were 104%, 103% and 103% for formaldehyde levels of 0.85, 0.12, and 0 mg/m^3, respectively. In addition, no significant changes in the concentration of inhaled histamine required for a 20% fall in peak expiratory flow measured immediately after exposures were noted in relation to formaldehyde levels. The PC_{20} for subjects exposed to 0, 0.12, and 0.85 mg/m^3 formaldehyde was 0.29 ± 0.3, 0.36 ± 0.53, and 0.26 ± 0.31, respectively.

Sauder et al. (1987) exposed 9 nonsmoking volunteers with asthma to 3 hour exposures of clean air or 3 ppm formaldehyde on 2 days separated by 1 week. Spirometric measurements were obtained at 0, 15, 30, 60, 120 and 180 minutes and a methacholine inhalation challenge was conducted at 180 minutes immediately after the lung function test. Pulmonary function tests including FVC, FEV1, $FEF_{25-75\%}$, specific airway conductance, and functional residual capacity indicated no difference between formaldehyde and clean air exposures. Paired t-tests were used to evaluate the ratio of each time point to the measurement at time = 0 for the formaldehyde

exposure subtracted by the ratio for the clean air exposure at the same time point. Response to methacholine challenge also was not affected by formaldehyde exposure.

Krakowiak et al. (1998) exposed 10 subjects exposed to formaldehyde occupationally (aged 23-52 years) and 10 volunteers with no occupational exposure to formaldehyde (aged 19−49 years) to 0.5 mg/m^3 formaldehyde for 2 hours. The group with formaldehyde exposure had bronchial asthma diagnosed by the doctor in the workplace and included 7 males and 3 females, some of whom smoked. The nonasthmatic group included only nonsmoking males. Exposure to formaldehyde did not result in changes in FEV1, PEF or the dose of histamine causing a 20% fall in FEV1 measured before, immediately after and up to 24 hours after the exposure period in either group.

Small but statistically significant deficits in pulmonary function due to acute formaldehyde exposure (2 or 3 ppm) have been reported in healthy volunteers during exercise (Green et al., 1987, 1989; Sauder et al., 1986). Although changes in lung function parameters averaged over experimental groups were generally small, some individuals exhibited clinically significant deficits, even after only 2 hours of exposure (Green et al., 1987). Nine healthy nonsmoking subjects, aged 26 ± 3.6 years, were exposed to clean air for 3 hours on day 1 and then 3 ppm formaldehyde for 3 hours on day 2 (Sauder et al., 1986). In addition, exercise for 8 minutes on a bicycle was included prior to each spirometry measurement during the exposure at 30, 60, 90, 120, 150, and 180 minutes. Deficits in FEV_1 and $FEF_{25–75\%}$ after the first 30 minutes of formaldehyde exposure compared to clean air were 2% ($p < 0.05$) and 7% ($p < 0.01$), respectively. Spirometric measures were not different after 60 and 180 minutes of exposure, even when assessed as absolute rather than relative measurements. The authors reported that the range of individual responses was -5% to +1% for FEV1 and -14% to +2% for $FEF_{25–75\%.}$

Another study compared the responses of healthy and asthmatic subjects exposed to clean air and 3 ppm formaldehyde for 1 hour with 15 minute exercise segments at 15 and 45 minutes using the same analytical methods as described by Sauder et al. (1986, 1987) (Green et al., 1987). Among the 22 healthy subjects, small but statistically significant decrements were observed during 3 ppm formaldehyde exposures, compared to the clean air exposures, at 47 and 55 minutes. Decreases of 2.1 to 3.8% were observed for FEV1, FVC, and FEV3. Decreases in $FEF_{25–75\%}$ also were observed (5.7 to 10.9%), but the changes were not statistically significant. No changes in mean specific conductance or airway reactivity measured by methacholine challenge were observed in either the healthy subjects or 16 subjects with a clinical history of asthma. No changes in pulmonary function measures were observed in the asthmatic group. Individual variability in responsiveness was noted by the authors. Thirteen percent (5 of

38 subjects) demonstrated formaldehyde-induced clinically significant deficits when exposed at 3 ppm during exercise (defined by Green et al. (1987) as a decrease in FEV_1 > 10% of control).

The authors followed up with an additional study to evaluate the effects of formaldehyde alone or in combination with respirable carbon particles (mass median aerodynamic diameter [GSD] 1.4 μm [1.8 μm]). A total of 29 healthy subjects were exposed according to a randomized block design for 2 hours each to clean air, 3.0 ppm formaldehyde, 0.5 mg/m^3 activated carbon aerosol and a mixture of 3 ppm formaldehyde and 0.5 mg/m^3 activated carbon aerosol. Exposures occurred at the same time of day and were separated by one week. Spirometric measurements were obtained prior to the beginning of exposure, and at 20, 50, 80, and 110 minutes during the exposure. In addition, a 15 minute bicycle ergometer exercise was completed at 15, 45, 75, and 105 minutes. The subject also measured peak flow at the end of the 2 hour exposure, on the hour for 8 hours, and 12 and 16 hours post exposure using Wright peak flow meters. A statistically significant decrease in $FEF_{25-75\%}$ (< 6% mean decrease) related to formaldehyde was observed among the 24 subjects who were able to complete the exercise protocol at 50 ($p < 0.05$) and 80 minutes ($p < 0.01$), and a decrease in peak flow was evident at 110 minutes $p < 0.03$). Formaldehyde exposure also resulted in decreased specific airways conductance at 120 minutes ($p < 0.01$). No formaldehyde effects on FVC, FEV_1, or FEV_3 were observed for formaldehyde alone, however statistically significant decreases in FVC (mean 2.5−4.5% decrease) and FEV_3 (mean decrease 3%) were reported at 20 and 50 minutes, and peak flow at 110 minutes. The combined exposure also significantly increased coughing at 20 and 80 minutes ($p < 0.05$). Formaldehyde alone did not have an effect on cough but statistically significant increases in headache, eye, nose and throat irritation, and chest discomfort occurred at all time points. The authors concluded that formaldehyde exposure during exercise resulted in an acute, transient effect on both large and small airways among healthy individuals. In addition, a combined exposure to formaldehyde and carbon particles increased coughing and small decrements in pulmonary function, suggesting that adhesion to particles increased delivery of formaldehyde to the lower respiratory tract.

Casset et al. (2006) evaluated the effect of formaldehyde exposure on the bronchial response to dust mite allergen in sensitized asthma patients. The study included 19 nonsmoking subjects (12 women and 7 men) ages 19-35 years with mild asthma. Subjects had positive skin prick tests and specific IgE to Dermatophagoides pteronyssinus (dust mites). Subjects had not had a respiratory tract infection in the two weeks prior to the testing. Individuals sensitized to pollen were studied outside the relevant pollen season and people sensitive to pet allergens did not have pets at home. The 30 minute crossover exposures to 100 μg/m^3 formaldehyde and air were randomly assigned with a three-week washout period in between. Subjects underwent mite

allergen challenge immediately after exposure to formaldehyde or air to determine the dose of allergen that resulted in a 20% reduction in FEV_1 (PD_{20}). The dose which induced early-phase bronchial response was significantly lower in the subjects exposed to formaldehyde (34.3 ng vs. 45.4 ng: $p < 0.05$). Late-phase bronchial response was measured over the 6 hours following the dust mite challenge by comparing FEV_1. The maximum percentage FEV1 reduction observed was significantly higher after exposure to formaldehyde 15% vs. 11% ($p < 0.05$) (Casset et al., 2006).

While the study by Casset et al. (2006) clearly showed that acute formaldehyde exposure (100 $\mu g/m^3$) enhanced both early-phase and late-phase bronchial responsiveness to mite allergen in mite-allergen sensitized people with asthma, a subsequent study with a similar protocol did not duplicate this finding. Ezratty et al. (2007) evaluated the response of asthmatics to inhaled allergen after a 60 minute exposure to 500 $\mu g/m^3$ (0.4 ppm) formaldehyde. The 12 subjects (7 men and 5 women) were 18−44 years old, were nonsmokers, and were diagnosed with intermittent asthma and allergy to pollen. No subjects had contracted an upper respiratory infection for at least 4 weeks before the study. The crossover exposures (60 minutes) to filtered air and 500 $\mu g/m^3$ formaldehyde occurred 2 weeks apart in random order. Allergen inhalation challenge, using an extract of 5 grass pollen allergens, was conducted immediately after each exposure and the dose producing a 15% decrease in FEV1 was determined (PD_{15}FEV1).

Responsiveness to methacholine was determined 8 hours after the allergen inhalation challenge ended. Lung function measurements were taken using a spirometer before, during and 8 hours after the end of the allergen challenge. In addition, PEF and FEV1 were measured with a portable spirometer every 15 minutes during exposures to filtered air or formaldehyde and every hour until the methacholine challenge. The authors reported that pulmonary function, expressed as percent predicted, was not affected by the formaldehyde exposure, although the data were not presented. The median PD_{15}FEV1 for the allergen challenge was 0.80 (0.15-2.0) index of reactivity (IR) after formaldehyde exposure and 0.25 (0.10-2.0) IR after the filtered air exposure ($p = 0.06$). The ratio of response after formaldehyde exposure compared to filtered air exposure was 1 in 7 subjects and higher in 5 subjects. The PD_{20} for methacholine challenge was 0.23 (0.01-3.6) and 0.17 (0.03-4) ($p = 0.42$). The PD_{20} for methacholine for formaldehyde compared to filtered air was lower in 3 subjects, higher in 4 subjects, and not changed in 5 subjects.

There are multiple potential explanations for these seemingly conflicting findings in Casset et al. (2006 and Ezratty et al. (2007) which include the small sample size of these studies, the differencing in the particular allergen tested and difference in the protocols. It is possible that the 12 subjects studied by Ezratty et al. (2007) may not have included individuals who were especially susceptible to formaldehyde. In the Casset et al. (2006) study, the investigators made

specific mention of the size of the particles used for the allergen challenge. They commented in the discussion that dosimetric models of inhaled formaldehyde show that the flux is very large in the first bronchial generations and then decreased rapidly. Casset et al. (2006) used an aerosol with large particles (Mass Median Aerodynamic Diameter of 11.1 μm) specifically because these large particles are deposited in the large airways where formaldehyde flux is higher. Ezratty et al. (2007) did not report the size of the particle used in their challenge but both studies did report the types of dosimeters jet nebulizers that were used and these were different.

In a study unrelated to formaldehyde, Praml et al. (2005) compared the physical and biologic doses of methacholine for different nebulizers including the type used by Ezratty et al. (2007). Praml et al. (2005) compared the airway responsiveness of 34 subjects using two types of nebulizers and found that in 17 subjects, neither system caused a 20% decrease in FEV1, while among 8 subjects, both systems were able to provoke a 20% decrease. The remaining 9 subjects responded to only one type of nebulizer. Using the same protocol, the same methacholine agent produced different results based on the type of nebulizer. It may be that the results of Casset et a. (2006) and Ezratty et al. (2007) which did have other differences can be explained thusly. It may also be that a difference in particle size plays a role if the pollen allergens used in Ezratty et al. (2007) were smaller and penetrated the lung beyond the upper lung where formaldehyde exposures are greater.

In general, acute formaldehyde exposures (0.5−3 ppm) have not induced significant pulmonary deficits in healthy, nonexercising volunteers (Kulle et al., 1987; Schachter et al., 1986; Schachter et al., 1987; Witek et al., 1986; Day et al., 1984; Andersen and Molhave, 1983). However, it is unclear whether the data analysis in these reports had the statistical power to substantiate the small deficits reported in occupational and student studies. All five reports had relatively small study groups of healthy individuals (n = 19 [Kulle et al., 1987], n = 16 [Andersen and Molhave, 1983], n = 15 [Schachter et al., 1986], n = 15 [Schachter et al., 1987], n = 15 [Witek et al., 1986], and n = 9 [Day et al., 1984]). The studies exposed a small number of diverse individuals, often including males and females of varying age, and some included current smokers. Three studies report the absolute values of the lung function parameters without adjustment to individual expected function or the unexposed baseline for each individual (Kulle et al., 1987; Andersen and Molhave, 1983; Day et al., 1984). As discussed, this decreases the power of the study to detect formaldehyde-induced changes in pulmonary function. In contrast, Witek et al. (1986) and Schachter et al. (1986, 1987) report lung function as a percent of baseline (although not normalized for age, gender and height). Each study showed an increase in FEV_1 in formaldehyde-exposed individuals at rest and increases in maximal expiratory flow (MEF) at 50% of expired vital capacity (MEF50%) (Witek et al., 1986; Schachter et al., 1986). However,

in both reports the SDs of changes in lung function parameters are quite large, nearly equaling the reported value and exceeding it in several cases. The absence of normalized raw data, combined with large individual variation, limit the interpretation of these studies.

Small but statistically significant deficits in pulmonary function (e.g., decreased FEV_1, FVC_1, $FEV_{3,}$ specific airways conductance) due to formaldehyde exposure (2 or 3 ppm) have been reported in healthy volunteers in controlled human exposure studies using exercise (Green et al., 1987, 1989; Sauder et al., 1986;). Although changes in lung function parameters averaged over experimental groups were generally small, some individuals exhibited clinically significant deficits, even after only 2 hours of exposure (Green et al., 1987). This differential response suggests susceptibility in certain subjects (Green et al., 1987). Other studies that included an exercise component did not report a difference in response among healthy volunteers (Schachter et al., 1986; Kulle et al., 1987). Acute controlled studies that evaluated responses among asthmatics reported no changes in pulmonary function associated with formaldehyde exposure (Sheppard et al., 1984, Ezratty et al., 2007; Harving et al., 1990; Green et al., 1987, 1989; Sauder et al., 1987; Witek et al., 1987, 1986; Krakowiak et al., 1998). These findings suggest that a brief exposure to formaldehyde may not trigger a response in the airways of asthmatic individuals in the absence of allergen. However, the large variation in pulmonary response among the individuals (healthy and asthmatic) that participated in the experimental exposure studies suggests that some individuals may be more sensitive to formaldehyde.

4.1.1.3. *Asthma*

A large number of studies have investigated the potential association between formaldehyde exposure and a continuum of adverse health effects ranging from decrements in pulmonary function to asthma. In general, epidemiologic studies of adults have reported varied results between null findings and positive findings but have not consistently distinguished between studies in which formaldehyde may be causing an increase in the incidence of asthma (e.g., phenotypic switching), increasing the prevalence of asthma, initiating an asthma attack or worsening the severity of an attack. Formaldehyde may itself be an allergen or it may potentiate the ability of other allergens to cause atopic switching or increase the sensitivity of atopic individuals. Thus formaldehyde exposure among nonatopic individuals could theoretically cause atopic switching in the presence or absence of allergens possibly resulting in a diagnosis of asthma. Formaldehyde could also cause an asthma attack or potentiate the influence of other stimuli on the risk of asthma attacks. Demonstration of a clear association of formaldehyde exposure, or the lack of an association, at one particular time does not necessarily imply that exposure to formaldehyde is causing or not causing adverse outcome at other times.

The National Research Council concluded in its report on Formaldehyde that, "Formaldehyde has been shown to cause bronchial asthma in humans" (NRC, 1981), citing numerous studies demonstrating the induction of asthma following exposure to formaldehyde (Hendrick and Lane, 1975, 1977; Laffont and Noceto, 1961; Nova and Touraine, 1957; Paliard et al., 1949; Popa et al., 1969; Sakula, 1975; Schoenberg and Mitchell, 1975; Turiar, 1952; Vaughan, 1939). In a subsequent review article on formaldehyde and the health effects that have been associated with it, Stenton and Hendrick (1994) reported on formaldehyde and asthma in occupational settings and starkly describe the "…first detailed case report of formaldehyde asthma confirmed by specific inhalation challenge test occurring in a nursing sister on a renal dialysis unit. Her symptoms were suggestive of late asthmatic reactions occurring 4 to 5 hours after heavy exposures. The occurrence of late reactions was confirmed in a series of challenge tests" (Stenton and Hendrick, 1994; Hendrick 1997). The results of the challenge tests are illustrated in Figure 4-1.

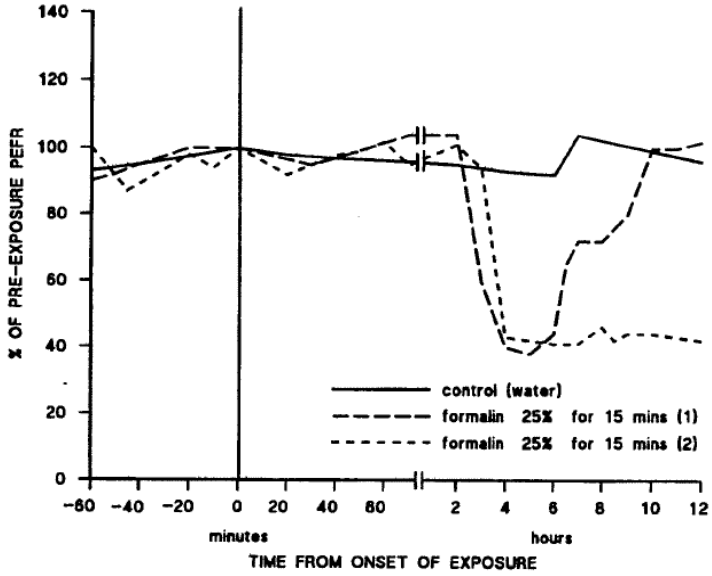

Figure 4-1. Delayed asthmatic reaction following the inhalation of formaldehyde after "painting" 100% formalin for 20 minutes. Challenge 2 was premedicated with inhaled betamethasone 200 µg.

Source: Stenton and Hendrick (1994).

Five years later, the two nurses were re-challenged with the nurse who had left the dialysis unit having no response to the subsequent challenge while the nurse who had remained working in the unit developed mild late asthmatic response with peripheral blood eosinophilia

(Stenton and Hendrick, 1994; Hendrick et al., 1982). Stenton and Hendrick (1994) concluded that these studies "provide clear evidence of formaldehyde's ability to induce asthma" but no indication of the exposure concentrations to induce it. In a follow-up study of dialysis unit staffers exposed to formaldehyde as a sterilizing agent, 8/28 people reported respiratory symptoms and a prolonged increase in circadian rhythm of peak expiratory flow rate was seen in one subject (Hendrick and Lane, 1983) implying an increase in airway responsiveness (Stenton and Hendrick, 1994). It should be noted, however, that while there did appear to be a clear response to formalin, it is not known what contribution to the response was attributable to formaldehyde and what contribution might have been attributable to methanol. Other cases of formaldehyde asthma have been described. Nordman et al. (1985) describe 12 cases and refers to several other case reports (Popa et al., 1965; Sakula 1975; Alanko et al. 1977). While the evidence of a causal association between formaldehyde and asthma is clear, the above studies do not offer information on the concentrations at which adverse effects would be expected in a population. While formaldehyde exposure is generally considered an etiologic factor for the development of asthma in occupational settings it appears to be a rare occurrence.

Numerous epidemiologic studies have investigated adverse effects in populations. Decreased peak expiratory flow rates (PEFR) are an important component in the diagnosis of asthma and there is extensive evidence of formaldehyde-induced decrements in PEFR (see Section 4.1.1.2). However, the diagnosis of asthma is both a more serious health condition and diagnostically more complex than decreased PEFR alone and is evaluated here as a distinct endpoint. While epidemiologic studies have investigated the potential association between formaldehyde exposure and a continuum of adverse health effects from pulmonary function to asthma, few nonoccupational studies have evaluated the potential effects of formaldehyde exposure on the risk of asthma onset (Delfino 2002).

However, residential formaldehyde exposure was reported to be associated with an increased risk of incident asthma in a population-based case-control study of 192 children aged 6 months to 3 years (Rumchev et al., 2002). The study was comprised of 88 children discharged from the emergency department of a children's hospital in Perth, Australia, with a primary diagnosis of asthma and 104 controls from the same community identified through the health department. Information about the child's respiratory condition and risk factors for asthma was obtained via a questionnaire compiled by the parent. Seasonal (winter, summer) in-home formaldehyde measurements taken in the living room and subject's bedroom were used to assess exposure (8-hour passive sampler). The odds ratios (ORs) for risk of asthma diagnosis by formaldehyde exposure level category (10-29, 30-49, 50-59 and >60 $\mu g/m^3$) were adjusted for measured indoor air pollutants, allergy levels of house dust mite, relative humidity, indoor

temperature, family history of asthma, atopy, age, sex, socioeconomic status, smoking, presence of pets, air conditioning, humidifier and gas appliances. Of these, age, allergic sensitization to common allergens, and family history of allergy were independent risk factors for asthma (ORs of 1.09, 2.57, and 2.66, respectively). Coexposures to other indoor air pollutants were also controlled for including benzene, toluene and ethylbenzene (Rumchev et al., 2004).

Categorical analysis of the data indicates the ORs for asthma were increased in the two highest formaldehyde exposure groups, reaching statistical significance for household exposures > 60 $\mu g/m^3$ (49 ppb) (OR of 1.39) (Rumchev et al., 2002). Analysis of the data with formaldehyde as a continuous variable provides a statistically significant increase in the risk of asthma (3% increase in risk per every 10 $\mu g/m^3$ increase in formaldehyde level. The paper states this effect as OR 1.003 (95% CI 1.002-1.004) which appears at odds with a 3% increased in risk per every 10 $\mu g/m^3$ but this must be the effect per 1 $\mu g/m^3$ and can be confirmed by comparing the per unit effect to the plotted results[1]. All analyses controlled for other indoor air pollutants, allergen levels, relative humidity, and indoor temperature as well as other risk factors.

While the study by Rumchev et al. (2002) focused on formaldehyde controlling for other indoor air pollutants, a subsequent report described the specific effects of those indoor air pollutants (Rumchev et al., 2004). This paper evaluated the risk of asthma incidence with 10 VOCs. The highest odds ratios were increased risks of asthma diagnosis associated with benzene, toluene, and ethylbenzene and were statistically significant associations. Compared to the effects observed for formaldehyde, the strength of the associations appear to be stronger on a per 10 $\mu g/m^3$ basis. The strength of these effects is an important consideration as the relative strength of the VOC effects appears to be larger than that attributable to formaldehyde if the effects of the measured indoor air pollutants had not been controlled for in the formaldehyde analysis (Rumchev et al., 2002). However, as these indoor air pollutants had been controlled for, the reported effect of formaldehyde should be independent of the effect of benzene and other VOCs in the absence of residual confounding. If two factors both cause the same outcome and are statistically associated, then they may mutually confound. In Rumchev et al. (2004) on page 750, the investigators assessed whether the effect of the VOCs were confounded by formaldehyde and stated that the results showed that exposure to VOCs still had a highly significant effect on asthma even when formaldehyde was controlled for. This finding further substantiates the formaldehyde finding since mutual confounding was not identified.

[1] In order to confirm that the effect size is 1.003 per unit change in exposure and 1.03 per 10 units, EPA compared these results to the plotted results in Rumchev et al. (2002). A line drawn across the plot at OR = 1.003 per one unit change in exposure estimates the non-linear categorical results well. At 60 $\mu g/m^3$, the extrapolated linear effect would be OR = 1.2.

Several other nonoccupational studies have evaluated the association between formaldehyde exposure and the prevalence of asthma among children (Garrett et al., 1999; Tavernier et al., 2006; Gee et al., 2005; Krzyzanowski et al., 1990; Palczynski et al., 1999). Three studies (Tavernier et al., 2006; Gee et al., 2005; Garrett et al., 1999) were performed by matching children with and without asthma and comparing the levels of formaldehyde in their homes. Gee et al. (2005) selected 100 cases with current asthma and 100 controls from 2 primary care facilities in an area of England with low socioeconomic status. Cases were identified through a screening questionnaire that had been validated with diagnoses by physicians. Cases and controls (aged 4−16 years) were matched by age and sex. Median formaldehyde levels were 0.03 ppm in living rooms and 0.04 ppm in bedrooms. Univariate comparisons found no differences in formaldehyde levels between cases of current asthma and controls without asthma. Notably, no association was observed for pollutant indicators of environmental tobacco smoke and current asthma, a recognized risk factor. A subsequent study of the same children in the same homes conducted a more thorough evaluation of risk factors (Tavernier et al. (2006). Again, a one-week average formaldehyde concentration in the living room or bedroom was not found to be associated with current asthma in multivariate analyses adjusted for several indoor variables. Respirable particulates, tobacco specific particles, volatile organic compounds, and nitrogen dioxide also were not associated with current asthma. Tavernier et al. (2006) did not report the measured levels of formaldehyde, but gave the OR for the highest tertile of exposure in the bedroom compared with the lowest tertile of exposure as 0.99 (95% CI: 0.39−2.50). The odds ratio for the second tertile compared to the lowest tertile was 1.22 (95% CI: 0.49-3.07). The width of the confidence intervals indicates that study did not have adequate statistical power to detect low level risks and suggests that these findings would still be consistent with a two-fold increase in risk.

Garrett et al. (1999) reported on the risk of allergy and asthma-like respiratory symptoms due to formaldehyde exposure in a cross-sectional survey of 80 households in rural Victoria, Australia with children, aged 7−14 years, with (n = 53) or without (n = 88) doctor-diagnosed asthma. Households were recruited via schools, medical centers, and advertisements in the local press. The study was designed to include asthmatic children in half of the households and the study recruited 43 households with at least one child with asthma diagnosed by a doctor and 37 households with no asthmatic children. Formaldehyde exposure was characterized by 4 seasonal in-home sampling events in 1994 and 1995 (4-day passive samples) in bedrooms of all participating children and in living rooms, kitchens, and outdoors. Median indoor formaldehyde concentrations were 15.8 $\mu g/m^3$ (12.6 ppb) with a maximum of 139 $\mu g/m^3$ (111 ppb). The median outdoor concentration was 0.7 $\mu g/m^3$ with a range of < 0.3−15.3 $\mu g/m^3$.

Information on asthma respiratory symptoms during the previous year was obtained through an interview with a parent after sampling was completed. An erratum to the original paper reported that the column headers in two tables were switched but that the summary statistical and conclusions in the 1999 report were correct as published. The proportion of asthmatic children by the highest formaldehyde level measured over four seasons was 0.16, 0.39, and 0.44 for <20 μg/m^3, 20-50 μg/m^3, and >50 μg/m^3, respectively (test for trend, $p < 0.02$). However, in logistic regression models, the ORs for the association did not remain statistically significant after controlling for parental allergy and asthma (ORs and 95% CIs were not provided).

A large, representative study of 202 households (mean formaldehyde level of 26 ppb) found that among children aged 6−15 years old and exposed to environmental tobacco smoke, the prevalence of physician-diagnosed asthma was 45.5% for those with measured levels of formaldehyde in the kitchen >60 ppb ($N = 11$). The prevalence of asthma dropped to 0% for levels 41−60 ppb ($N = 12$) and 15.1% for levels ≤40 ppb ($N = 106$) (chi-squared trend test $p < 0.05$). No trend in asthma prevalence was seen for children who were not exposed to environmental tobacco smoke (Krzyzanowski et al., 1990).

A study performed by Tuthill (1984) measured formaldehyde exposure for children grades K through 6 by using a combination of proxy variables. Overall, there was no association, but some individual variables did show an increased risk. For example, the reported risk ratio for having new construction or remodeling performed in the house in the past 4 months was 2.5 (95% CI: 1.7−3.9). The risk ratio for having new or upholstered furniture in the house (brought into the house within the past 4 months) was 2.2 (95% CI: 1.2−3.9).

A study in Poland randomly selected 120 households with children 5−15 years of age in 10 year old apartment houses (Palczynski et al., 1999). Using self-reported asthma prevalence as an outcome, study investigators found no association with levels of formaldehyde (mean 25.9 μg/m^3, range 2.0−66.8 μg/m^3) measured using 24-hour samples in the children. Among adults, the authors reported a higher prevalence of allergic diseases in the highest formaldehyde exposure group but that the group was too small for statistical evaluation. However, the prevalence of allergic asthma was higher among adults exposed to 25.1-50 μg/m^3 compared to <25 μg/m^3 and exposed to environmental tobacco smoke ($p = 0.03$).

Delfino et al. (2003) conducted a panel study of 22 Hispanic children with a minimum one year history of doctor diagnosed asthma, aged 10−16 years, and living in Los Angeles. The participants were nonsmokers from nonsmoking households, and lived and went to school within 3 miles of a central site monitor. The children recorded the severity of asthma symptoms in daily diaries for 3 months. The mean outdoor 24-hour levels of formaldehyde were 7.21 ppb (range 4.27−14.02 ppb). A positive association between asthma symptom scores (comparing children

who reported bothersome or more severe symptoms, including those that interfered with their daily activities, versus those with no symptoms or symptoms that were not bothersome) and increasing levels of formaldehyde measured on the previous day was observed (OR 1.37 [95% CI: 1.04−1.89]). The generalized estimating equation models adjusted for the occurrence of respiratory infections. Notably a test for effect modification revealed a stronger association with formaldehyde among children who did not regularly take anti-inflammatory medications.

Other studies of residential exposure to formaldehyde have investigated asthma prevalence and symptoms in adults (Norback et al., 1995; Matsunaga et al., 2008). A cross-sectional study by Norback et al. (1995) reported mean levels of formaldehyde, measured on one day for 2 hours, were 29 μg/m^3 (range <5−110 μg/m^3) in the bedrooms of individuals experiencing nocturnal breathlessness compared with formaldehyde levels of 17 μg/m^3 (<5−60 μg/m^3) among those without nocturnal breathlessness. The study sample was 88 individuals from a community in Sweden who responded to a screening questionnaire, had lived in the same home during the study period, and agreed to a medical interview and home sampling (58% of recruited). The eligible population was residents of Uppsala, Sweden who had answered yes (N = 74) to one of 3 questions regarding attacks of asthma in the previous 12 months, nocturnal breathlessness in the past 12 months, or current use of asthma medications or a random sample of those who had answered no (N = 80) to all three questions. The OR for nocturnal breathlessness was 12.5 (95% CI: 2.0−77.9) per a 10-fold increase in the indoor concentration in logistic regression models adjusted for age, sex, current smoking, wall to wall carpets and presence of house dust mites. The effect was substantially stronger in magnitude than the associations observed for toluene, terpenes, and volatile organic compounds. Therefore, the association with formaldehyde is likely not entirely explained by confounding by the volatile organic compounds

A recent cross sectional study (Matsunaga et al., 2008) found no association between a 24-hour personal sample for formaldehyde and prevalence of asthma when pregnant women with an exposure ≥47 ppb were compared to those with exposure to <18 ppb. The adjusted odds ratio from the logistic regression model was 2.65 (95% CI: 0.63-11.11). The small number of women identified with asthma resulted in wide confidence intervals and low statistical power. The authors analyzed baseline data collected in 2001 and 2003 from 998 participants in the Osaka Maternal and Child Health Study in Japan. The prevalence of current asthma, defined as a self report of medical treatment for asthma in the last 12 months, was 2.1% (N = 21). A prior diagnosis or treatment prior to the last 12 months was not assessed. However, they did report an increased risk of atopic eczema treated in the last 12 months. Median formaldehyde levels were 24 ppb with a maximum of 131 ppb. The odds ratio from atopic eczema for women exposed to

≥47 ppb formaldehyde was 2.25 (95% CI 1.01-5.00) in multiple logistic regression models adjusted for age, gestation, parity, family history of asthma, family income, education, mite antigen level in house dust, and season. The association was significantly higher among those with no family history of allergy.

The association between formaldehyde and asthma also has been studied by examining occupational exposures (Fransman et al., 2003; Malaka and Kodama, 1990) and school-related exposures (Zhao et al., 2008; Smedje and Norback, 2001; Norback et al., 2000). The two occupational studies examined the respiratory health of plywood workers (Fransman et al., 2003; Malaka and Kodama, 1990). The most recent of these was conducted at a plywood mill in New Zealand by Fransman et al. (2003). Of an estimated 170 workers approached by site managers or team leaders, 112 workers agreed to participate (66%). Personal samples of formaldehyde exposure were taken for 22 workers and job titles were categorized into low and high exposure groups using the median formaldehyde concentration. The geometric mean level of formaldehyde was 0.08 mg/m^3 (65 ppb) and the majority of samples were above the limit of detection which was reported to be 0.03 mg/m^3 (24 ppb). Formaldehyde exposure was categorized into low and high groups for 38 and 11 workers who had the same job title as those who had carried samplers. Compared with those with low levels of formaldehyde exposure, the odds ratio for asthma defined as woken by shortness of breath in the last 12 months, asthma attack in the last 12 months, or current asthma medication among workers with high levels of exposure was 4.3 ([95% CI]: 0.7−27.7]). An association was not seen when examining formaldehyde exposure and use of asthma medication. Asthma prevalence also was evaluated in relation to terpene, inhalable dust, abietic acid and endotoxin, and a higher odds ratio was suggested only for terpene categorized into low and high exposure (OR = 2.0 [95% CI]: 0.6-6.8).

The second study of plywood workers was completed in Indonesia (discussed in 4.1.1.2). Background levels of formaldehyde ranged from 0.003 to 0.07 ppm. The highest concentration of formaldehyde detected in an air sample was in the particleboard unit (range 1.16 to 3.48 ppm). Asthma, which was defined as “have you ever had an attack of wheezing that made you feel short of breath,?” was found to be positively associated with formaldehyde exposure (Malaka and Kodama, 1990). The estimated odds ratio, controlling for age, smoking status, and dust for the 93 exposed and 93 unexposed workers was 6.31 ($p < 0.01$).

Studies of exposure to formaldehyde at schools have been performed in China (Zhao et al., 2008) and in Sweden (Smedje and Norback, 2001). In a cross-sectional study from China (Zhao et al., 2008), the 7 day mean level of formaldehyde in 31 classrooms at 10 junior high schools was reported to be 2.3 µg/m^3 (range 1.0−5.0 µg/m^3) indoors and 5.8 µg/m^3 (range 5.0−7.0 µg/m^3) outdoors. The prevalence of cumulative and doctor diagnosed asthma reported

by the 1993 children (90% of eligible) was 1.8% and 1.2%, respectively. In models controlling for outdoor concentrations of formaldehyde, nocturnal attacks of breathlessness were significantly associated with indoor formaldehyde (OR 2.72, 95% CI: 1.03-7.18). In models controlling for indoor concentrations of formaldehyde, cumulative asthma was associated with outdoor formaldehyde levels (OR 4.61, 95% CI 1.09-19.5). An increased risk for daytime attacks of breathlessness also was observed (OR 1.29, 95% CI 0.99-1.68). The 3 level hierarchical logistic models also adjusted for age, sex, parental asthma or allergy and home environmental factors (environmental tobacco smoke etc), and indoor and outdoor concentrations of sulfur dioxide, nitrogen dioxide, and ozone. Moreover, new furniture in the home (a potential source of formaldehyde exposure) was associated with wheeze or whistling in the chest (OR 1.76, 95% CI 1.10-2.81) and daytime attacks of breathlessness (OR 1.31, 95% CI 1.0-1.72). In Sweden (Smedje and Norback, 2001), the mean level of formaldehyde measured indoors in school classrooms in 1993 and 1995 were higher than those measured by Zhao et al. (2008) (mean 4, range <5.0−72 μg/m^3).

The Swedish investigators (Smedje and Norback, 2001) conducted a 4 year follow-up study among 1732 students from 39 schools aged 7-13, who completed a mailed questionnaire in 1993. Both questionnaires were completed by 1347 students, 66% of those invited in 1993. In 1993, 6.6% reported having physician diagnosed asthma (*N* = 89) and 34% (*N* = 589) reported a history of atopy defined by an affirmative answer regarding either childhood eczema, allergy to pollens or pet dander. The 4 year incidence of physician diagnosed asthma was 4.5% (*N* = 56). This study did not report an association between formaldehyde exposure and the incidence of asthma (OR 1.2 [95% CI: 0.8−1.7]) among the whole study population. However, when the investigators stratified on history of atopy, they reported that among 22 children without a history of atopy, a new diagnosis of asthma was significantly more likely at higher concentrations of formaldehyde (OR 1.7 per 10 μg/m^3 [95% CI: 1.1−2.6]) and at higher total concentrations of mold (OR = 4.7 per 10-fold increased in total molds [95% CI: 1.2-18.4] in the classroom air. The finding for adverse effects of formaldehyde and mold did not appear to control for the other exposure and no information on the potential correlation between the two exposures was provided.

In order to evaluate the potential for confounding of the reported formaldehyde association by the reported mold association, the magnitude of effects must be compared on an appropriate scale since the magnitude of an odds ratio depends on the magnitude of the change in exposure level that is expected to produce increased risk. Standardizing the units to the reported geometric mean standard deviation, the result for formaldehyde (GSM = 2.3 μg/m^3) is

OR^2 = 1.13 per GSD and the result for mold is OR^3 = 1.02 for a comparison of risks at the GSM to 10*GSM and OR^4 = 1.06 for a comparison of risks at the minimum value of total molds ($5*10^3/m^3$) to 10*minimum. As it appears that the magnitude of the formaldehyde effect is substantially stronger than that of the mold effect (following standardization of exposure increment) it can be concluded that the reported formaldehyde effect could not have been the spurious result of uncontrolled confounding by mold. Unfortunately the logistic regression models did not account for the correlated formaldehyde concentrations for children by classroom.

A recent meta-analysis of formaldehyde exposure and asthma in children (McGwin et al., 2010) identified seven peer-reviewed studies providing quantitative results and summarized those findings. Odds ratios and confidence intervals were abstracted and effect estimates were standardized to odds ratios per 10 $\mu g/m^3$. Funnel plots were used to assess publication bias and did not show such a bias. Fixed- and random-effects models were used to calculate pooled ORs and 95% confidence intervals following a test of heterogeneity. A fixed-effect model assumes that all the individual studies provided estimates of the same effect or slope while the random-effect model allows for different effects or slopes in the source studies that may reflect difference in baseline risk factors within in the study populations. The authors preferred the fixed-effect model when heterogeneity was lower and the random-effect model was preferred when the data were more heterogeneous. Both models were presented as the degree of heterogeneity, measured by the Q test and I^2 statistic, which indicated the presence of moderate heterogeneity. However, the Q test value of 14.28 ($p < 0.0001$) and the I^2 statistic of 51% met the authors definition of sufficiently heterogeneous to prefer the random-effect model results.

Of the seven studies that were included in the meta-analysis, six reported increased risks of asthma associated with exposure to formaldehyde. The results of the random-effect model results showed an overall effect estimate of OR = 1.17 (95% CI: 1.01-1.036) (see Figure 4-2). The three studies with the highest statistical weights based on the inverse of the variance of the study ORs were for the studies by Rumchev et al. (2002), Garrett et al. (1999) and Krzyzanowski et al. (1990). Higher weights are reflected by narrower confidence intervals in these studies which implied that they were able to estimate effects with greater precision and so were assigned greater weight in the meta-analysis. The authors (McGwin et al., 2010) noted that an influence plot revealed that the study by Rumchev et al. (2002) may have had 'undue influence on the study data' and recomputed the random effects model without that study. The authors suggest that one difference is that this study is unique in focusing on very young children. Excluding

[2] OR per GSD = exp[ln(OR per $\mu g/m^3$)/10 $\mu g/m^3$ * 2.3 $\mu g/m^3$] = exp[ln(1.7)/10*2.3] = 1.13.
[3] OR per GSD = exp[ln(OR per 10-fold increase)/(9*GSM)*1.6 $\mu g/m^3$] = exp[ln(4.7)/162*1.6] = 1.02.
[4] OR per GSD = exp[ln(OR per 10-fold increase)/(9*Minimum)*1.6 $\mu g/m^3$]=exp[ln(4.7)/45*1.6] = 1.06.

Rumchev et al. (2002), the OR = 1.24 (95% CI: 1.07-1.45) was somewhat higher than the OR = 1.17 for all the studies.

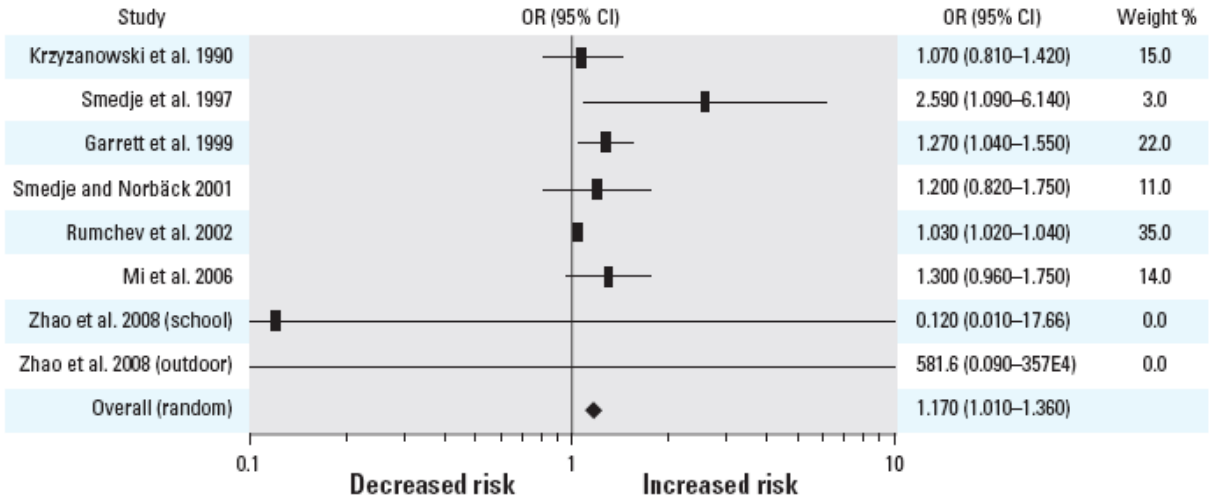

Figure 2. Forest plot of the relative risk estimates and their 95% CIs from the studies included in the meta-analysis of the association between formaldehyde exposure and asthma in children based on a random-effects model.

Figure 4-2. McGwin Forest plot of relative risk estimates and 95% CIs from studies included in a meta-analysis of formaldehyde exposure and asthma in children based on the random effects models.

Source: McGwin et al. (2010).

Separate random-effects were fit for the six studies in which the ORs were for self-reported asthma yielding an OR = 1.26 (95% CI: 0.97−1.64) and for the two studies that used diagnosed asthma OR = 1.12 (0.88−1.44). Meta-analytic results stratified by study design yielded an OR = 1.25 (95% CI: 1.08−1.44) for the cross-sectional studies. This systematic review of the literature on asthma and formaldehyde provide evidence of a concentration-dependent increased risk of asthma (prevalence and incidence) associated with increased concentrations of formaldehyde.

Garrett et al. (1999) also evaluated the prevalence and severity of allergic sensitization to 12 common allergens and reported increased prevalence with increasing formaldehyde concentration in the home. A respiratory symptom score, developed using responses by parents to a validated respiratory questionnaire during an interview, also was increased. The frequency of each respiratory symptom reported during the past year was categorized into four groups

(none, 1−3 times, 4−12 times, and >12 times) with a score of 0−3, and scores for the eight symptoms (cough, cough in the morning, shortness of breath, waking due to shortness of breath, wheeze/whistling, asthma attacks, chest tightness, and chest tightness in the morning) were combined to construct a total symptom score for each child. Mean respiratory symptom scores increased with categories of the highest of four seasonal 4-day measurements of formaldehyde in a multiple regression model after adjusting for parental asthma and allergy and interactions. For the atopy endpoints, severity/prevalence of allergic sensitization to 12 common allergens was increased in the medium (20−50 μg/m^3) and high (>50 μg/m^3) exposure groups relative to the low (<20 μg/m^3) exposure group, based on the highest of four seasonal 4-day formaldehyde measurements in the home ($p < 0.001$). The proportion of atopic children by three categories of average formaldehyde levels in bedrooms was 0.50, 0.59 and 0.74 for <10 μg/m^3, 10−30 μg/m^3, and >30 μg/m^3, respectively (test for trend, $p = 0.06$). The proportion by highest formaldehyde level was 0.33, 0.64, and 0.75 for <20 μg/m^3, 20−50 μg/m^3, and >50 μg/m^3, respectively (test for trend, $p < 0.001$). In logistic regressions, the crude association for atopy with an increase in bedroom formaldehyde concentration per 10 μg/m^3 was OR = 1.34 which increased when adjusted for parental asthma and gender to an odds ratio of 1.40 per 10 μg/m^3 (95% CI: 0.98−2.00). Thus, parental asthma was not a confounder of the association between formaldehyde and prevalence of atopy. The adjusted odds ratio for atopy with an increase in highest recorded formaldehyde per 20 μg/m^3 was 1.42 (95% CI 0.99−2.04). Passive smoking, the presence of pets, indoor nitrogen dioxide concentrations, airborne fungal spores and house-dust-mite allergens also did not influence the effect estimates and were unlikely to be confounders. The association between formaldehyde concentrations and severity of allergic sensitization was analyzed using two measures, number of positive skin prick tests and the ratio of wheal diameters after skin pricks with allergens compared to the histamine wheal size. Average levels of both measures of severity were higher in the two higher formaldehyde groups compared to the lowest group ($p < 0.05$). Further, both measures were linearly related to increasing formaldehyde categories in regression models controlling for parental asthma and allergy and sex. The authors reported that 74% of the children had lived in the residences for at least 5 years and 36% had lived there since birth. While the statistical analysis did not specifically account for correlations in children living in same home (siblings), this methodological limitation would not have induced a bias in the estimate of the effects, rather, the likely impact would have been to underestimate the true variability in the effect estimate due to the unlikely assumption that siblings can be treated as completely independent individuals.

Two controlled exposure studies in humans investigated whether exposure to a low level of formaldehyde would enhance inhaled allergen responses among already atopic individuals

(Casset et al., 2006; Ezratty et al., 2007). These studies are also described in Section 4.1.1.2.2 on pulmonary function. Casset et al. (2006) evaluated the effect of formaldehyde exposure on the bronchial response to dust mite allergen in sensitized asthma patients. The study included 19 nonsmoking subjects (12 women and 7 men) ages 19-35 years with mild asthma. Subjects had positive skin prick tests and specific IgE to Dermatophagoides pteronyssinus (dust mites). Subjects had not had a respiratory tract infection in the two weeks priors to the testing. Individuals sensitized to pollen were studied outside the relevant pollen season and people sensitive to pet allergens did not have pets at home. The 30 minute crossover exposures to 100 $\mu g/m^3$ formaldehyde and air were randomly assigned with a three-week washout period in between. Subjects underwent mite allergen challenge immediately after exposure to formaldehyde or air to determine the dose of allergen that resulted in a 20% reduction in FEV_1 (PD_{20}). The dose which induced early-phase bronchial response was significantly lower in the subjects exposed to formaldehyde (34.3 ng vs. 45.4 ng: $p < 0.05$). Late-phase bronchial response was measured over the 6 hours following the dust mite challenge by comparing FEV_1. The maximum percentage FEV1 reduction observed was significantly higher after exposure to formaldehyde 15% vs. 11% ($p < 0.05$) (Casset et al., 2006).

While the study by Casset et al. (2006) clearly showed that acute formaldehyde exposure (100 $\mu g/m^3$) enhanced both early-phase and late-phase bronchial responsiveness to mite allergen in mite-allergen sensitized people with asthma, a subsequent study with a similar protocol did not duplicate this finding. Ezratty et al. (2007) evaluated the response of asthmatics to inhaled allergen after a 60 minute exposure to 500 $\mu g/m^3$ (0.4 ppm) formaldehyde. The 12 subjects (7 men and 5 women) were 18−44 years old, were nonsmokers, and were diagnosed with intermittent asthma and allergy to pollen. No subjects had contracted an upper respiratory infection for at least 4 weeks before the study. The crossover exposures (60 minutes) to filtered air and 500 $\mu g/m^3$ formaldehyde occurred 2 weeks apart in random order. Allergen inhalation challenge, using an extract of 5 grass pollen allergens, was conducted immediately after each exposure and the dose producing a 15% decrease in FEV1 was determined (PD_{15}FEV1). Responsiveness to methacholine was determined 8 hours after the allergen inhalation challenge ended. Lung function measurements were taken using a spirometer before, during and 8 hours after the end of the allergen challenge. In addition, PEF and FEV1 were measured with a portable spirometer every 15 minutes during exposures to filtered air or formaldehyde and every hour until the methacholine challenge. The authors reported that pulmonary function, expressed as percent predicted, was not affected by the formaldehyde exposure, although the data were not presented. The median PD_{15}FEV1 for the allergen challenge was 0.80 (0.15−2.0) index of reactivity (IR) after formaldehyde exposure and 0.25 (0.10−2.0) IR after the filtered air exposure

(p = 0.06). The ratio of response after formaldehyde exposure compared to filtered air exposure was 1 in 7 subjects and higher in 5 subjects. The PD_{20} for methacholine challenge was 0.23 (0.01−3.6) and 0.17 (0.03−4) (p = 0.42). The PD_{20} for methacholine for formaldehyde compared to filtered air was lower in 3 subjects, higher in 4 subjects, and not changed in 5 subjects.

There are multiple potential explanation for these seemingly conflicting findings in Casset et al. (2006 and Ezratty et al. (2007) which include the small sample size of these studies, the differencing in the particular allergen tested and difference in the protocols. It is possible that the 12 subjects studied by Ezratty et al. (2007) may not have included individuals who were especially susceptible to formaldehyde. In the Casset et al. (2006) study, the investigators mad specific mention of the size of the particles used for the allergen challenge. They commented in the discussion that dosimetric models of inhaled formaldehyde show that the flux is very large in the first bronchial generations and then decreased rapidly. Casset et al. (2006) used an aerosol with large particles (Mass Median Aerodynamic Diameter of 11.1 μm) specifically because these large particles are deposited in the large airways where formaldehyde flux is higher. Ezratty et al. (2007) did not report the size of the particle used in their challenge but both studies did report the types of dosimeters jet nebulizers that were used and these were different. In a study unrelated to formaldehyde, Praml et al. (2005) compared the physical and biologic doses of methacholine for different nebulizers including the type used by Ezratty et al. (2007). Praml et al. (2005) compared the airway responsiveness of 34 subjects using two types of nebulizers and found that in 17 subjects, neither system caused a 20% decrease in FEV1, while among 8 subjects, both systems were able to provoke a 20% decrease. The remaining 9 subjects responded to only one type of nebulizer. Using the same protocol and the same methacholine agent produced different results based on the type of nebulizer. It may be that the results of Casset et al. (2006) and Ezratty et al. (2007) which did have other differences can be explained thusly. It may also be a difference in particle size plays a role if the pollen allergens used in Ezratty et al. (2007) were smaller and penetrate the lung beyond the upper lung where formaldehyde exposure are greater.

Multiple occupational cases reports have documented that formaldehyde can cause the onset of asthma and an epidemiologic study of residential exposures has shown that the risk of incident physician-diagnosed asthma is associated with formaldehyde in a concentration-response relationship after controlling for multiple personal characteristics and other coexposures. The regression slope was statistically significant and increased odds ratios were reported at formaldehyde concentrations of 50−59 $\mu g/m^3$ becoming statistically significant at 60 $\mu g/m^3$ (OR = 1.39). The findings of Rumchev et al. (2002; 2004) are consistent with a causal association of formaldehyde on the incidence of asthma.

Other studies of residential exposure to formaldehyde that evaluated the prevalence of asthma diagnosed by a doctor among children support this finding (Krzyzanowski et al., 1990; Garrett et al., 1999). Two studies reporting null results evaluated self-reports of asthma, a less precise measure of asthma (Gee et al., 2005; Tavernier et al., 2006; Palczynski et al., 1999). Tavernier et al. did not report the range of formaldehyde levels evaluated so the variation of concentrations evaluated is not known. Among the 187 children aged 15 years or less studied by Palczynski et al., only 9 were defined as having asthma. Studies using other designs also have reported an association of asthma among children (self-reports of physician diagnosed asthma and respiratory symptoms) and formaldehyde concentrations outdoors or in school classrooms (Zhao et al., 2008; Smedje and Norback, 2001). While Smedje and Norback did not observe an association of asthma incidence with formaldehyde exposure in a four year follow up of students who had completed a mailed questionnaire, a subgroup with no history of atopy reported a new diagnosis of asthma associated with formaldehyde. Mean formaldehyde concentrations in the school classrooms were 8 $\mu g/m^3$ (Geometric mean [GSD] 4 $\mu g/m^3$ [2.3]).

Many of these studies were evaluated quantitatively in a meta-analysis by McGwin et al. (2010). McGwin et al indicated a significant positive association between formaldehyde exposure and childhood asthma both with the Rumchev study (2002) and without it. However, the magnitude of the effect reported by Rumchev et al. (2002) may be smaller than that of other studies in a linear model. As peak expiratory flow rate is a diagnostic criterion of physician-diagnosed asthma incidence, the results of the residential epidemiology study by Krzyzanowski et al. (1990) showing statistically significant decrements in PEFR associated with increased formaldehyde concentration are strongly supportive. Formaldehyde exposure has also been associated with an increased severity of asthma symptoms in children (Garrett et al. 1999; Delfino et al., 2003).

Studies of exposure among adults are suggestive of an association with asthma. Residential exposures at average concentrations of 29 $\mu g/m^3$ were associated with nocturnal breathlessness (Norback et al., 1995) and occupational levels above 1 ppm were associated with self-reported asthma (Malaka and Kodama, 1990). A null study in New Zealand included very few participants resulting in wide confidence intervals (Fransman et al., 2003).

Garrett et al. observed an increase in the prevalence and severity of allergic sensitization to common allergens associated with formaldehyde at concentrations of 20 $\mu g/m^3$ and above. The role of formaldehyde in exacerbating allergic responses to common allergens among atopic individuals was demonstrated by Cassett et al. (2006) using formaldehyde concentrations of 100 $\mu g/m^3$. Another study using different allergens, dosimeters, and study protocol did not report an effect of formaldehyde on allergic responses (Ezratty et al., 2007). Finally, the report

by Smedje and Norback (2001) of increased incidence of asthma over a four year follow-up of a cohort of school children among children without a history of atopy is consistent with a role of formaldehyde in increasing sensitivity to allergens.

4.1.1.4. *Respiratory Tract Pathology*

Formaldehyde-induced respiratory tract pathology includes inflammation, rhinitis, goblet cell hyperplasia, metaplastic changes, squamous cell hyperplasia, and impaired mucociliary transport. Formaldehyde may bind to the trigeminal nerve and trigger the release of neurogenic mediators of inflammation that result in tissue edema, lacrimation, mucus production and leukocyte infiltration. How much inflammation, hyperplasia, and metaplastic change are due to sensory irritation-induced inflammatory responses compared with formaldehyde-induced direct cell damage cannot be distinguished. Increased mucus flow and metaplastic changes may progress in relation to the concentration and duration of exposure to protect the underlying tissue. When the exposure exceeds protective and defensive mechanisms, permanent damage results (Swenberg et al., 1983). Nonetheless, these changes serve as a sensitive indicator of formaldehyde exposure, since they occur before gross cellular damage and focal lesions (Monticello et al., 1989), and potentially suggest a point at which the concentration and duration of exposure exceed the protective nature of local responses (increased mucus flow, goblet cell hyperplasia, squamous metaplasia, etc.) (Swenberg et al., 1983). A number of human studies have reported nasal lesions associated with exposure to formaldehyde (Pazdrak et al., 1993; Ballarin et al., 1992; Boysen et al., 1990; Holmström et al., 1989c; Edling et al., 1988), while other studies have documented changes in mucociliary clearance and activity (Holmström and Wilhelmsson, 1988; Andersen and Molhave, 1983). These studies are summarized below.

4.1.1.4.1. *Nasal lesions.*

Ballarin et al. (1992) did a case-control study of 15 workers from a plywood factory where urea-formaldehyde glue is used. Mean levels of formaldehyde exposure (8-hour average) were estimated to be 0.09, 0.1, and 0.39 mg/m^3 in three regions of the facility (sawmill, shearing press, and warehouse, respectively). Nasal respiratory samples were obtained. Stained cells were scored for histopathology. Cytology examination revealed increased squamous metaplasia cells in 10 of 15 (67%) factory workers (with an average severity score of 2.3) compared with one of 15 (6%) controls (with an average histology severity score of 1.6). In addition, one formaldehyde exposed worker (n = 15) exhibited mild dysplasia and had the highest severity score (3.0). Authors suggest that these results may be due to chronic irritation of the nasal respiratory mucosa. This small study reported only incidence of lesions and did not score based

on severity of lesions. The lesion incidence was not reported in relation to dose, so no dose-response relationship could be determined, precluding the establishment of a point of departure (POD).

Holmström et al. (1989c) collected nasal biopsy samples from 36 workers not exposed to formaldehyde and 70 workers exposed to formaldehyde at a median concentration of 240 ppb. Nasal biopsy samples were scored on a 0−8 range with normal respiratory epithelium as 0 and carcinoma as 8. Observed histologic changes included loss of cilia, goblet cell hyperplasia, and cuboidal and goblet cell metaplasia replacing normal columnar epithelium. The incidence associated with each histologic change was not reported and cannot be compared between formaldehyde-exposed and control individuals. Moreover, these biologically relevant changes were not analyzed independently in the analysis. The mean scores were 1.56 (range, 0−4) for the control group and 2.16 (range, 0−4) for the formaldehyde-exposed group. Although the range of scores in the controls and formaldehyde-exposed groups were the same (0−4), the difference in mean scores (1.56 versus 2.16) was statistically significant ($p < 0.05$); scores were worse in the formaldehyde-exposed group. The authors reported no correlation between the duration of exposure and histologic changes and no correlation between smoking habits and biopsy scores. The loss of cilia, goblet cell hyperplasia, and cuboidal and squamous cell metaplasia replacing the columnar epithelium were increased in the group exposed to formaldehyde and is a biologically relevant change. This study provides a lowest-observed-adverse-effect level (LOAEL) of 0.240 ppm for nasal histopathology.

Edling et al. (1988) collected nasal biopsy samples from workers ($n = 75$) exposed to formaldehyde at three plants (workers in two of these plants were also exposed to wood dust) compared with a referent group ($n = 25$). Concentrations ranged from 0.1 to 1.1 mg/m^3 (TWA) with peaks of 5 mg/m^3. Nasal histology was scored from 0 to 8 by increasing severity, from normal respiratory epithelium (0) to carcinoma (8). A normal respiratory epithelium was noted in 3 of 75 workers. A loss of cilia and goblet cell hyperplasia (scores of 2) was reported in eight workers. Mixed cuboid/squamous epithelium (metaplasia), stratified squamous epithelium, and keratosis were reported in 58 of 75 workers (those with scores of 3, 4, and 5 were combined). Dysplasia (score of 6) was reported in 6 of 75 formaldehyde-exposed workers. None of the workers had lesions that warranted a histologic score higher than 6. Histologic scores did not correlate with duration of exposure but could not be confirmed due to poor reporting. Data from the referent group were not included. A POD could not be determined from this study.

Boysen et al. (1990) collected nasal biopsy samples from workers exposed to air ($n = 37$) or to formaldehyde ($n = 37$) and sometimes wood dust. The exposed workers were classified into two exposure groups, 0.5−2 ppm and >2 ppm. Nasal biopsy samples were assessed by using

a histopathology score range of 0−5, based on the pathology of pseudostratified columnar epithelium (0) to dysplasia (5). Mean pathology scores for the control were decreased compared with the formaldehyde-exposed group (1.4 and 1.9, respectively) but did not reach statistical significance. Little quantitative pathology data were provided, although qualitative histology revealed a range of observed effects from deciliated epithelial cells to mixed stratified cuboidal, squamous epithelium to dysplasia. None of the control samples received histologic severity scores of 4 or 5, indicating that keratinizing stratified squamous epithelium and dysplasia were not observed in controls. A wider variety of histopathologic lesions were reported in exposed workers compared with controls, and a greater number of exposed workers had histologic changes compared with controls. Incidence data for each type of histopathology were not reported, but the authors wrote that the degree of metaplastic alterations was more pronounced among the exposed workers. An upper range for the high concentration group (>2 ppm) was not reported, and median concentrations were not provided.

Pazdrak et al. (1993) exposed human subjects (six men, three women) to 0.4 ppm formaldehyde in a chamber for 2 hours. Approximately half of the subjects suffered from skin hypersensitivity to formaldehyde, while the other subjects were healthy. An evaluation of nasal lavage pretest and following formaldehyde exposure revealed that the hypersensitive and healthy groups had similarly elevated eosinophil counts at 0 hours after exposure (from 42×10^3 cells/mL to 72×10^3 cells/mL for healthy subjects [$p < 0.05$] and from 39×10^3 cells/mL to 69×10^3 cells/mL for hypersensitive subjects [$p < 0.05$]). Similar eosinophil levels were also seen in both groups at 3 and 18 hours. Both groups had equivalent increases in lavage albumin and total protein levels following exposure, but basophil counts were unchanged. Based on evidence of formaldehyde-induced inflammation, these data provide a LOAEL of 0.4 ppm for nasal histopathology.

4.1.1.4.2. *Mucociliary clearance.*

In addition to abnormal nasal histopathology, changes in mucociliary clearance were also observed in some of these studies at similar exposure concentrations. The mucociliary apparatus is an important barrier to infection and exogenous agents and, thus, is considered as a potential adverse effect. These effects may be due to direct interaction of formaldehyde with the mucus itself or to SI-induced inflammation in the nasal tissue that affects mucus production and creation of an effective mucosal barrier.

Andersen and Molhave (1983) reviewed five controlled human studies, one of which (Andersen and Lundqvist, 1974) examined mucus flow rate in 16 individuals acutely exposed to 0, 0.3, 0.5, 1, or 2 ppm formaldehyde for 4−5 hours in a chamber. Mucus flow rate was

decreased in the anterior and middle third of the ciliated mucosa at 0.3 ppm, but statistical significance was not determined. This study included smokers and nonsmokers. The small sample size, potential confounder effect from smoking, and lack of dose-response relationship preclude the establishment of a POD.

Holmström and Wilhelmsson (1988) demonstrated reduced mucociliary clearance and nasal mucosal swelling in 70 workers exposed to a mean formaldehyde concentration of 0.21 ppm, compared with a referent group of store clerks (n = 36) and was further averaged over years of exposure. Mucosal swelling and mucociliary activity was measured in the nasal turbinates. The authors also reported symptoms not only during the weekdays, but also over weekends and vacation periods. Formaldehyde-exposed subjects self-reported significantly more nasal discomfort, eye discomfort, deeper airway discomfort, and frequent headache than the referent group. Groups exposed to formaldehyde had more pronounced mucosal swelling (10.7 nasal resistance score) compared with the reference group (6.5 nasal resistance score). This difference persisted when data were normalized for differential nasal congestion in the subjects. Decreased mucociliary activity was seen in 3% of controls and 20% of formaldehyde-exposed subjects and reached statistical significance ($p < 0.05$). It is not clear whether impaired mucociliary clearance was a consequence of altered cell morphology or increased mucus viscosity. These data provide a LOAEL of 0.21 ppm based on impaired mucociliary clearance.

Thus, mild nasal epithelial lesions observed in formaldehyde-exposed workers have been observed consistently at levels of about 0.20 ppm to about 2 ppm (Boysen et al., 1990; Holmström et al., 1989; Edling et al., 1988). Of these, Holmström et al. (1989) and Edling et al. (1988) do not appear to be confounded by exposure to wood dust. Nasal biopsy pathology from formaldehyde-exposed workers is consistent with irritant and reactive properties of formaldehyde (Ballarin et al., 1992; Boysen et al., 1990; Holmström et al., 1989; Edling et al., 1988; Berke, 1987). Moreover, these findings are supported by results from animal toxicity and pharmacokinetic and anatomical airflow studies, indicating that, at concentrations less than 1 ppm, inhaled formaldehyde gas does not reach lower regions of the respiratory tract. Of the available human studies that evaluated histopathology, Holmström and Wilhelmsson (1988) appears to be the most robust and sensitive. The study was carefully designed and included a large sample of formaldehyde-exposed subjects who were considered separately from workers exposed to combinations of exposures (formaldehyde and wood dust). Study subjects had been exposed to formaldehyde regularly for many years. The authors reported not only weekday exposures but effects reported on weekends and on vacation. Total exposure was carefully calculated and averaged. The data were controlled for potential confounders, such as smoking. The endpoint of reduced mucociliary clearance has been substantiated by Andersen and Molhave

(1983) and Holmström et al. (1989). Animal studies have also reported formaldehyde-induced changes on the nasal mucosa and are highlighted in Section 4.2.1.2.

4.1.1.5. *Immunologic Effects*

Numerous studies have examined the immunologic responses of individuals exposed to formaldehyde. This section will discuss four specific areas related to immunotoxicity after exposure to formaldehyde: increased upper respiratory tract (URT) infections, systemic immune dysfunction, sensitization and atopy, and production of formaldehyde-protein complexes. Some studies report increased incidence of URT infections after exposure to formaldehyde (Lyapina et al., 2004; Krzyzanowski et al., 1990; Holness and Nethercott, 1989). This effect appears to occur independently of systemic immune changes and may be due to damage to the mucosal barrier, thus facilitating pathogen access. A number of studies have investigated the hypothesis that formaldehyde may induce systemic immunomodulation (Ohtani et al., 2004a, b; Erdei et al., 2003; Thrasher et al., 1990, 1987; Pross et al., 1987). Some studies have also evaluated immune system effects by investigating the role of reactive oxygen species (ROS) from respiratory burst associated with immune cells (Lyapina et al., 2004; Gorski et al., 1992) and by assessing chromosomal damage in immune cells (Orsière et al., 2006; Yu et al., 2005). In addition to the effects of formaldehyde on asthmatics and the potential for formaldehyde exposure to exacerbate asthmatic responses (Pourmahabadian et al., 2006; Herbert et al., 1994; Malaka and Kodama, 1990; Alexandersson and Hedenstierna, 1989; Alexandersson et al., 1982), reviewed in Section 4.1.1.2, numerous studies have investigated whether formaldehyde may directly induce sensitization and atopic responses by measuring immunoglobulin E (IgE) levels associated with formaldehyde exposure (Ohmichi et al., 2006; Vandenplas et al., 2004; Doi et al., 2003; Baba et al., 2000; Palczynski et al., 1999; Krakowiak et al., 1998; Wantke et al., 1996a, b; Liden et al., 1993; Salkie, 1991; Grammer et al., 1990; Kramps et al., 1989). Findings are largely negative and suggest that formaldehyde-induced IgE production is not likely. Lastly, studies have investigated the production of formaldehyde-specific antibodies, formaldehyde-albumin complexes, and formaldehyde-heme complexes (Kim et al., 2001; Carraro et al., 1997; Grammer et al., 1993, 1990; Dykewicz et al., 1991; Thrasher et al., 1990). Heme complex formation is not a strict immunologic endpoint but may trigger antibody formation and thus it will be discussed in this section. This section will thus summarize the human studies that have specifically addressed the increased incidence of URT infections, immunotoxic endpoints, atopy and sensitization, and formation of formaldehyde-heme and formaldehyde-albumin complexes.

4.1.1.5.1. *Increased URT infections.*

Three studies have investigated the possibility that formaldehyde exposure leads to increased URT infections (Lyapina et al., 2004; Krzyzanowski et al., 1990; Holness and Nethercott, 1989). Lyapina et al. (2004) studied 29 workers who were occupationally exposed occupationally to formaldehyde for an average of 12.7 years through contact with carbamide-formaldehyde glue. The mean values of the average shift concentrations of formaldehyde in the application of carbamide-formaldehyde glue was found to be 0.71 ppm TWA with a range of 0.32 to 1.57 ppm. The workers were divided into two subgroups, one (n = 12) that suffered from either a long history (with clinical findings) of chronic mucous inflammation of the URT with multiple relapses and a second group (n = 17) whose URT inflammations were short, acute, and predominantly viral. Twenty-one healthy subjects served as controls. A statistically significant association of self-reported chronic bronchitis and decreased resistance to URT infection was reported in all the exposed workers compared with controls (p = 0.02). Of the workers, 41% had a history of chronic respiratory infection and frequent long-lasting infectious inflammatory relapses (group 1a). Another group (group 1b) consisted of 17 exposed workers, 12 of whom had no history of recurrent viral infections of the URT. There was a statistically significant association of frequency and duration of inflammatory relapses between groups 1a and 1b. No dates were provided regarding when these measurements were made or over what period of time they were calculated.

Krzyzanowski et al. (1990) measured formaldehyde levels in homes and recorded, by way of a questionnaire, health histories from adult and child residents. Formaldehyde levels were reported from samples taken for two 1-week periods in various rooms of the home (kitchen, living room, subject's bedroom). The average formaldehyde level was 26 ppb in 202 homes, and levels were stratified into homes with exposure levels below 40 ppb, between 40 and 60 ppb, and above 60 ppb. Incidences of doctor-diagnosed chronic bronchitis were more prevalent in children (under age 15) living in homes with higher formaldehyde (>60 ppb) readings in the kitchen ($p < 0.001$). This effect was more pronounced ($p < 0.001$) in children simultaneously exposed to environmental tobacco smoke. The prevalence of chronic cough was also increased in adults living in homes with measurable levels of formaldehyde, but data were not shown. Holness and Nethercott (1989) assessed chronic bronchitis in 87 funeral workers, where the average formaldehyde exposure was reported at 0.38 ± 0.19 ppm. Chronic bronchitis was observed in 20 funeral workers (n = 87) exposed to formaldehyde compared with 3 cases of chronic bronchitis in nonexposed referent controls (n = 38).

These studies suggest that exposure to formaldehyde may be associated with increased incidence of chronic bronchitis. The mechanism for this association has not been elucidated.

Pathogens may gain access to the URT via a compromised mucosal barrier, as has been shown in histopathology studies (see Section 4.1.1.4).

4.1.1.5.2. *Immune function.*

A number of studies have evaluated the ability of formaldehyde to induce systemic immunotoxic effects (Erdei et al., 2003; Thrasher et al., 1990, 1987; Pross et al., 1987). Some studies have reported altered innate immune responses associated with formaldehyde exposure in immunologically compromised children (Erdei et al., 2003), while others have noted adaptive immune response suppression associated with formaldehyde exposure (Thrasher et al., 1990, 1987) and changes associated with alterations to a predominant T—lymphocyte helper 2 (Th2) pattern (Ohtani et al., 2004a, b). In contrast, Pross et al. (1987) did not observe formaldehyde-associated changes in systemic immune function.

Erdei et al. (2003) found that *Haemophilus influenzae* humoral biomarker (H.in.IgG), *Klebsiella pneumoniae* biomarker (K.pn.IgG), and elevated monocyte concentrations were significantly associated with high formaldehyde concentrations in asthmatic children, compared with nonsensitive children. Briefly, Erdei et al. (2003) compared the immune system responses in 9- to 11-year-old Hungarian school children whose respiratory systems were immunologically compromised (chronic respiratory disease, asthma) and normal children who were exposed to indoor air pollutants, including formaldehyde. In the homes of the children with the highest levels of pollutants, 49.3% of formaldehyde measurements exceeded the Hungarian indoor standard of 0.01 ppm, while 20% exceeded the World Health Organization's (WHO's) suggested indoor level of 0.09 ppm. The authors excluded from consideration all measurements that exceeded WHO's air quality guidelines in one unidentified city to prevent a "city-related bias," since these measurements occurred entirely in that city. The average formaldehyde concentration in the 123 homes tested was 14 ppm with a range of 0.5 to 46 ppm. H.in.IgG and K.pn.IgG were significantly associated with high formaldehyde concentrations ($p < 0.013$ and $p < 0.049$, respectively) in sensitive children compared with nonsensitive children. These markers were also correlated with high levels of nitrogen dioxide, the number of cigarettes smoked, and exposure to paint, volatile organic compounds, and solvents. Additionally, indoor formaldehyde exposure was significantly associated with increased monocyte concentrations ($p < 0.017$) that are important to the innate immune response (inflammation) in diseased tissue. The authors concluded that the elevation of immune biomarkers in sensitive children with respiratory disease is likely the result of high concentrations of toxic indoor air pollutants, including formaldehyde.

Thrasher et al. (1987) assessed the effects of formaldehyde exposure on cellular immunity and antibody formation in eight exposed and eight unexposed individuals. The exposed group consisted of three males and five females. Seven of the exposed individuals had resided in mobile homes for periods ranging from 2 to 7 years; the eighth was a laboratory worker who resided in a newly decorated, energy-efficient apartment. Air monitoring in four of the homes revealed formaldehyde vapor concentrations ranging from 0.07 to 0.55 ppm. Venous blood samples were collected from all subjects and T- and B-cells were counted and monitored for blastogenesis. When IgG and IgE antibodies to formaldehyde were monitored in serum, no IgE antibodies to formaldehyde were detected in exposed or control subjects. IgG antibody titers in exposed subjects ranged from 1:8 to 1:256 but essentially were undetected (1:4) in seven of the controls. T- and B-cell numbers were significantly lower ($p < 0.05$) in mobile home residents (48 and 12.6%, respectively) compared with those of control subjects (65.9 and 14.75%, respectively). As determined by incorporation of ^{3}H-thymidine into 48-hour unaltered lymphocytes, phytohemagglutinin-stimulated T- and B-cell blastogenesis was significantly depressed ($p < 0.01$) in cells of mobile home residents compared with those of control subjects (17,882 and 28,576 cpm, respectively). Thrasher et al. (1987) concluded that exposure to formaldehyde decreases the proportion of peripheral T cells.

In a later study, Thrasher et al. (1990) evaluated five groups of subjects with varying levels and durations of formaldehyde exposure. The groups consisted of (1) asymptomatic chiropractic students exposed during anatomy classes (controls with only intermittent exposure to formaldehyde), (2) mobile home residents, (3) office workers, (4) patients with multiple symptoms who had been removed from the source of formaldehyde for at least a year, and (5) occupationally exposed patients. All groups were assessed for immunologic function via white cell, lymphocyte, and T-cell counts, T-helper/suppressor ratios and B-cell counts. When compared with controls (chiropractic students), the patient groups had significant elevations in formaldehyde antibody titers and B-cell titers.

Ohtani et al. (2004a, b) reported effects of exposure to formaldehyde and diesel exhaust particles on cytokine production by human monocyte-derived dendritic cells (MoDCs) and T cells in vitro. Dendritic cells were stimulated with CD40 ligand and interferon (IFN)-γ, T cells with anti-CD3/CD28 antibodies. Cytokine proteins and mRNA levels were measured in supernatants by enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR), respectively. Formaldehyde and diesel exhaust particles significantly increased tumor necrosis factor (TNF)-α levels and suppressed interleukin (IL)-12p40 protein and mRNA levels in MoDCs. The same treatment suppressed protein synthesis and mRNA expression of IFN-γ and IL-10 in T cells. The authors concluded that their findings support a role of formaldehyde

and diesel exhaust particles in altering the immune response to a Th2-dominant pattern that furthers allergic inflammation. Further details, such as exposure concentrations and experimental protocols, are not available.

In contrast, Pross et al. (1987) concluded that formaldehyde does not induce altered immune activity. The authors evaluated the immunologic response of asthmatic subjects exposed to UFFI off-gas products. Subjects consisted of 23 individuals with a history of asthmatic symptoms attributed to UFFI and 4 individuals with asthma unrelated to UFFI off-gas products. All subjects were exposed in an environmental chamber according to the following sequence: (1) room air (placebo) for 30 minutes; (2) 1 ppm formaldehyde gas for 3 hours; (3) UFFI particles (4 μm diameter, 0.5 particles/mL) for 3 hours, commencing 48 hours after formaldehyde gas exposure; and (4) UFFI off-gas products for 3 hours, commencing 48 hours after UFFI particle exposure. There was a significant increase in the percentage and absolute number of eosinophils and basophils in the subjects who lived in UFFI homes but no differences between exposure groups with respect to lymphocyte subpopulations either before or after UFFI exposure. However, when T8 suppressor cells were counted, values in the UFFI-exposed group pre-exposure and postexposure, a small but statistically significant ($p < 0.01$) increase in T8 cell count was observed. The biological significance of this increase in T8 cell count in exposed asthmatics is not known. Pross et al. (1987) concluded that short-term exposure to formaldehyde was not immunosuppressive and did not result in systemic immune reactivity.

Respiratory burst from immune cells creates ROS that can incur further cellular damage. Several studies have evaluated, either directly or indirectly, the potential role of ROS as potential mediators of formaldehyde-associated effects, particularly those caused by immune cells. Gorski et al. (1992) measured chemiluminescence resulting from the release of free radicals from granulocytes of healthy and formaldehyde-sensitive (based on anamnesis and a positive patch test) subjects. Thirteen subjects with contact dermatitis who were occupationally exposed to formaldehyde and five healthy volunteers participated in the study. All underwent skin-prick tests for common allergens as well as a histamine inhalation provocation test. Subjects were exposed to 0.5 mg/m^3 (0.41 ppm) formaldehyde for 2 hours, and the PEFR was measured immediately before exposure, after 60 and 120 minutes of exposure, and 6 and 21 hours after completion of exposure. Peripheral blood granulocyte chemiluminescence was measured in the presence of luminol. Free radical production was increased significantly within 30 minutes of beginning the exposure in subjects with allergic dermatitis and remained elevated for 24 hours compared with baseline values. Gorski et al. (1992) concluded that granulocyte chemiluminescence did not increase in healthy, formaldehyde-exposed patients but was

diagnostic for formaldehyde-sensitive patients. These results also suggest a putative role for oxidative damage associated with formaldehyde exposure, particularly in sensitized individuals.

Lyapina et al. (2004) also reported effects of formaldehyde exposure on neutrophil respiratory burst activity (NRBA), the capacity of polymorphonuclear leukocytes to produce reactive oxygen radicals in response to chemical or microbial stimuli using flow cytometry. Briefly, Lyapina et al. (2004) studied 29 workers who were occupationally exposed to formaldehyde for an average of 12.7 years through contact with carbamide-formaldehyde glue with a mean value of the average shift concentration of formaldehyde reported as 0.71 ppm TWA with a range of 0.32 to 1.57 ppm. The workers were divided into two subgroups, one (n = 12) that suffered from either a long history (with clinical findings) of chronic mucous inflammation of the URT with multiple relapses, and a second group (n = 17) whose URT inflammations were short, acute, and predominantly viral. Twenty-one healthy subjects served as controls. A suite of hematological tests and flow cytometric analysis for respiratory burst activity were performed. Although no significant difference was observed in the spontaneous and stimulated NRBA (median percentage of oxidizing cells) between the 29 exposed workers with URT inflammation and the healthy controls (0.83 versus 1.35, respectively), a separate comparison of the NRBA of 12 workers with chronic, repeating URT infections and 17 workers with short, infrequent episodes of URT inflammations was significant (0.45 versus 1.00, p = 0.037). When the NRBA of the group with chronic URT infections (n = 12) was separately compared with that of the healthy controls (n = 21), the results were also significant (0.45 versus 1.35, p = 0.012). Individuals with chronic URT infections have reduced NRBA that could be due to formaldehyde exposure. Neutrophils respond to tissue damage or local invasion of microorganisms and act to phagocytize foreign cells. If neutrophilic activity is hampered or altered by formaldehyde exposure, then the ability to fight infection will be diminished, leading to prolonged infection. However, no dose-response pattern of formaldehyde exposure could be determined from this study.

Other investigators have reported chromosomal damage in immune cells due to formaldehyde (Orsière et al., 2006; Yu et al., 2005). Yu et al. (2005) evaluated chromosomal damage in lymphocytes from 151 exposed and nonexposed workers from a plywood factory detected by comet assay. The authors reported that chromosomal damage was statistically elevated in lymphocytes from formaldehyde-exposed workers compared with controls. However, no information on exposure duration or levels was provided. Orsière et al. (2006) studied DNA damage in lymphocytes from 59 hospital employees with formaldehyde exposures from pathology and anatomy laboratories in five hospitals. Controls were 37 workers from the same hospitals, matched on gender, age, and smoking habits, with no known exposure to

genotoxic agents. Study participations were excluded if workers had a history of radio- or chemotherapy or had used therapeutic medications that were known to be mutagenic. Occupational exposure was determined through 15-minute and 8-hour personal air sampling during a typical workday. Mean formaldehyde concentrations were 2 ppm (range: <0.1−20.4 ppm) for 15-minute sampling and 0.1 ppm (range: <0.1−0.7 ppm) for 8-hour sampling. No change in DNA damage was found between the beginning and end of the workday among exposed workers (3.9 ± 0.6 versus 3.6 ± 0.5 relative light units/ng DNA). However, exposed workers had significant elevations in the binucleated micronucleated cell rate (BMCR) per 1,000 cells compared with controls (16.9 ± 9.3 versus 11.1 ± 6.0%; $p < 0.001$) suggesting a clastogenic response, but BMCR did not appear to be correlated with formaldehyde concentration. Linear regression analysis showed that the effect for exposure remained after adjusting for gender, age, smoking, and drinking habits. For 18 exposed and 18 control workers who underwent cytokinesis-blocked micronucleus assay (CBMA) combined with fluorescent in situ hybridization (FISH) with pan-centromeric DNA probe, results showed that the frequency of micronuclei (MN) containing only one centromere (C1+MN) was elevated among the exposed compared with unexposed workers (11.0 ± 6.2% versus 3.1 ± 2.4%; $p < 0.001$). The effect of exposure remained significant after controlling for gender, age, smoking, and drinking habits. Results from Yu et al. (2005) and Orsière et al. (2006) suggest that formaldehyde exposure may promote chromosomal damage leading to micronucleated lymphocytes.

Compromised lymphocyte function may significantly contribute to altered immune status. The mechanism underlying this effect has not been elucidated.

4.1.1.5.3. *Sensitization and atopy.*

Numerous studies have documented formaldehyde-induced exacerbation of asthmatic responses (Garrett et al., 1999; Kriebel et al., 1993; Delfino et al., 2003; Norback et al., 195; Cassett et al., 2006; also see Section 4.1.1.2). The mechanism of this effect has not been clarified and has led investigators to assess the potential for formaldehyde to directly induce formation of formaldehyde-specific antibodies, leading to allergic responsiveness. One case report showed systemic allergic reactions (e.g., anaphylaxis) to formaldehyde in a patient undergoing hemodialysis (Maurice et al. [1986] referenced in Thrasher et al. [1990]). Some studies have evaluated the potential association of formaldehyde-specific IgE in already-sensitized individuals (Baba et al., 2000; Palczynski et al., 1999). Other studies have investigated whether formaldehyde can directly induce IgE in nonsensitized individuals. Most of the studies have not identified presence of formaldehyde-specific IgE (Ohmichi et al., 2006; Krakowiak et al., 1998; Grammer et al., 1993, 1990; Kramps et al., 1989; Thrasher et al., 1987)

and are summarized below. A few studies (Vandenplas et al., 2004; Doi et al., 2003; Liden et al., 1993) reported positive IgE against formaldehyde, associated with exposure, but the IgE titers were either transient (Vandenplas et al., 2004) or were positive in a small subset of previously sensitized subjects (2 of 15) (Liden et al., 1993). Doi et al. (2003) detected IgE against formaldehyde in two asthmatic children (out of 122 asthmatic children), but the response severity did not correlate with exposure level.

Palczynski et al. (1999) evaluated whether exposure to formaldehyde might facilitate specific sensitization to common allergens. The study population was comprised of residents of apartments built in 1989−1990. Only households with children from 5−15 years were eligible for the study. A random sample of 120 apartments was selected in which lived a total of 465 persons aged 5−65 years. Individual demographic characteristics and medical histories were determined by questionnaire. Residents were tested, using the skin-prick method, for allergen response to a variety of materials, such as household dust, pollens, and feathers. Total serum IgE levels were measured, and the presence of formaldehyde-specific IgE antibodies was determined. Measured mean levels of formaldehyde were 21.05 ± 8.94 ppb. No significant relationship between respiratory allergy prevalence and indoor exposure to formaldehyde was detected. Significant increases in serum IgE levels were found in children exposed to both environmental tobacco smoke and formaldehyde.

Baba et al. (2000) investigated whether production of formaldehyde-specific IgE could be detected in adult asthmatics. Formaldehyde exposure levels were not documented. Formaldehyde-IgE was detected in two asthmatic patients (n = 80), one male and one female, but the titer of IgE did not parallel the severity of the asthmatic responses and could not be linked to formaldehyde exposure. Thus, formaldehyde-specific IgE-mediated allergy was rare in adult chronic asthmatics.

Several studies have examined serum for formaldehyde-specific IgE antibodies in groups of formaldehyde-exposed humans (Ohmichi et al., 2006; Krakowiak et al., 1998; Wantke et al., 1996a, b; Salkie, 1991; Grammer et al., 1990; Kramps et al., 1989). While formaldehyde-specific IgE was reported in one study (Wantke et al., 1996a), results from most other studies failed to find a consistently strong association between formaldehyde-specific IgE or IgG antibodies in groups of formaldehyde-exposed humans.

Wantke et al. (1996a) detected elevated levels of formaldehyde-specific IgE as determined by Radio Allergo Sorbent Test or RAST, which detects allergen-specific IgE) in 24 of 62 8-year-old children who were students in three particleboard-paneled classrooms in which the estimated formaldehyde air concentrations were 0.075, 0.069, and 0.043 ppm. In a health survey, the children reported headaches (29/62), fatigue (21/62), dry nasal mucosa (9/62), rhinitis

(23/62), cough (15/62), and nosebleeds (14/62). The number of children with symptoms in each classroom decreased with decreasing formaldehyde concentration (49, 47, and 24, respectively, for the 0.075, 0.069, and 0.043 ppm classrooms). However, the investigators reported that elevated levels of specific IgE did not correlate with the number and severity of symptoms. When the children were evaluated after 3 months in a new school that did not have particleboard paneling and had lower ambient formaldehyde concentrations (0.029, 0.023, and 0.026 ppm), the number of children reporting symptoms decreased significantly from earlier figures, and, when measured in 20 of the children, the mean serum levels of formaldehyde-specific IgE declined significantly compared with premoving mean levels.

In contrast, a study by Krakowiak et al. (1998) measured serum IgE levels in asthmatic and healthy subjects as part of a larger study to characterize the mechanism of formaldehyde-induced nasal and bronchial response in asthmatic subjects with suspected formaldehyde allergy. Ten subjects reported to have formaldehyde rhinitis and asthma and 10 healthy subjects underwent a 2-hour inhalation challenge in an exposure chamber with formaldehyde at a concentration of 0.5 mg/m^3 (0.41 ppm). Formaldehyde-specific serum IgE antibodies were measured, and cellular, biochemical, and mediator changes were assessed in nasal lavage before, immediately after, and at 4 and 24 hours after challenge. Challenges with formaldehyde caused only transient symptoms of rhinitis in both groups. Furthermore, none of the subjects thought to have occupational asthma developed clinical symptoms of bronchial irritation. No specific IgE antibodies to formaldehyde were detected in persons with occupational exposure to formaldehyde. No differences in the nasal response to formaldehyde were found between subjects reported to have occupational allergic respiratory diseases and healthy subjects ($p > 0.05$). The study showed that inhaled formaldehyde at a level as low as 0.5 mg/m^3 did not induce a specific allergic response either in the upper or in the lower part of the respiratory tract. In addition, it demonstrated that there was no difference in nasal response to formaldehyde between asthmatic subjects occupationally exposed to formaldehyde and healthy subjects.

Similarly, formaldehyde-specific IgE antibodies were detected in only 1 serum sample (out of 86) from four groups of formaldehyde-exposed subjects (Kramps et al., 1989). The subject with detected formaldehyde-specific IgE displayed allergic symptoms. The groups included (1) 28 subjects living or working in places with formaldehyde-containing construction materials (e.g., chipboard) and estimated formaldehyde concentrations ranging from 0.08 to 0.37 ppm, (2) 18 occupationally exposed subjects from an anatomy laboratory and in other unspecified industries where air concentrations were not measured, (3) 12 hospital attendants who worked with formaldehyde-sterilized hemodialysis equipment, and (4) 28 hemodialysis

patients coming into contact with equipment sterilized with formaldehyde. Other subjective symptoms, such as headache, eye irritation, and respiratory complaints, were reported by 24/28 subjects in the construction material group and confirm that formaldehyde is an irritant (reviewed in Section 4.1.1.1). Durations of exposure or length of employment were not reported for the subjects in this study.

Grammer et al. (1990) studied the immunologic nature of formaldehyde sensitivity in 37 workers who were examined by a group of physicians in response to complaints of formaldehyde-related illness. Air sampling of formaldehyde ranged from 0.003 to 0.078 ppm, but specific levels were not tied to specific workplace areas. Blood samples were collected and assayed for IgE and IgG activity against formaldehyde-human serum albumin (formaldehyde-HSA) and HSA alone. None of the workers had IgG activity against formaldehyde-HSA. Five workers had comparable IgE activity against both formaldehyde-HSA and HAS that was more than twice the normal control serum levels. No IgE antibodies were detected in the other 32 workers. The authors concluded that there was no evidence of an immunologically mediated response to formaldehyde in this group of workers.

Formaldehyde-specific IgE was not detected in any of a group of 45 medical students before or after the students attended a 4-week anatomy dissecting course (Wantke et al., 1996b). Estimates of ambient air concentrations of formaldehyde ranged from 0.059 to 0.219 ppm (0.124 ± 0.05 ppm; mean ± SD). However, the survey revealed frequencies of irritation symptoms that were consistent with other studies (e.g., itching of the skin in 33/45 students, headache in 15/45, and burning eyes in 13/45).

Similarly, Ohmichi et al. (2006) were unable to correlate formaldehyde exposure with specific IgE production among eight students attending a gross anatomy laboratory. Formaldehyde exposure was estimated to range from 0.33 to 1.47 ppm during the laboratory sessions. The sample size was small, and IgE levels varied substantially (ranging from <19 to >5,000 international units/mL). Compared with IgE levels taken 90 minutes prior to the start of the first session, IgE levels measured shortly after the last session and up to 23 days following the last session showed no association with exposure.

Salkie (1991) investigated the prevalence of formaldehyde-specific IgE in practicing pathologists who complained of formaldehyde sensitivity. Exposure levels were not reported. Serum samples were assayed for total IgE and formaldehyde-specific IgE. Of the 46 subjects, 29 self-reported atopy that was confirmed in 12 subjects by positive IgE. Moreover, 29 subjects complained of formaldehyde-specific sensitivity. However, zero subjects had formaldehyde-specific IgE, and there was no evidence that atopic individuals were more sensitive to

formaldehyde than nonatopic individuals. The authors noted that atopic individuals may have selectively reduced their exposure to formaldehyde.

Vandenplas et al. (2004) evaluated a case study of a 31-year-old male who was accidentally exposed to formaldehyde for 2 hours. The exposure level was not provided. The subject had smoked a pack of cigarettes a day for 13 years and was admitted to the emergency room for asthmatic symptoms. Eight days following exposure, increased levels of formaldehyde-specific IgE antibodies were detected but could not be detected in subsequent assessments.

A clinical study by Liden et al. (1993) evaluated IgE-specific antibodies against formaldehyde in 23 subjects who had previously tested positive for skin sensitization by skin prick test. Subjects were exposed to formaldehyde by skin patch (1% formaldehyde in water). Ten of the subjects were classified as atopic Though 15 of 23 of the sensitized subjects were also sensitive to formaldehyde applied by skin patch, formaldehyde-IgE was positive in 2 of 15 individuals who were not classified as atopic. No dose-response relationship could be determined from the study design of this study.

Doi et al. (2003) conducted a clinical study in 155 children of which 122 were asthmatics. No specific exposure to formaldehyde was documented. IgE against formaldehyde was determined in blood. Formaldehyde-specific IgE was found in two asthmatic children. Thus, while several studies have documented formaldehyde-specific IgE, the occurrence is rare and may be transient. Asthmatic children may be more predisposed to form formaldehyde-specific IgE than nonatopic individuals or adults. The formation of formaldehyde-specific IgE is quite rare.

4.1.1.5.4. *Formaldehyde-albumin and formaldehyde-hemoglobin complexes.*

Numerous studies have shown that formaldehyde can bind to blood proteins such as hemoglobin (Hb) and human serum albumin (HSA) forming formaldehyde-Hb (Bono et al. 2006) and formaldehyde-HSA complexes (Carraro et al., 1997; Grammer et al., 1993, 1990; Dykewicz et al., 1991; Thrasher et al., 1990). Kim et al. (2001) failed to identify IgE against formaldehyde-HSA complexes in one case-control subject following industrial occupational exposure to formaldehyde. These complexes may serve to traffic formaldehyde throughout the bloodstream and throughout the body. While formaldehyde may be too small to engender an immune response, these complexes may be able to trigger formaldehyde-protein-specific antibodies, leading to an immune response, including sensitization.

Thrasher et al. (1990) evaluated five groups of subjects as follows with varying levels and durations of formaldehyde exposure: asymptomatic chiropractic students exposed during

anatomy classes (controls with only intermittent exposure to formaldehyde), mobile home residents, office workers, patients with multiple symptoms who had been removed from the source of formaldehyde for at least a year, and occupationally exposed patients. All groups were assessed for production of IgG, IgM and IgE class of antibodies against formaldehyde-HSA. The level of all classes of autoantibodies was significantly elevated in patients exposed long-term to formaldehyde. From the data, Thrasher et al. (1990) concluded that exposure to formaldehyde stimulates IgG antibody production to formaldehyde-HSA.

Grammer et al. (1990) studied the immunologic nature of formaldehyde sensitivity in 37 workers who were examined by a group of physicians in response to complaints of formaldehyde-related illness. Air sampling of formaldehyde ranged from 0.003 to 0.078 ppm, but specific levels were not tied to specific workplace areas. Blood samples were collected and assayed for IgE and IgG reactivity against HSA alone and formaldehyde-HSA. None of the workers had IgG activity against formaldehyde. Five workers had IgE against both HSA alone and against formaldehyde-HSA complexes. No IgE antibodies were detected in the other 32 workers. The authors concluded that there was no evidence of an immunologically mediated response to formaldehyde in this group of workers.

Grammer et al. (1993) described the evaluation of a worker with bronchospasm symptoms caused by formaldehyde exposure. The worker was evaluated by means of ELISA, cutaneous tests, and methacholine and formaldehyde inhalation challenges. The ELISA showed that the worker had positive IgE and IgG titers to formaldehyde-HSA. The worker also had a positive cutaneous test for formaldehyde-HSA but a negative methacholine challenge at 25 mg/mL and negative formaldehyde inhalation challenges at exposure concentrations of 0.3, 1, 3, and 5 ppm for 20 minutes. The worker might have developed a positive response if a higher concentration of formaldehyde had been used for the challenge, but it is more probable that the worker's symptoms were not caused by immunologically mediated asthma.

Dykewicz et al. (1991) evaluated whether IgE or IgG antibodies to formaldehyde were related to formaldehyde exposure or to respiratory symptoms arising from such an exposure. The authors studied 55 potentially exposed subjects (hospital histology technicians, internal medicine residents, pathology residents, current smokers, and persons with known workplace exposure to formaldehyde) and compared them to controls with no history of formaldehyde exposure. Reported workplace formaldehyde concentrations were 0.2−0.64 ppm for pathology residents, 0.64 ppm for histology technicians, and 0.6−11 ppm for miscellaneous formaldehyde exposure scenarios. Workplace air concentrations were not measured for the other occupations. Occupational exposure to formaldehyde averaged 12.45 years for histology technicians, 0.38 years for medical residents, 3.21 years for pathology residents, and 18.34 years for five

subjects exposed to formaldehyde in miscellaneous workplaces. Blood samples were analyzed for IgE and IgG reactivity with formaldehyde-HSA complexes. Three subjects had IgE against formaldehyde-HSA; these three and two others had low levels of anti-formaldehyde-HSA IgG. The presence of IgG and IgE antibodies to formaldehyde was not clearly related to formaldehyde exposure or pack-years of smoking. One subject had both IgE and IgG antibodies and also suffered from eye and respiratory symptoms when exposed to formaldehyde at his workplace. However, the authors concluded that they could not establish a relationship between IgE and IgG levels and formaldehyde exposure. This study has several limitations. First, the volunteers (hospital workers) may not be representative of exposed workers in the general population. One of the exposure groups comprised cigarette smokers. Although the study focused on formaldehyde-HSA antibodies, which would be unaffected by the other chemicals, respiratory symptoms among smokers would reflect exposures to the constituents of cigarette smoke. Dykewicz et al. (1991) concluded that immunologically mediated asthma caused by formaldehyde is extremely rare and may not exist at all.

Carraro et al. (1997) reported development of a reliable assay to effectively measure formaldehyde-HSA complexes in smokers, ex-smokers, and nonsmokers. A correlation between formaldehyde-HSA antibodies and smoking status was detected with highest percentage of individuals for polyclonal antibodies detected in smokers and lowest percentage detected in nonsmokers . This study did not correlate formaldehyde exposure and formaldehyde-HSA antibodies.

Given that formaldehyde is a sensory irritant that is particularly bothersome to individuals with compromised lung function or asthma, numerous studies have assessed the ability of formaldehyde to induce immunotoxic effects (Wieslander et al . 1997; Norback et al . 1995; Grammer et al . 1993; Pross et al . 1987). Some studies have documented increased rates of chronic bronchitis and upper respiratory tract infections associated with exposure to formaldehyde, which suggests a possible immunomodulatory effect (Gorski and Krakowiak 1991; Krzyzanowski et al . 1990; Malaka and Kodama 1990; Holness and Nethercott 1989; Tuthill 1984). However, of the numerous articles that have investigated systemic immunomodulatory effects due to formaldehyde (Lyapina et al., 2004; Gorski et al., 1992; Thrasher et al., 1990, 1988, 1987; Pross et al., 1987), few have reported significant immune modulation related to formaldehyde exposure (Thrasher et al., 1990, 1988, 1987; Pross et al., 1987). Significant decreases in specific adaptive immune cell populations do not appear correlated to formaldehyde exposure (Erdei et al., 2003; Gorski et al., 1992; Thrasher et al., 1990, 1987; Pross et al., 1987). Thus, the tendency for increased infection rates associated with formaldehyde may not be related to altered immune function. Perhaps altered mucociliary

clearance and disturbed mucosal barrier may provide greater access for pathogens and result in greater infection rates. Moreover, formaldehyde has been associated with exacerbation of asthmatic or atopic responses, particularly in sensitized individuals. However, this effect does not appear to occur by increased IgE or formaldehyde-specific IgE levels (Ohmichi et al., 2006; Palczynski et al., 1999; Krakowiak et al., 1998; Wantke et al., 1996b; Salkie, 1991; Grammer et al., 1990; Kramps et al., 1989). Thus, formaldehyde-associated enhanced allergic responses does not appear to be due to direct induction of sensitization and may not occur via an immunologic mechanism. Lastly, the formation of formaldehyde-heme and formaldehyde-HSA complexes has been well documented (Grammer et al., 1993, 1990; Dykewicz et al., 1991; Thrasher et al., 1990) and may serve as a biomarker of exposure (Carraro et al., 1997). Moreover, these complexes may provide a means by which formaldehyde travels throughout the bloodstream and may drive antibody formation that may lead to immune activation.

4.1.1.6. *Neurological/Behavioral*

There is some suggestion of neurological impairment in humans following occupational exposure to formaldehyde; the data are limited and the results from several studies are potentially confounded by exposure to other solvents. Two studies of histology technicians with occupational exposure to formaldehyde and other solvents found neurological deficits and poorer performance on neurocognitive tests associated with formaldehyde exposure (Kilburn et al., 1987, 1985). In another study, Kilburn and Warshaw (1992) found no change from initial performance, for as long as 4 years, in follow-up evaluations of histology technicians with continuing exposure to formaldehyde. In a preliminary report from a prospective study, Weiskopf et al. (2009) found a strong association between duration of formaldehyde exposure and death from amyotrophic lateral sclerosis (ALS). In a controlled exposure study, Bach et al. (1990) found that, when workers with chronic formaldehyde exposure were challenged with an acute formaldehyde exposure, they exhibited poorer performance on some neurocognitive tests compared with workers without chronic exposure undergoing the same acute challenge conditions. In another controlled exposure study, Lang et al. (2008) found equivocal changes in reaction time following an acute exposure.

4.1.1.6.1. *Epidemiological studies.*

Kilburn et al. (1985) reported that a group of 76 female histology technicians displayed statistically significantly greater frequencies of neurobehavioral deficits (lack of concentration and loss of memory, disturbed sleep, impaired balance, variations in mood, and irritability), than did a referent group of 56 unexposed female clerical workers. The technicians had been

employed from 2 to 37 years (mean 12.8 years). Analysis of workplace air samples indicated the presence of several solvents, ranging from 0.2 to 1.9 ppm for formaldehyde, 3.2 to 102 ppm for xylene, 2 to 19.1 ppm for chloroform, and 8.9 to 12.6 ppm for toluene. Subsequently, Kilburn et al. (1987) administered a battery of 10 tests to 305 female histology technicians to assess various aspects of cognitive and motor function. The researchers analyzed the results by regression analysis with age, years of smoking, and hours per day of exposure to formaldehyde and other solvents as explanatory variables. Increased daily hours of exposure to formaldehyde were significantly correlated with decreased performance in several tests (including several types of memory, dexterity, and balance), whereas hours of daily exposure to other solvents were only correlated with decreased performance in a single memory test. In a later prospective study of performance, 318−494 histology technicians were tested in a battery of neurobehavioral tests, and testing for a subset of subjects was repeated yearly for up to 4 years. No statistically significant decrement in performance was found when initial test results were compared with retest results to evaluate effects of continuing occupational exposure to formaldehyde (or other solvents) or possible effects of aging (Kilburn and Warshaw, 1992). Kilburn (1994) later reported that three anatomists and one railroad worker, occupationally exposed to airborne formaldehyde for 14−30 years, each showed impaired performance on several neurobehavioral tests (e.g., choice reaction time, abnormal balance, digit symbol, and perceptual motor speed).

Weisskopf et al. (2009) evaluated the association between chemical exposure and death from ALS, using the cohort of 987,229 people from the prospective Cancer Prevention Study II of the American Cancer Society. From 1989−2004, 1,156 deaths from ALS were identified from mortality records from the National Death Index. Exposure assessment occurred prior to follow-up and was based on a questionnaire; participants were asked about current exposure to 12 types of chemicals and whether they had been regularly exposed in the past. Exposure was evaluated by duration (in years), as information regarding exposure levels was not available. After controlling for a number of potentially confounding factors (including age, sex, smoking status, military service, education, alcohol intake, occupation, vitamin use, and exposure to other chemicals), it was found that exposure to formaldehyde for a known duration was statistically significantly associated with increased risk of death from ALS ($p < 0.0001$) with a relative risk (RR) of 2.47 (95% CI: 1.58−3.86) based on 22 deaths. Weisskopf et al. (2009) reported that the association had a strongly significant dose-response relationship, with increased duration of exposure associated with increased RR of ALS mortality with a reported p value for continuous trend of 0.0004. Multivariate adjusted rate ratios were 1.5 for known formaldehyde exposures less than 4 years, 2.1 for 4−10 years, and 4.1 for >10 years. Although the authors indicated that

these results need independent verification, the results of this study of the nearly one million people followed for 15 years is unlikely to be biased due to its longitudinal design.

4.1.1.6.2. *Controlled exposure studies.*

Bach et al. (1990) examined whether cognitive and motor performance of humans responded acutely to formaldehyde exposure and whether previous chronic exposure to formaldehyde affected the responses observed following acute exposure. Thirty-two men with at least 5 years of occupational exposure to formaldehyde and 29 matched controls were exposed to formaldehyde at concentrations of 0.04, 0.21, 0.48, or 1.10 mg/m^3 (32, 170, 390, or 890 ppb) for 5.5 hours. During the exposure period, symptoms were assessed by using a standardized questionnaire, and subjects were evaluated in four tests designed to estimate several aspects of cognitive function. Testing was performed once prior to exposure and twice during the exposure period. The authors noted that the typical dose-related symptoms of respiratory irritation were not seen in this study. Previously unexposed subjects reported more headaches, "heavy head," and physical tiredness than the exposed workers ($p < 0.025$). In both occupationally exposed and unexposed subjects, decreased performance in an addition test was significantly correlated with increasing concentration of formaldehyde (decreased number of additions, $p < 0.025$; increased number of errors, $p < 0.05$). Compared with previously unexposed subjects, occupationally exposed subjects showed significantly decreased performance in three other tests (digit symbol test [pooled exposure groups, $p < 0.025$]; digit span [total digit sum], $p < 0.025$; graphic continuous line test, $p < 0.05$), although the effect was not dose related. The study did not adjust for several potential confounders, including prior exposure to other chemical agents, and the age and health status of the cases and controls. Authors concluded that their data indicated that acute exposure to formaldehyde might cause acute effects on CNS functions at exposures of 0.40 and 1.2 mg/m^3 (equivalent measured doses of 390 or 890 ppb, with a NOAEL of 170 ppb), but that more investigation was needed to verify their results.

In a study evaluating chemosensory irritation, Lang et al. (2008) assessed possible changes in reaction time during an acute (4-hour) exposure to formaldehyde concentrations between 0−0.5 ppm (some exposure sessions also included short concentration peaks of up to 1 ppm) with or without a masking agent (ethyl acetate). Twenty-one healthy volunteers were exposed once per day to each of 10 different exposure combinations in random order (for a total of 10 sessions per subject). Reaction time was tested before and after each exposure session. Significant increases in reaction time were seen at 0.3 ppm formaldehyde, with or without masking agent, but not at 0.5 ppm. The significance of these findings is unclear.

Performance of 16 healthy volunteers on addition, multiplication, and card punching tasks was measured by Andersen and Molhave (1983) before and during a 5-hour exposure to formaldehyde at concentrations up to 2 mg/m^3. The authors reported that formaldehyde exposure had no effect on performance, but results were not presented.

4.1.1.6.3. *Summary.*

The limited information currently available from human studies does not permit a definitive conclusion regarding an association between formaldehyde exposure and human neurotoxicity. There is, however, sufficient information to raise a serious concern for this type of effect, and additional studies are needed.

4.1.1.7. *Developmental and Reproductive Toxicity*

Epidemiologic studies suggest a convincing relationship between occupational exposure to formaldehyde and adverse reproductive outcomes in women. Several of these studies deal with spontaneous abortion following maternal occupational formaldehyde exposure (Taskinen et al., 1999, 1994; John et al., 1994; Seitz and Baron, 1990; Hemminki et al., 1985, 1982; Axelsson et al., 1984), but not all reported a significant association between exposure and spontaneous abortion. A study of fecundability found an increase in time to pregnancy among female workers exposed to formaldehyde (Taskinen et al., 1999). Three studies that examined the effect of occupational exposures on the incidence of congenital malformation produced mixed results (Dulskiene and Gražulevičiene, 2005; Taskinen et al., 1994; Hemminki et al., 1985). A population-based, semiecologic study found an association between atmospheric formaldehyde exposure and low birth weight (Gražulevičiene et al., 1998).

4.1.1.7.1. *Spontaneous abortion.*

Several epidemiologic studies report a relationship between occupational exposure to formaldehyde and increases in risk of spontaneous abortion following maternal occupational formaldehyde exposure (Taskinen et al., 1999, 1994; John et al., 1994; Seitz and Baron, 1990; Axelsson et al., 1984). Increased RRs were in the range of 1.7 to more than 3.0. However, other studies (Hemminki et al., 1985, 1982) found no association between occupational formaldehyde exposure and spontaneous abortion. Paternal occupational exposure to formaldehyde was not related to spontaneous abortion (Lindbohm et al., 1991).

The earliest report of an association between spontaneous abortion and formaldehyde exposure comes from a Swedish cohort study of female laboratory workers (Axelsson et al., 1984). Subjects were women born in 1935 or later and worked in a university laboratory during 1968−1979. There were 745 women who responded to a mailed questionnaire (response

rate = 95%), 556 of whom reported on 1,180 pregnancies that resulted in 997 births. Exposure to formaldehyde was estimated based on answers to the questionnaires. Formaldehyde exposure was reported only in connection with 10 pregnancies, of which 5 went to term, 3 were reported as miscarriages, and 2 were terminated by induced abortion. Excluding the latter, the spontaneous abortion rate among women exposed to formaldehyde in the first trimester was 3/8 (37.5%) compared with 14/148 (9.5%) in the population of laboratory workers not exposed to any solvent in the first trimester.

While not computed by the authors, the OR can be calculated as 5.7 (95% CI: 1.2−26.6). The exposure assessment on which this result is based was methodologically weak but unlikely to be a source of bias. Given the exploratory nature of this study, potential confounders were not controlled for, but no other coexposure was more strongly related to the increased risk of miscarriage, so this result is not likely to be explained by confounding. Selection bias is also an unlikely explanation given the high participation rate. However, although this association of an increased risk of pregnancy loss with formaldehyde exposure is statistically significant, the CI is wide and chance may be a possible explanation for this finding.

In a 1988 Health Hazard Evaluation, the National Institute for Occupational Safety and Health (NIOSH) investigated complaints of adverse reproductive outcomes at a plant where work pants were cut and sewn with a fabric that was treated with a resin that releases formaldehyde (Seitz and Baron, 1990). In a NIOSH laboratory, the fabric released 163 to 1,430 μg of formaldehyde/gram of cloth. TWA personal breathing space formaldehyde levels ranged from trace to 0.46 ppm, while workstation values ranged from 0.32 to 0.70 ppm. The investigators studied the outcomes by using a mailed questionnaire. The response rate for current employees was 98%. There were 296 pregnancies among a cohort of 188 women. The investigators found increased rates of spontaneous abortion, premature birth, and congenital malformations. The crude rate of spontaneous abortion was 21% among women working at the plant while pregnant (4 of 19 pregnancies), 15% among women employed elsewhere while pregnant (11 of 71 pregnancies), and 5% among women at home while pregnant (10 of 206 pregnancies). The investigators did not explain how workers employed elsewhere or at home during pregnancy were categorized compared with current workers, nor did they calculate RRs. As calculated from data presented in Table 5 of the monograph, the crude OR (not corrected for multiple observations per woman) for those pregnant while currently working at the plant compared with all others was 3.2 (95% CI: 0.8−12). There were also excess congenital malformations (13 versus 2%) and premature births (13 versus 4%) among the live births (both based on two births each in the exposed group) from the women who were pregnant while

employed at the textile plant compared with women who stayed at home. After the NIOSH investigation, changes were made in the plant to improve ventilation.

Because the report provides insufficient details of the methodology and the fact that there was no personal exposure classification in this study, it is difficult to validate the findings in this report. The results did not take into account other potential risk factors for spontaneous abortion or correct for multiple pregnancies per woman. Furthermore, the marked differences between the "home" and "work" pregnancies were difficult to interpret.

A cohort study of effects of paternal occupational exposures in Finland by Lindbohm et al. (1991) found that exposure to formaldehyde had little effect on the rate of spontaneous abortions among 99,186 pregnancies listed in the national hospital discharge register. The analysis was limited to births/spontaneous abortions in 1976 and from May 1980 to April 1982. Spontaneous abortion incidence came from the hospital discharge register and data from outpatient clinics. There were 808 pregnant wives among potentially formaldehyde-exposed fathers. Exposure to formaldehyde was based on employment information listed in the Finnish 1980 census. Compared with pregnancies among wives of unexposed spouses, the age and socioeconomic level-adjusted ORs were 1.1 for low paternal exposure to formaldehyde and 1.0 for moderate to high paternal exposure. Paternal occupational exposures to ethylene oxide, gasoline/benzene, and rubber industry chemicals were associated with spontaneous abortion. The authors hypothesized that the mode of action (MOA) for spontaneous abortion following male exposure to chemicals is genetic damage to germ cells.

The indirect exposure assessment was a substantial limitation of this study. Some confounders in a study of this type could not be controlled for (smoking history, previous spontaneous abortions, alcohol use), and census data could not provide completely accurate information, potentially masking associations between paternal formaldehyde exposure and spontaneous abortion.

A case-control study by Taskinen et al. (1994) of effects of maternal occupational exposure to chemicals used in laboratories in Finland indicated that exposure to formalin, which is a 37% aqueous solution of formaldehyde, was related to an increased risk of spontaneous abortion. The investigators studied subjects from payrolls of Finnish state-employed laboratory workers, the laboratory workers' union, and a register of workers occupationally exposed to carcinogens. These records were cross-referenced with the hospital discharge register. The investigators selected women who had a single spontaneous abortion during the period 1973−1986 and two controls who had delivered a baby without malformations. The final sample size was 208 cases and 329 controls after refusals and other exclusions. The response rate was 82.4%.

Information on occupational exposure, health status, medication, contraception, and pregnancy history came from mailed questionnaires. Industrial hygienists' construction of an exposure index was based on the subjects' descriptions of their work assignments, use of solvents including estimates of quantity used, and use of a fume hood. ORs were adjusted for employment, smoking, alcohol consumption, parity, previous miscarriage, birth control failure, febrile disease during pregnancy, and other organic solvents found in laboratory work. Spontaneous abortion was associated with 3−5 days per week of formalin exposure (OR 3.5 [95% CI: 1.1−11.2]). A contemporaneous study of formaldehyde concentrations in similar Finnish workplaces (pathology and/or histology laboratories) reported workroom air to range from 0.01 to 7 ppm with a mean of 0.45 ppm formaldehyde (Heikkilä et al., 1991 [as cited in Taskinen et al., 1994]) and that the highest exposures occurred during emptying of sample containers, dish washing, and preparation of formaldehyde solution.

Although the results of this study indicate an increased risk between spontaneous abortion and exposure to formaldehyde/formalin, the women were also exposed to several chemicals concurrently, of which toluene (OR 4.7 [95% CI: 1.4−15.9]) and xylene (OR 3.1 [95% CI: 1.3−7.5]) were also significantly associated with the incidence of spontaneous abortion. However, the investigators reported that the women were more likely to be coexposed to formalin and xylene, which would make confounding by toluene less likely, and, since xylene was not as strongly associated with the outcome as was formaldehyde, it too is unlikely to fully explain the reported relationship between formaldehyde and increased risk of spontaneous abortion. While it is possible that exposure misclassification may have occurred because of the indirect assessment of workplace chemical exposure, an overall conclusion is that, since the exposure assessment was conducted by industrial hygienists, it is unlikely that this form of bias will have impacted the results of the study to any great extent.

In a U.S. study (John et al., 1994), the results of a case-control study of cosmetologists also supported an association between spontaneous abortion and the use of formaldehyde-based disinfectants. The study population came from the 1988 North Carolina cosmetology license registry. Women on this list who were 22−36 years of age were screened to find those who were recently pregnant. The cases were full-time cosmetologists who experienced a spontaneous abortion before gestational week 20 during 1983−1988. The most recent spontaneous abortion was used as the reference case. Controls were full-time cosmetologists who delivered a live infant during the same time period.

Information was based on mailed questionnaires. Women were not told the purpose of the study in order to avoid selection and recall bias. Of 8,356 women who received the screening questionnaires, 72.5% responded. Of those, 1,696 qualified for the study and 73.6%

completed a more detailed questionnaire. Among them, 96 women were "absolutely sure" they had a spontaneous abortion and qualified as cases. There were 1,058 live births that qualified as controls. Exposure assessment included identification of disinfectants used as well as types of chemicals used on hair, use of gloves, hours worked, number of procedures involving chemicals, and use of manicure products. Presence of formaldehyde in the cosmetology profession in general was confirmed in two NIOSH hazard reports (Almaguer and Klein, 1991; Almaguer and Blade, 1990). ORs were adjusted for age, smoking, pregnancy characteristics, other jobs, hours worked, education (cosmetology school), hours standing per week, number of chemical procedures per week, hair dyes per week, bleachings per week, permanents per week, use of gloves, beauty salon characteristics, and use of alcohol or formaldehyde disinfectants.

Among women who worked full time in cosmetology (61 cases and 315 controls), the crude odds ratio for use of formaldehyde-based disinfectants was 2.0 (95% CI: 1.1-3.8). In models adjusted for maternal characteristics and other workplace exposures, the odds ratio remained elevated (OR = 2.1 [95% CI: 1.0−4.3]) indicating a lack of confounding by other covariates. Other chemical exposures were also associated with spontaneous abortion, including number of chemical services per week, hair dyes, bleaches, and alcohol-based disinfectants. Strengths of this study include adjustment for important confounding risk factors for spontaneous abortion, detailed collection of interview-based information, a favorable response rate, and the fact that the index population had a high likelihood of formaldehyde exposure. These data provide overall support for an association between formaldehyde exposure and spontaneous abortion.

In a retrospective cohort study by Taskinen et al. (1999) of female woodworkers in Finland, exposure to formaldehyde was associated with delayed conception and spontaneous abortion. The subjects, recruited from a woodworkers' union and other businesses involving wood processing, were linked to a national register of births. Women were included if they were born between 1946 and 1975, had a live birth at age 20−40 years during 1985−1995, had worked in the wood processing industry for at least 1 month, and had first employment in the wood processing industry beginning at least 6 months before the index pregnancy. The first pregnancy that fulfilled the above criteria was the index pregnancy. There were 1,094 women with these criteria. Information about personal characteristics, pregnancies, and exposures were collected from mailed questionnaires for which the response rate was 64%. After other exclusions (primarily infertility history, unknown time to pregnancy, and contraceptive failure), the final sample included 602 women.

Estimates of mean daily exposure to formaldehyde were based on measurements taken at the women's factories of employment during the early 1990s. Where measurements were

unavailable, measurements from equivalent industries were used. An exposure index representing a TWA exposure was established for every person in the study based on the concentration of workplace formaldehyde multiplied by the proportion of the workday exposed to formaldehyde. The investigators categorized TWA formaldehyde exposure into categories of low (mean of 18 ppb), medium (mean of 76 ppb), and high (mean of 219 ppb) exposure.

Time-to-pregnancy data were analyzed by a discrete proportional hazards regression procedure with, as the outcome, a fecundability density ratio (FDR), in which a ratio of average incidence densities of pregnancies for exposed women was compared with that of the employed, unexposed women. As explained by Taskinen et al. (1999), an FDR significantly below unity suggests that conception was delayed. The age-, employment-, smoking-, alcohol consumption-, parity-, and menstrual irregularity-adjusted FDR was 0.64 (95% CI: 0.43−0.92) for women exposed to high formaldehyde levels compared with the unexposed controls, indicating that there was a substantial delay in time to conception in this group of women. Among a subset of women with high exposure who did not use gloves, the FDR was even lower (0.51 [95% CI: 0.28−0.92]), suggesting that these results might be explained in part through dermal contact with formaldehyde or might indicate an individual's failure to follow appropriate precautions, which might have increased inhalation exposures in other ways. Exposure to solvents, wood dust and other dusts, and phenols was not associated with decreased fecundability.

The investigators further conducted an analysis of the risk of spontaneous abortion after carefully including only women who had the same workplace during the year of the spontaneous abortion as they had during the beginning of the time-to-pregnancy period. Spontaneous abortion was associated with formaldehyde exposure in the low exposure group (OR = 2.4 [95% CI: 1.2−4.8]), in the medium exposure group (OR = 1.8 [95% CI: 0.8−4.0]), and in the high exposure group (OR = 3.2 [95% CI: 1.2−8.3]). Endometriosis was also associated with the highest formaldehyde level (OR = 4.5 [95% CI: 1.0−20.0]).

This study by Taskinen et al. (1999) was a well-designed population-based case-control study that appears to have been well executed and appropriately analyzed. The study population of Finnish women was well defined and adequately selected so as to allow for meaningful comparisons of health effects between individuals with different levels of exposure to formaldehyde. The participation rate was 64%, which is low enough to raise a concern about the potential for selection bias. However, the authors noted that selection bias has not influenced the results of other reproductive epidemiology studies reporting results on smoking, irregular menstruation, and earlier miscarriages, which are known to lengthen the time to pregnancy (Bolumar et al., 1996; Sallmén et al., 1995; Baird and Wilcox, 1985). Furthermore, there is no evidence to support conjecture that an individual's decision to participate in this study would be

differential with respect to their workplace formaldehyde exposures while being nondifferential with respect to the other exposures of interest, including organic solvents, wood dust, and phenols. Since the women who chose to participate in this study were not likely to be aware of the specific hypotheses under investigation, nor could they have known the formaldehyde exposures that were independently estimated by an industrial hygienist, selection bias is not a likely explanation for the findings of adversity.

Data on pregnancy history, including spontaneous abortions, were collected by questionnaire. Spontaneous abortion is the most common adverse outcome of pregnancy (Klein et al., 1989), and retrospective self-report of spontaneous abortion has been found to match well with prospectively collected reproductive histories (Wilcox and Horney, 1984). Many spontaneous abortions, however, are missed with self-reporting with the magnitude likely exceeding 25%, but only rarely do women self-report false positive events (Wilcox and Horney, 1984). The effect of such an undercount is to cause a bias towards the null when the likelihood of undercounting is unrelated to formaldehyde exposure. The implication is that the observed association of increased risk of spontaneous abortion associated with occupational exposure to formaldehyde may be an underestimation of the true risk.

Two studies (Hemminki et al., 1985, 1982) specifically assessed the effects of formaldehyde exposure and reported no significant increase in the risk of spontaneous abortion. Hemminki and colleagues (1982) conducted a retrospective cohort study of nurses who were potentially exposed to chemical sterilizing agents, including formaldehyde, ethylene oxide, and glutaraldehyde. The risk of having a spontaneous abortion among the women on the sterilizing staff was compared with that among the control population of nursing auxiliaries whom the supervisory nurses thought to be unexposed to the chemical sterilizing agents during the previous three decades. However, no measurements of the chemical sterilizing agents were taken. Information about exposure to chemical sterilizing agents was obtained from the supervising nurses. When the women were conducting sterilizing procedures during their pregnancies, the frequency of spontaneous abortion was 15.1% compared with 4.6% for the nonexposed pregnancies among the sterilizing staff. The increased frequency of spontaneous abortion correlated with exposure to ethylene oxide but not with exposure to glutaraldehyde or formaldehyde. The investigators reported that ethylene oxide concentrations have been measured in many sterilizing units in Finnish hospitals; 8-hour weighted mean concentrations have ranged from 0.1 to 0.5 ppm with peak concentrations up to 250 ppm (measurements by the Finnish Institute of Occupational Health) (Hemminki et al, 1982). No measurements of glutaraldehyde concentrations were available. Hemminki et al. (1982) reported that exposure to formaldehyde in the sterilization units may be minimal, particularly when gas chambers are used.

The range of formaldehyde concentrations measured in sterilizing units has been reported as 0.03−3.5 ppm.

It is not clear that the unexposed women who served as controls were an appropriate comparison group to the sterilizing staff. The investigators reported that, among the sterilizing staff, those women who were unexposed during pregnancy experienced a rate of spontaneous abortion of 4.6% but that, among the comparison population of nursing auxiliaries who were presumed to be unexposed, the rate of spontaneous abortion was 10.5%. Had the nursing auxiliaries been an appropriate comparison group, it would be expected that their rate of spontaneous abortion would be similar to the unexposed sterilizing staff. Given this anomaly in study design and the unknown concentrations of formaldehyde exposure that were assessed as positive or negative by supervisory nurses regarding occupational exposures in the previous 30 years, it is concluded that this report of no association between formaldehyde exposure and the risk of spontaneous abortion does not temper the conclusion that formaldehyde exposure has been shown to increase the risk of spontaneous abortion.

A second study by the same lead author (Hemminki et al., 1985) used a different study design to reassess the hypothesis that chemical exposures common in the field of nursing could be risk factors for spontaneous abortion. This case-control study found no increase in the risk of spontaneous abortion associated with exposure to formaldehyde. The head nurses at each hospital were asked by the investigators whether each case or control had been exposed to formaldehyde during a given 3-month period corresponding to the first trimester of a study participant's pregnancy during 1973−1979. Formaldehyde exposure was assessed as positive or negative for either use as a sterilizing agent or use of sterilized instruments. The reported crude OR for formaldehyde exposure was 0.6; no CIs were provided. From the data reported in Table 2 in Hemminki et al. (1985), the unadjusted OR and its CI can be computed post hoc as OR (0.70 [95% CI: 0.28−1.73]). The authors acknowledged that the study failed to distinguish between sterilizing work and the use of sterilized instruments, where only very small exposures could be expected. Given the likelihood of extreme exposure misclassification and the presentation of only crude results without control of potential confounding for formaldehyde, these results do not appear to be exculpatory of a true causal association between formaldehyde exposure and the risk of spontaneous abortion.

A meta-analysis of formaldehyde exposure and spontaneous abortion was conducted by Collins et al. (2001b). However, the published results should be interpreted with caution. This meta-analysis included one very large null study of paternal formaldehyde exposure along with seven studies of maternal exposure. The two null studies by Hemminki et al. (1985, 1982) were also included without consideration of the potentially extreme exposure misclassification that

may have attenuated any true adverse effect. Nevertheless, the overall reported meta-analytic RR for parental formaldehyde exposure based on eight maternal and paternal exposure studies was 1.6 (95% CI: 0.9−2.7). For case-control studies the RR was 1.8 (95% CI: 0.7−4.8), and for cohort studies the RR was 1.7 (95% CI: 1.2−2.3). Collins et al. (2001b) argued that the method of exposure evaluation may have influenced the observed results; they stated that several of the studies whose exposures were based on the investigator's judgment were likely misclassified, which may have obscured the true relationship, while other studies that assessed exposure based on self-reporting could have suffered from recall bias. They report that RRs were higher for studies based on self-reported exposures (RR = 1.9 [95% CI: 1.3−2.6]) than those based on objective exposure assessments (RR = 1.5 [95% CI: 0.6−3.7]) and suggested that this difference might reflect recall bias in the exposure assessment. However, for recall bias to have been operable in these studies, the women who provided self-reported data on pregnancy history and occupational exposure would have had to appreciate that the hypothesis of interest was the specific effect of formaldehyde on the risk of spontaneous abortion. In the specific case of the study by Taskinen and colleagues (1999), the investigator also looked at the effects of other exposures, such as organic solvents, dust, and phenols, and did not report adverse effects. It is therefore unlikely that the women providing exposure data were doing so in a manner indicative of recall bias. If the supposition of nondifferential misclassification error in exposure is indeed correct, the observed results of the meta-analysis would likely have been biased towards the null. Therefore, the true RR for maternal formaldehyde could be higher than Collins et al. (2001b) reported and would likely be statistically significant. Had the study of paternal exposure been set aside, the meta-analysis almost surely would have shown a statistically significant increase in the risk of spontaneous abortion associated with maternal formaldehyde exposure. This single study reported a null finding based on exposure assessment from census records of employment, and, as the largest of the studies in the meta-analysis, it contributed the greatest weight.

Lastly, Collins and coworkers (2001b) suggested that there were potential confounding factors in each of the workplaces that might have produced the observed findings of increased risk of spontaneous abortion associated with formaldehyde. While each of these occupational studies focused on women who were coexposed to formaldehyde and other chemicals, the occupational groups were quite different and had different sets of coexposures. The woodworkers in the Taskinen et al. (1999) study were potentially coexposed to organic solvents related to painting and lacquering, dusts, and phenols, none of which was shown to be an independent predictor of adverse risk. The cosmetologists studied by John et al. (1994) were coexposed to hair dyes, bleach, alcohol-based disinfectants, and chemicals specific to services, such as fingernail sculpturing, but, in analyses that were specifically adjusted for other work

exposures and their potentially confounding effects, the investigators reported an OR of 2.1 (95% CI: 1.0−4.3) for the use of formaldehyde-based disinfectants. The laboratory workers studied by Axelsson et al. (1984) were potentially coexposed to a wide range of solvents, but the miscarriage rate was highest among those exposed to formaldehyde, and, for a potential confounder to entirely explain an observed effect of another exposure, it must be more strongly associated with the adverse outcome.

It does not appear that the collective results of formaldehyde exposures associated with increased risk of spontaneous abortion—often in spite of exposures being crudely measured—can be explained by information bias or confounding.

The findings by Taskinen et al. (1999) of reduced fertility and increased risk of spontaneous abortion are internally consistent and coherent with other reports of increased risk of pregnancy loss associated with exposure to formaldehyde (John et al., 1994; Taskinen et al., 1994; Seitz and Baron, 1990; Axelsson et al., 1984). Absent evidence of alternative explanation for these findings, it is concluded that exposure to formaldehyde is associated with pregnancy loss and diminished fertility.

4.1.1.7.2. ***Congenital malformations.***

Only three studies have reported on the epidemiologic evidence of an association between formaldehyde exposure and the risks of births having congenital malformations. In the earliest study by Hemminki et al. (1985), the investigators presented an analysis of 34 congenital malformations from the Finnish Register of Congenital Malformations and compared them with a group of 95 controls from those used in the larger study. An association was found between formaldehyde exposure and malformations based on three exposed cases (OR = 1.8).

The case-control study by Taskinen et al. (1994) of effects of occupational exposure to chemicals used in laboratories in Finland examined the potential effects of exposure to formalin on both spontaneous abortions and congenital malformation. The investigators reported on a study of 36 laboratory workers with a child registered in the Finnish Register of Congenital Malformations and 105 controls. There was no association between formalin and congenital malformations.

A Lithuanian study (Dulskiene and Gražulevičiene, 2005) for which only a brief summary is available in English investigated the risk of congenital heart malformations as a result of exposure to 43 different agents. The number of births included in the study was not given in the English abstract. Exposure to residential ambient formaldehyde concentrations of >2.42 μg/m^3 (0.002 ppm) was associated with a 24% increase in the risk of congenital heart malformations (OR = 1.24 [95% CI: 0.81−2.07]). The details of this study are unavailable in

English translation, making it impossible to critically analyze details, such as coexposure and other possible confounders.

4.1.1.7.3. *Low birth weight.*

A case-control study by Gražulevičiene et al. (1998) examined the association of low birth weight (<2,500 grams) and air pollutants, including formaldehyde, particulates, sulfur dioxide, lead, ozone, and nitrogen dioxide, measured in 12 areas in the city of Kaunas, Lithuania. This city has conducted environmental pollutant measurements since 1993, and the investigators classified formaldehyde exposure based on the area of residence of the study subjects. Formaldehyde levels in the 12 districts of Kaunas in 1994 ranged from 1.36 to 5.28 $\mu g/m^3$ (0.0011−0.0043 ppm), with a citywide average of 3.14 $\mu g/m^3$ (0.0026 ppm). Information on infants came from a birth registry. There were 244 cases of low birth weight and 4,089 normal controls born in 1994. Personal data came from record-based prenatal interviews, and pregnancy data came from hospital records.

The crude RR of low birth weight among women exposed to the highest airborne formaldehyde level was 1.68 (95% CI: 1.24−2.27). After adjustment for age, occupation, hazardous work, education, marital status, smoking, hypertension, and other air pollutants, the OR was still elevated but no longer statistically significant (OR 1.37 [95% CI: 0.90−2.09]). Although formaldehyde exposure was the only single air pollutant associated with low birth weight, factors such as smoking, marital status, and pregnancy-related factors had more of an impact on birth weight. Total suspended particulates (OR 2.58 [95% CI: 1.34−4.99]) and hazardous work (OR 2.62 [95% CI: 1.12−6.10]), which was not defined by the authors, were also related to low birth weight.

Aside from studies of birth weight deficits from tobacco smoke and occupational exposure, the literature on exposure to ambient air pollutants to support the investigators' hypothesis is limited. The strength of the association between total suspended particulates and low birth weight supports the idea that incidence of birth weight <2,500 grams may be related to atmospheric pollution, although this finding may not be specific to formaldehyde. Because of the large number of variables evaluated in the analysis, large fluctuations in the atmospheric formaldehyde measurements, coexposure to other pollutants, and geographic variability of low birth weight, it is difficult to estimate the impact of formaldehyde alone on low birth weight.

4.1.1.7.4. *Summary.*

Although all studies on potential developmental toxicity of formaldehyde have limitations and do not uniformly report positive results, the associations between spontaneous

abortion, delayed conception, or reproductive outcomes and formaldehyde exposure in multiple studies cannot be dismissed, because several studies report concordant findings across several populations and study methodologies. The results of most of the studies with positive findings were adjusted for many potentially confounding factors that may be related to spontaneous abortion and infertility, including smoking and alcohol use, pregnancy and reproductive history, and other chemical exposures.

The association between fertility and formaldehyde (Taskinen et al., 1999) stands out because of its strong quantitative statistical analysis, adequate sample size, and rigorous exposure assessment. This study was designed to specifically assess the effect of formaldehyde on reproductive outcomes. Furthermore, it was the only study with an exposure assessment based on quantitative measurements from the subject's workplace. Moreover, the investigators conducted a multivariable survival analysis that approximates a longitudinal life table or person-year analysis while simultaneously adjusting for important confounders. The findings were strengthened by statistically significant associations between formaldehyde and spontaneous abortion and endometriosis. The fact that the use of gloves may reduce the reproductive effect of formaldehyde supports the dose-response relationship in this study, and the lack of an association between time to pregnancy and any other workplace exposures strengthens the specificity of formaldehyde effects. The results also support associations reported between formaldehyde and increased risk of spontaneous abortion because subfertility and spontaneous abortion are biologically linked (subclinical pregnancy losses are increased among women with fertility problems) (Gray and Wu, 2000; Hakim et al., 1995), and both subfertility and spontaneous abortion may be related to sensitivity to environmental agents (Correa et al., 1996).

4.1.1.8. *Oral Exposure Effects on the Gastrointestinal Tract*

No human epidemiology studies exist to determine an association between oral exposure of formaldehyde and adverse health effects in the gastrointestinal (GI) tract.

4.1.1.9. *Summary: Noncarcinogenic Hazard in Humans*

Formaldehyde has clearly and consistently been shown to be a potent sensory irritant with a variety of adverse health effects. Eye, nose, and throat irritation as a result of formaldehyde exposure has been documented in a wide range of epidemiologic studies (Ritchie and Lehnen, 1987; Hanrahan et al., 1984; Liu et al., 1991; Kriebel et al., 1993; Horvath et al., 1988; Holmström and Wilhelmsson, 1988; Akbar-Khanzadeh et al., 1994). Workers chronically exposed to formaldehyde have exhibited signs of reduced lung function consistent with BC, inflammation, or chronic obstructive lung disease (Malaka and Kodama, 1990; Herbert et al.,

1994; Alexandersson and Hedenstierna, 1989). A well-conducted residential epidemiology study has convincingly shown a concentration response for decreased pulmonary function among children with increased formaldehyde exposures (Krzyzanowski et al., 1990). Several cross-sectional studies have described associations between increased concentrations of formaldehyde and increased prevalence of asthma (Garrett et al., 1999; Krzyzanowski et al., 1990; Norback et al., 1995; Zhao et al., 2008). In addition, a case-control study that focused on risk factors for the initial physician diagnosis of asthma, which is indicative of atopic switching, has shown concentration-dependent adverse effects associated with formaldehyde exposure (Rumchev et al., 2002).

Results of research on the effects of formaldehyde on tissue histology suggest that formaldehyde is also responsible for reduced mucociliary clearance and the induction of histopathologic lesions in the nose Pazdrak et al., 1993; Holmström et al., 1989; Holmström and Wilhelmsson, 1988; Boysen et al., 1990). In addition, there is evidence of neurological impairment in several studies of formaldehyde-exposed histology technicians, but confounding exposures to other neurotoxic solvents and inconsistent results prevent drawing definitive conclusions concerning the neurotoxicity of formaldehyde from these studies (Kilburn et al.,1985, 1987, 1994; Kilburn and Warshaw, 1992).

Finally, there is epidemiologic evidence that formaldehyde is associated with adverse reproductive outcomes. Four of six occupational studies found an increased risk of spontaneous abortion among formaldehyde-exposed women (Taskinen et al., 1999, 1994; John et al., 1994; Seitz and Baron, 1990; Axelsson et al., 1984). Results of other studies suggested associations among formaldehyde and congenital malformations, low birth weight, and endometriosis (Hemminki et al., 1985; Gražulevičiene et al., 1998; Taskinen et al, 1999). The strongest evidence of an association between formaldehyde and an adverse reproductive outcome came from a well-conducted study of infertility in women employed in the wood processing industry (Taskinen et al., 1999). This study found a greater than threefold increased risk of spontaneous abortion, a nearly 50% decrease in a measure of delayed conception indicating reduced fertility, and increased time to pregnancy associated with average daily formaldehyde exposures of 0.15−1 ppm.

4.1.2. Cancer Health Effects

The potential for an association between formaldehyde exposure and human cancer has been studied by examining mortality statistics for occupationally exposed individuals as well as in case-control studies of specific cancers. Studies which provide evidence for various respiratory tract cancers, lymphohematopoietic cancers, brain cancer, pancreatic cancer, and

other cancers have been published for a range of exposure environments. The following discussion examines the evidence for each cancer type and site. An evaluation is provided regarding the strength of the available epidemiologic evidence for the association between formaldehyde exposure and each cancer.

4.1.2.1. *Respiratory Tract Cancer*

4.1.2.1.1. *Nasopharyngeal Cancer (NPC).*

Nasopharyngeal cancer is a very rare form of cancer. In the United States, the incidence rate has been estimated at 0.7 cases per 100,000 person-years (Lee and Ko, 2005). In contrast, incidence rates for lung cancer are approximately 100 times higher (60-65 per 100,000 person-years). The most common form of nasopharyngeal cancer arises from the epithelial cells lining the nasopharynx. This presentation constitutes between 75 and 100% of all nasopharyngeal cancers. There are two types, squamous cell carcinoma, and nonkeratinizing carcinoma. In the U.S., the 5-year survival rate for nasopharyngeal cancer is about 25% (Burt et al., 1992). Certain exposures have been implicated in its etiology, including Epstein-Barr virus (EBV), wood dust and particles and substances (including formaldehyde) applied to wood as a preservative or insecticide, exhaust fumes, nickel dust from smelting and refining operations, and nitrosamines. The major epidemiologic studies of formaldehyde exposure in relation to nasopharyngeal cancer are summarized in Table 4-2. This table includes cohort studies that reported data pertaining to nasopharyngeal cancer with risk estimates based on more than two observed cases. Table 4-3 summarizes the case-control studies with detailed job history data allowing for characterization of likelihood of formaldehyde exposure

4.1.2.1.1.1. ***Cohort studies of nasopharyngeal cancer.***

Several studies examined exposure to formaldehyde in relation to solid tumor-related mortality risk at 10 production facilities included in the cohort study conducted by the National Cancer Institute (NCI) (Hauptmann et al. 2004; Blair et al., 1987, 1986). This cohort consisted of 25,619 workers from 10 manufacturers of formaldehyde, formaldehyde resins, molding compounds, plastic products, film or plywood who were first employed prior to 1966. Occupational histories from company records and exposure to formaldehyde was estimated for each individual job category from work histories, with calendar-time and plant-specific estimates based on assessments of job titles and tasks associated with those jobs using plant visits by industrial hygienists and monitoring data (Blair et al. 1986; Stewart et al. 1986; Blair and Stewart 1990). Exposures were categorized by highest peak exposure, average intensity of exposure, cumulative exposure and duration of exposure. The highest peak exposure categories were defined as nonexposed, low (>0− <2.0 ppm), medium (2.0− <4.0 ppm), and high (> 4.0 ppm).

Table 4-2. Major cohort studies of formaldehyde exposure and nasopharyngeal cancer (with 2 or more cases)

Reference	Study design	Exposure assessment	Results (number of observed deaths)				
Hauptmann et al. (2004) [Extension of NCI study by Blair et al., 1987, 1986)], United States	Retrospective cohort mortality study of 25,619 workers employed at 10 formaldehyde plants in the U.S. followed from either plant start-up or first employment through 1994. The 10 plants produced formaldehyde (3 plants), molding compounds (3 plants), photographic film (2 plants), plywood (1 plant), and formaldehyde resins (6 plants).	Time-dependent exposure estimates[a] based on job titles, tasks, visits to plants by study industrial hygienists, and monitoring data measurements. Peak exposure = short-term excursions >8-hour TWA formaldehyde intensity and knowledge of job tasks. Workers contributed pre-exposure person time to nonexposed category. RRs were from Poisson regression models, using a 15-year lag to account for tumor latency.	Overall				
			Nonexposed	SMR	1.56	(95% CI: 0.39−23)	(2)
			Exposed	SMR	2.10	(95% CI: 1.05−21)	(8)
			Peak exposure (ppm)				
			0	RR[b]	1.00	(referent)	(2)
			>0 to <2.0		*N*/A		(0)
			2.0 to <4.0		*N*/A		(0)
			4.0 or greater		1.83	Not provided	(7)
			Trend p < 0.001 (Trend on categorical data)				
			Average intensity of exposure (ppm)				
			0	RR[b]	1.00	(referent)	(2)
			≤0.5		*N*/A		(0)
			0.5 to <1.0		0.38	Not provided	(1)
			1.0 or greater		1.67	Not provided	(6)
			Trend p = 0.066 (Trend on continuous data among exposed only)				
			Cumulative exposure (ppm-years)				
			0	RR[b]	2.40	Not provided	(2)
			>0 to <1.5		1.00	(referent)	(3)
			1.5 to <5.5		1.19	Not provided	(1)
			5.5 or more		4.14	Not provided	(3)
			Trend p = 0.025 (Trend on continuous data among exposed only)				
			Duration (years)				
			0	RR[b]	1.77	Not provided	(2)
			>0 to <5		1.00	(referent)	(4)
			5 to <15		0.83	Not provided	(1)
			15 or more		4.18	Not provided	(2)
			Trend p = 0.147 (Trend on continuous data among exposed only)				

Table 4-2. Major cohort studies of formaldehyde exposure and nasopharyngeal cancer (with 2 or more cases)

Reference	Study design	Exposure assessment	Results (number of observed deaths)				
Marsh et al. (2002), Connecticut, United States	Retrospective cohort mortality study of 7,328 workers hired up to 1984 and followed until 1998 in one plant from Hauptmann et al. (2004). Mortality was compared with death rates in two Connecticut counties and the U.S.	Worker-specific exposure[a] from job exposure matrix based on available sporadic sampling data from 1965−1987, job descriptions, and verbal job descriptions by plant personnel and industrial hygienists. Exposures ranked on a 7-point scale with exposure range assigned to each rank. 17% of jobs validated with company monitoring data; remaining 83% based on professional judgment. Assumed pre-1965 exposure levels same as post-1965 levels.	Overall				
			U.S. referent	SMR	4.94	(95% CI: 1.99−10)	(7)
			County referent		5.00	(95% CI: 2.01−10)	(7)
			Short-term worker (<1 year)		5.35	(95% CI: 1.46−14)	(4)
			Long-term worker (<1 year)		4.59	(95% CI 0.95−13)	(3)
			Formaldehyde exposure	SMR	6.03	(95% CI: 2.42−12.42)	(7)
			Duration of formaldehyde exposure (years)				
			0 to <1	SMR	5.84	(95% CI: 1.59−15)	(4)
			1−9		3.17	(95% CI: 0.08−18)	(1)
			10+		12.5	(95% CI: 1.51−45)	(2)
			Cumulative exposure (ppm-years) county				
			0 to <0.004	SMR	3.97	(95% CI: 0.10−22)	(1)
			0.004−0.219		5.89	(95% CI: 1.22−17)	(3)
			0.22+		7.51	(95% CI: 1.55−22)	(3)
			Average intensity exposure (ppm)				
			0 to <0.03	SMR	2.41	(95% CI: 0.06−13)	(1)
			0.03−0.159		15.3	(95% CI: 4.16−39)	(4)
			0.16+		4.13	(95% CI: 0.50−15)	(2)
			Duration of exposure to >0.2 ppm (years)				
			Unexposed	SMR	3.01	(95% CI: 0.36−11)	(2)
			0 to <1		4.81	(95% CI: 0.58−17)	(2)
			1−9		4.04	(95% CI: 0.10−231)	(1)
			10+		27.6	(95% CI: 3.34−100)	(2)

Table 4-2. Major cohort studies of formaldehyde exposure and nasopharyngeal cancer (with 2 or more cases)

Reference	Study design	Exposure assessment	Results (number of observed deaths)				
Marsh et al. (2002), Connecticut, United States (continued)			Duration of exposure to ≥0.7 ppm (years)				
			Unexposed	SMR	3.64	(95% CI: 0.99−9.31)	(4)
			<1		9.51	(95% CI: 1.15−34)	(2)
			1+		11.1	(95% CI: 0.28−62)	(1)
Hayes et al. (1990), United States	Proportionate mortality cohort, *n* = 4,046 male embalmers and funeral directors, died 1975−1985.	Exposure presumed.	Overall	PMR	2.16	(95% CI: 0.59−5.54)	(4)
Hansen and Olsen (1995), Denmark	Proportionate incidence study of 2,041 men with cancer who died between 1970 and 1984, identified from the Danish Cancer Registry and matched with the Danish Supplementary Pension Fund, whose longest work experience occurred at least 10 years before the cancer diagnosis. The SPIR measured the proportion of cases in formaldehyde-associated companies relative to the proportion of cases among all employees in Denmark.	Linked companies through tax records to the national Danish Product Register.	Overall	SPIR	1.3	(95% CI: 0.03−3.2)	(4)

[a]Exposure estimates by Hauptmann et al. (2004) were 10 times higher than those of Marsh et al. (2002, p. 259).
[b]Adjusted for calendar year, age, sex, race, and pay category (salaried versus wage). Confidence intervals not provided by authors, but were described as including 1.0.

Table 4-3. Case-control studies of formaldehyde exposure and nasopharyngeal cancer

Reference, study area	Study design	Exposure assessment	Results			
Olsen et al. (1984) Denmark	Population-based, *n* = 314 cases from Danish Cancer Registry during 1970−1982. Three controls/case sampled with cancer of the colon, rectum, breast, and prostate by age, sex, and year of diagnosis of cases	Employment histories after 1964 from files maintained by Danish Cancer Registry evaluated by industrial hygienists.	Men	OR	0.7	(95% CI: 0.3−1.7)
			Women	OR	2.6	(95% CI: 0.3−22)
Vaughan et al. (1986a), Washington	Population-based, *n* = 27 incident cases (1980−1983) from a 13-county area (Washington State Cancer Surveillance System) and 552 matched controls from random digit dialing in same area, for occupational exposures. Adjusted for cigarette smoking, alcohol consumption, gender, and age.	Interview-based information on lifetime occupational exposure to formaldehyde with cases, next of kin, and controls. Exposure from available hygiene data, NIOSH and other data, and NCI job exposure linkage system. Exposure score based on sum of no. years spent per job weighted by estimated formaldehyde level.	Intensity			
			Low	OR	1.2	(95% CI: 0.5−3.3)
			Medium/high		1.4	(95% CI: 0.4−4.7)
			No. years exposed			
			1−9	OR	1.2	(95% CI: 0.5−3.1)
			10 or more		1.6	(95% CI: 0.4−5.8)
			Exposure score (no lag)			
			5−19	OR	0.9	(95% CI: 0.2−3.2)
			20 or more		2.1	(95% CI: 0.6−7.8)
			Exposure score (15 year lag)			
			5−19	OR	1.7	(95% CI: 0.5−5.7)
			20 or more		2.1	(95% CI: 0.4−10)
Vaughan et al. (1986b), Washington	Same cases and controls as Vaughan et al. (1986a). Adjusted for ethnic origin and cigarette smoking.	Same as Vaughan et al. (1986a). Also included residential history in past 50 years, and use of particleboard or plywood.	Years of residence in mobile home			
			1−9	OR	2.1	(95% CI: 0.7−6.6)
			10 or more		5.5	(95% CI: 1.6−19)
			Years of exposure to particleboard			
			1−9	OR	1.4	(95% CI: 0.5−3.4)
			10 or more		0.6	(95% CI: 0.2−2.3)

Table 4-3. Case-control studies of formaldehyde exposure and nasopharyngeal cancer (continued)

Reference, study area	Study design	Exposure assessment	Results			
Vaughan et al. (1986b), Washington (continued)			Exposure source			
			Occupation only	OR	1.7	(95% CI: 0.5−5.7)
			Mobile home		2.8	(95% CI: 1.0−7.9)
			Both		6.7	(95% CI: 1.2−39)
Roush et al. (1987), Connecticut	Population-based, *n* = 173 male cases from the Connecticut Tumor Registry who died of any cause from 1935−1975. 605 male controls randomly selected from state death certificates during same time period. results adjusted for age at death, year at death, and availability of occupational information (Roush et al., 1987).	Four exposure categories based on probability and duration were classified by an industrial hygienist according to job title, industry, specific employment and year of employment. Exposure categories: I, probably exposed most of working life; II, probably exposed most of working life and probably exposed 20+ years before death; III, probably exposed most of working life and probably to high level in some year; IV, probably exposed most of working life and probably exposed to high level 20+ years before death.	Exposure category			
			I	OR	1.0	(95% CI: 0.6−1.7)
			II		1.3	(95% CI: 0.7−2.4)
			III		1.4	(95% CI: 0.6−3.1)
			IV		2.3	(95% CI: 0.9−6.0)
West et al. (1993), Phillipines	Hospital-based, *n* = 104 non-Chinese incident cases from the Philippine General Hospital, matched with 104 hospital and 101 community controls. Adjusted for years since first exposure to dust and exhaust fumes.	Personal interview, including job history. Industrial hygienists blinded to case-control status reviewed and rated jobs as likely or unlikely to be exposed. Analysis by length of exposure, length of exposure lagged 10 years, time since first exposure, and age at first exposure, based on date of interview or death.	Length of exposure (years)			
			<15	RR	2.7	(95% CI: 1.1−6.6)
			15 or more		1.2	(95% CI: 0.5−3.2)
			Length of exposure lagged 10 years (years)			
			<15	RR	1.6	(95% CI: 0.7−3.8)
			15 or more		2.1	(95% CI: 0.7−6.2)

Table 4-3. Case-control studies of formaldehyde exposure and nasopharyngeal cancer (continued)

Reference, study area	Study design	Exposure assessment	Results
West et al. (1993), Phillipines (continued)			Years since first exposure <25 RR 1.3 (95% CI: 0.6−3.2) 25 or more 2.9 (95% CI: 1.1−7.6) Age at first exposure (years) <25 RR 2.7 (95% CI: 1.1−6.6) 25 or older 1.2 (95% CI: 0.5−3.3)
Armstrong et al. (2000), Malaysia	Hospital-based, *n* = 282 Chinese cases, individually matched to 282 controls by age and sex recruited through multistage area sampling. Adjusted for diet and smoking variables.	Personal interview, including detailed job history (description, time, machines, tools, substances used, exposures); exposure classification blinded to case-control status	Ever exposed OR 0.71 (95% CI: 0.34−1.43)
Vaughan et al. (2000), United States	Population-based, *n* = 196 incident epithelial cases from 5 U.S. cancer registries (1987−1993) matched with 244 controls from random digit dialing in the same geographic regions. Adjusted for age, sex, race, SEER site, cigarette usage, proxy status, and education.	Interviewed for lifetime occupational and chemical exposure. Exposure estimates by industrial hygienist without knowledge of case-controls status. Probability of exposure: definitely not or unlikely (<10%); possible (≥10% and <50%); probable (>50% and <90%); and definite ≥90%). Jobs with potential exposure assigned estimated concentration levels based on TWA: low (<10 ppm), moderate (≥10 and <50 ppm), and high (≥50 ppm).	Possible, probable or definite exposure (61 cases, 76 controls) Ever OR 1.6 (95% CI: 1.0−2.8) Duration (years) 1−5 OR 0.9 (95% CI: 0.4−2.1) 6−17 1.9 (95% CI: 0.9−4.4) 18 or more 2.7 (95% CI: 1.2−6.0) *Trend p = 0.014* Cumulative exposure (ppm-years) 0.05−0.40 OR 0.9 (95% CI: 0.4−2.0) 0.41−1.10 1.8 (95% CI: 0.8−4.1) ≥1.10 3.0 (95% CI: 1.3−6.6) *Trend p = 0.033*

Table 4-3. Case-control studies of formaldehyde exposure and nasopharyngeal cancer (continued)

Reference, study area	Study design	Exposure assessment	Results
Vaughan et al. (2000), United States (continued)			Probable or definite exposure (27 cases, 30 controls) Ever — OR — 2.1 (95% CI: 1.1−4.2) Duration (years) 1−5 — OR — 2.0 (95% CI: 0.8−5.0) 6−17 — 3.3 (95% CI: 0.9−12) 18 or more — 1.6 (95% CI: 0.5−5.6) *Trend p = 0.069* Cumulative exposure (ppm-years) 0.05−0.40 — OR — 1.9 (95% CI: 0.7−4.9) 0.41−1.10 — 2.6 (95% CI: 0.7−9.5) ≥1.10 — 2.2 (95% CI: 0.7−7.0) Definite exposure (10 cases, 2 controls) Ever — OR — 13.3 (95% CI: 2.5−70)
Hildesheim et al. (2001)	Population-based, *n* = 375 incident cases from two Taiwanese hospitals between 7/15/91 and 12/31/94. 325 controls came from a random sample of households from a national household registration system and were age, sex, and area-of-residence matched. Tumors were histologically confirmed. All subjects were tested for the EBV; subset analysis based on	In-person interviews collected information on risk factors and job history for jobs held >1 year, including length of time job held, type of industry, and tasks, tools, and materials used on the job. Industrial hygienist assigned Standard Industry Classification/ Standard Occupational Classification codes to jobs, assigning each a probability and intensity of exposure on a 0−9 scale. Exposure metrics were duration, average intensity (intensity scale), average	**All cases and controls** Ever — OR — 1.4 (95% CI: 0.93−2.2) Duration >0−≤ 10 — OR — 1.3 (95% CI: 0.69−2.3) > 10 — 1.6 (95% CI: 0.91−2.9) Cumulative exposure (ppm-yrs) > 0− <25 — OR — 1.3 (95% CI: 0.70−5.8) ≥ 25 — 1.5 (95% CI: 0.88−2.7)

Table 4-3. Case-control studies of formaldehyde exposure and nasopharyngeal cancer (continued)

Reference, study area	Study design	Exposure assessment	Results			
Hildesheim et al. (2001) (continued)	EBV positivity (360 cases and 94 controls).[a] Adjusted for age, sex, education, and ethnicity.	probability (probability scale), cumulative (average intensity), years since 1st exposure, and age at 1st exposure. Analysis of nonkeratinizing or undifferentiated tumors yielded similar results as overall analysis	**EBV positive cases and controls**			
			Ever	OR	2.7	(95% CI: 0.1.2−6.2)
			Duration			
			>0−≤ 10	OR	2.8	(95% CI: 0.83−9.7)
			> 10		2.6	(95% CI: 0.87−7.7)
			Cumulative exposure (ppm-yrs)			
			> 0− <25	OR	4.0	(95% CI: 0.92−17)
			≥ 25		2.2	(95% CI: 0.80−5.8)

[a]EBV seropositives defined as positive for one of the following anti-EBV antibodies known to be associated with nasopharyngeal cancer: viral capsid antigen IgA, EBV nuclear antigen 1 IgA, early antigen IgA, DNA binding protein IgG, and anti-DNase IgG.

Average intensity categories of exposure were defined as nonexposed, low (>0− <0.5 ppm), medium (0.5− <1.0 ppm), and high (≥1.0 ppm). Cumulative exposure was defined as nonexposed, low (>0− <1.5 ppm-years), medium (1.5− <5.5 ppm-years), and high (≥5.5 ppm-years). Duration of exposure was defined as nonexposed, low (>0 years− <5 years), medium (5− <15 years), and high (>15 years). The presence of formaldehyde-containing particulates and other potential chemical coexposures in the plants were indentified as suspected carcinogens including antioxidants, asbestos, carbon black, dyes and pigments, hexamethylenetetramine, melamine, phenol, plasticizers, urea, wood dust and benzene. Standardized mortality ratios (SMRs) were calculated using sex-, race, age-, and calendar-year-specific U.S. mortality rates. Relative risks based on internal comparisons within the cohort were estimated using Poisson regression, controlling for calendar year, age (5-year categories), sex, race, and pay category.

Subjects were initially followed to January 1, 1980, accruing approximately 600,000 person-years of follow-up (Blair et al., 1987). Information on potential formaldehyde exposures after 1980 was unavailable. Hauptmann et al. (2004) updated the cohort through December 31, 1994 reporting a total accrual of person-time of 865,708 person-years with a median duration of follow-up of 35 years. Cause of deaths was taken from death certificates. A total of 10 deaths from nasopharyngeal cancer were identified. Among them 8 were classified as ever exposed and 2 never exposed.

Hauptmann et al. (2004) reported an increased risk of nasopharyngeal cancer in exposed workers based on U.S. population death rates (standardized mortality ratio [SMR] = 2.1 [95% CI: 1.05−4.21]). This association was based on a total of 8 nasopharyngeal exposed cancer cases; however, 1 of these cases was reclassified to oropharyngeal cancer based on secondary sources of data and thus 7 nasopharyngeal cases were included in additional analyses using Poisson regression modeling of relative risks (RRs) with an internal referent group.

In addition to the SMR analysis, Hauptman et al. (2004) provided both RRs based on internal referent groups (unexposed employees, and the low exposed workers) and regression analysis of all exposed workers. Use of an internal referent group reduces potential selection bias due to the healthy worker effect and potential confounding from area- and employment-related factors. Both of these analyses were conducted for four different exposure metrics Exposure measures used in the Poisson regression were based on a standardized exposure assessment protocol (summarized in Table 4-2), and included measures of highest peak exposure, average exposure intensity, cumulative exposure, and duration of exposure to formaldehyde (Hauptmann et al., 2004). RRs for nasopharyngeal cancer increased with various exposure metrics with trend *p*-values (based on continuous exposure measures) < 0.001 and 0.025 for the peak exposure and cumulative exposure metrics, respectively (see Table 4-2).

These analyses also adjusted for potential confounders, including calendar year, age, sex, race, and pay category. The investigator evaluated exposures to 11 suspected carcinogens and other widely used chemicals in the plants including antioxidants, asbestos, carbon black, dyes and pigments, hexamethylenetetramine, melamine, phenol, plasticizers, urea, wood dust, and benzene. Hauptmann et al. (2004) reported that the relative risks for most cancers did not meaningfully change when adjusted for these exposures except for nasopharyngeal cancer and melamine exposure. While the RR for the highest exposure categories of peak and average intensity of formaldehyde exposure declined with adjusted for melamine exposure, Hauptmann et al. (2004) reported that the relative risks for cumulative exposure and duration of exposure were still elevated. The tests for trend in exposure-response remained highly statistically significant for peak ($p < 0.001$), average ($p = 0.021$), and cumulative ($p = 0.006$) exposure. There was no evidence of any differential measurement error that could have produced a spurious association; nondifferential measurement error would likely have led to an observed effect of formaldehyde that was less than that which would otherwise have been observed in the absence of measurement error.

Overall this study provides strong causal evidence of an association between formaldehyde exposure and the risk of nasopharyngeal cancer mortality. Although this is a rare cancer the cohort was large with sufficient follow-up to accrue 7 exposed cases of nasopharyngeal cancer compared to 2 unexposed cases making this cohort study the most statistically powerful of the cohort studies of nasopharyngeal cancer. The objective exposure assessment was of high quality which also increased the power of this study to detect an effect—even with a small number of cases. Potential sources of confounding or bias were also well controlled. Finally, evidence of a stronger association, with the high exposure groups for each of the 4 different exposure metrics, indicates the consistency of exposure-response relationship.

Following these reports of increased risk of nasopharyngeal cancer associated with formaldehyde exposure, a series of post hoc analyses of a subset of the NCI cohort were undertaken by Marsh and coworkers (Marsh et al., 2007a, b, 2002, 1996; Marsh and Youk, 2005). Briefly, these studies focused on the specific findings from a single plant in the NCI cohort (Wallingford, Connecticut) that generated the majority of the nasopharyngeal cancer cases. The Marsh et al. (2002) study had both a cohort analysis and a nested case-control analysis. The cohort study followed 7328 workers employed in the plant from 1941 to 1984, extending the follow-up period to 1998 and independently evaluated the exposure assessment (Marsh et al., 1996 present the earlier analysis with follow-up through 1984). Seven deaths from nasopharyngeal cancer were seen in this cohort, for SMRs of 4.94 (95% CI 1.99-10.19) using the United States population as the referent group and 5.00 (95% CI 2.01-10.30) using the local

county referent group. Nearly identical results using the national and local populations as the referent group shows that those background rates were very similar. Strong associations were seen with measures of ever exposure to formaldehyde (SMR = 6.03; 95% CI: 2.42-12.42), as well as duration of exposure (Greater than 10 years, SMR = 12.46; 95% CI: 1.51-45.02) and cumulative exposure (Greater than 0.22 ppm, SMR = 7.51; 95% CI: 1.55-21.93) (see Table 4-2 for additional detail). This study used a different exposure assessment and even computed an SMR based on local rates. Additionally the exposure response seen with both cum and duration of exposure adds strength to the observed associations. The nested case-control study ascertained data on smoking and date of hire, however, these data were not able to be modeled due to the limited number of nasopharyngeal cases (n = 7). This analysis supports the findings of the NCI study, although only a single plant was included in the study (Marsh et al., 2002). However, Marsh et al. (2002) interpret these data as providing evidence against the involvement of formaldehyde in nasopharyngeal cancer because elevated risks were seen in short-term (as well as in long-term workers) and in the lower exposure categories (as well as in the higher exposure categories) of the various formaldehyde measures.

Marsh and Youk (2005) further suggest that this interpretation of the Marsh et al. (2002) data weakens the support seen in the larger NCI study for a causal link between formaldehyde and nasopharyngeal cancer because the Hauptman et al. (2004) results are primarily driven by the results from the Wallingford, Connecticut plant. Marsh and Youk (2005) re-analyzed of nasopharyngeal cancer data from the Hauptmann et al. (2004) study. They compared the results from the Wallingford, Connecticut plant (Plant 1), which contributed five of the nine nasopharyngeal cancer deaths in the NCI study to the other nine plants. Marsh and Youk (2005) reported that when the SMR for nasopharyngeal cancers in Plants 2 to 10 combined in was not elevated (SMR = 0.65, 95% CI = 0.8 to 2.3, 4 deaths), in comparison with that of Plant 1 alone (SMR = 10.3, 95% CI = 3.8 to 22.5, 6 deaths). However, from Marsh and Youk (2005), the two plants with the highest exposures were Plants 1 and 2. The SMR for Plant 2 was elevated at 5.35 based on a single case (95% CI: 0.13-29.83). While this effect estimate is extremely unstable statistically, it is may be a reflection of the relatively higher formaldehyde exposures than at other plants.

In the most recent reports, Marsh et al. (2007a) provides additional data from a nested case-control study with the 7 nasopharyngeal cancer cases and 16 other pharyngeal cancer cases identified from the Wallingford, Connecticut cohort. Cases were matched to 4 controls in the cohort based on age, race, sex, and year of birth. Smoking history and other work history data (jobs held other than at the Wallingford plant) were obtained from interviews with 5 of the 7 cases and with 68 of 88 controls. The source of the interview differed between cases and

controls, with family member interviews used for most of the cases, and self-interviews used for most of the controls. Details of the content and structure of the interview were not provided, but the authors say that little useful information was obtained from these interviews (Marsh et al. 2007a, 2002). Additional sources of work history included data from the plant job application, city directories and genealogy-based search strategies. An association was seen between nasopharyngeal cancer risk and work in silversmithing occupations (including brass plating or other jobs relating to silver or brass, OR 14.4, 95% CI 1.3, 758; 4 exposed cases); the odds ratio for other types of metal work (steel work and welding) was 3.61 (95% CI 0.50-22.7; 3 exposed cases) and for the combined category of silversmithing and other metal work the OR was 7.31 (9% CI 1.08-82.1; 5 exposed cases). There are no prior citations of an association between silversmithing exposures and nasopharyngeal cancer in the medical literature, but Marsh et al. review the literature pertaining to related exposures (sulfuric acid mists, metal dusts) and respiratory and laryngeal cancer to support this association. However, the results for these exposures and laryngeal cancer are inconsistent, and data pertaining to these exposures and nasopharyngeal cancer are quite limited. Despite these limitations, Marsh et al. (2007) suggest that the observed associations between nasopharyngeal cancer and formaldehyde exposure in the Wallingford plant are due to these other occupational exposures. Marsh et al. (2007) do note that history of silversmithing and other metal work was not associated with formaldehyde exposure, and so was not a confounder of the formaldehyde results as reported for the Wallingford Plant. In addition, there was no evidence of confounding due to smoking history. Regardless, Marsh et al. (2007a) suggest that these other occupational exposures, rather than formaldehyde, could explain the risk of nasopharyngeal cancer seen in the Wallingford cohort.

Two cohort studies of professional groups, such as anatomists, pathologists, embalmers, and funeral directors, examined the risk of nasopharyngeal cancer and formaldehyde exposure (see Table 4-2). In general, measurements of formaldehyde concentrations were not available in studies of these groups but are generally below 1 ppm (IARC, 1995; Korczynski, 1994; Stewart et al., 1992; Moore and Ogrodnik, 1986). Hayes et al. (1990) conducted a study of 3,649 deceased white and 397 deceased nonwhite U.S. male embalmers and funeral directors who had died between 1975 and 1985, using records from local licensing boards, state funeral directors' associations in 32 states and the District of Columbia, the National Funeral Directors' Association, and state offices of vital statistics (n = 894). Expected deaths by cause were derived from 5-year age- and calendar-year-specific proportions of deaths among appropriate race groups from the U.S. population. No measured exposure data were available. Hayes et al. (1990) reported an excess risk of nasopharyngeal cancer among male professional embalmers and funeral directors, based on 4 deaths with 1.85 expected (PMR 2.16, 95% CI: 0.59−5.54). The

authors note that although these results are based on small numbers of deaths, the specific excesses of nasopharyngeal cancer that they observed were consistent with those reported by Vaughan et al. (1986a,b; and Blair et al., 1987) which showed increased risks of nasopharyngeal cancer associated with exposure to formaldehyde.

Hansen and Olsen (1995) studies workers in 265 Danish industries that produced more than one kg of formaldehyde per employee per year, where 2,041 of 91,182 cancer patients had at least 10 years of continuous formaldehyde-related work experience before diagnosis. The risk of cancer incidence was estimated from standardized proportionate incidence ratios as denominators were not available. The standardized proportionate incidence ratio (SPIR) is the proportion of cases of a particular cancer among exposed persons compared to the proportion of cases of a particular cancer among the general population. Hansen and Olsen (1995) reported an SPIR, based on 4 observed and 3.2 expected cases, of 1.3 (95% CI 0.03, 3.2).

Other studies also examined nasopharyngeal cancer risk in occupational cohorts, but are limited by the small number of expected cases. Pinkerton et al. (2004) updated of a cohort study of 11,030 workers (82% female) followed from 1955 or the beginning date of exposure through 1982 in three garment factories conducted by Stayner et al. (1988). Formaldehyde resins were used to treat fabrics in these factories beginning in 1955 and 1959. Although formaldehyde levels were available on a subset of the employees from monitoring data available from surveys completed in 1981 and 1984, they were not used in this analysis. The geometric mean 8-hour time weighted average exposure across all departments and plants was 0.09-0.20 ppm. The geometric mean formaldehyde concentration across all departments was estimated at 0.15 ppm. No cases of nasopharyngeal cancer were observed compared to an expected number of one case.

Gardner et al. (1993) studied a cohort of 14,017 workers exposed to formaldehyde in the British chemical industry and followed until the end of 1989. Exposure to formaldehyde was classified according to job title and assigned to categories including nil/background (<0.1 ppm), low (0.1-0.5 ppm), moderate (0.6-2.0 ppm) and high (over 2.0 ppm). Results were stratified by date of first employment and among men first employed before 1965, the SMR for cancer of the pharynx was 1.47 (95% CI: 0.59-3.03). No cases were reported among the men first employed after 1965. Although the cause-specific cancer mortality was for cancer of pharynx (ICD-9 146−9) and would include nasopharyngeal cancer, the authors stated separately that there were 1.3 cases of nasopharyngeal cancer that were expected but no deaths recorded for nasopharyngeal cancer. Coggon et al. (2003) updated this cohort study with additional follow-up through the end of 2000 and reported the effects of formaldehyde exposure on mortality from cancer among 14,014 workers. Coggon et al. (2003) reported only one case compared to an

expected number of two cases (SMR 0.5, 95% CI 0.07-3.55 as calculated in a meta-analysis by Bossetti et al., 2007).

Other cohort studies examined occupational formaldehyde exposure in relation to total cancer or lung cancer risk, but did not provide specific data for nasopharyngeal cancer risk (Andjelkovich et al., 1995; Bertazzi et al. 1989, 1986; Edling et al., 1987;). These studies are not discussed further in this section. A summary of studies of oral cavity cancers (including oropharynx) and laryngeal cancers is presented in Section 4.1.2.1.4.

4.1.2.1.1.2. ***Case-control studies of nasopharyngeal cancer.***

Eight case-control studies (Hildesheim et al., 2001; Vaughan et al., 2000; Armstrong et al., 2000; West et al., 1993; Roush et al., 1987; Vaughan et al., 1986a, b; Olsen et al., 1984) provided evidence of excess risks of nasopharyngeal cancer due to formaldehyde exposure (see Table 4-3). Olsen et al. (1994) conducted a population-based case-control study of nasopharyngeal cancer in Denmark. Cases of nasopharyngeal cancer (n = 266) were ascertained from the Danish Cancer Registry during 1970-1982. Controls were diagnosed with cancer of the colon, rectum, breast, and prostate and three controls were matched to each case by age, sex, and year of diagnosis. Employment histories after 1964 were evaluated by industrial hygienists who classified subjects as exposed or unexposed and wee blinded to case-control status. Among men the odds ration for occupational exposure to formaldehyde was OR = 0.7 (95% CI: 0.3-1.7); among women the OR = 2.6 (95% CI: 0.3-21.9).

Vaughan et al. (1986a) conducted a population-based case-control study of the pharynx, sinus and nasal cavity. Incident cases of nasopharyngeal cancer (n = 27) were matched to controls selected by random digit dialing in the same areas. Medical, occupational, and residential histories as well as information of smoking and alcohol consumption were obtained by telephone interview for cases and controls. When cases were deceased, next-of-kin were interviewed. Logistic regression was used to control for potential confounders. Exposure was evaluated from available hygiene data, NIOSH and other data, and NCI job exposure linkage system. Exposure scores were based on sum of number of years spent per job weighted by estimated formaldehyde level. Occupational formaldehyde exposures were evaluated by maximum exposure level, number of years exposed, an exposure score across all years and an exposure score that excluded the previous 15 years to account for the induction periods between possible etiologically relevant exposures and the detection of incident cancers. Exposure scores were based on a weighted sum of the years in a job with weights depending upon the estimated exposure level. In all but one of eight comparisons, the odds ratios were elevated; however, none to the odds ratios was statistically significant. Effect estimates were most elevated for

nasopharyngeal cancer among those with the highest exposures score. Ignoring the potential induction period, the OR = 2.1 (95% CI; 0.6-7.8). With the 15-year induction period the OR = 2.1 (95% CI: 0.4-10).

Vaughan et al. (1986b) evaluated the residential exposures to formaldehyde among the same nasopharyngeal cases as reported in Vaughan et al. (1986a). Logistic regression analyses controlled for smoking and ethnic origin (White, Black, Asian, other). Potential residential exposure to formaldehyde was estimated by utilizing residence in a mobile home with or without the presence of urea-formaldehyde foam insulation (UFFI) or particleboard or plywood as a surrogate for exposure. The authors found statistically significant OR of 5.5 (95% CI: 1.6-19.4) for subjects reporting residence of 10 or more years in a mobile home with UFFI before diagnosis. An elevated odds ratio was reported for less than 10 years of mobile home residence (OR = 2.1; 95% CI: 0.7-6.6). The authors also evaluated whether the risk associated with living in a mobile home was affected by also working in a mobile home. Occupational exposure to mobile homes without residential exposure had an OR = 1.7 (95% CI: 0.5-5.7). Residential exposure to mobile homes without occupational exposure had an OR = 2.8 (95% CI: 1.0-7.9). Both having residential and occupational exposure to mobile homes was statistically significantly associated with incidence of nasopharyngeal cancer (OR = 6.7, 95% CI: 1.2-38.9).

Roush et al. identified 173 cases of nasopharyngeal cancer in men from the Connecticut Tumor Registry who dies of any cause between 1935 and 1975. Male controls (n = 605) were selected from state death certificates during the same time period. Four exposure categories were created based on the probability and duration of formaldehyde exposure based on industrial hygienist evaluations of subject's job titles, industry, specific employment and duration of employment. Logistic regression adjusted for potential confounders including age at death, year of death. For the lowest exposure category defined as 'probably exposed most of working life,' the OR = 1.0 (95% CI: 0.6-1.7); for the second exposure category defined as 'probably exposed most of working life and probably exposed 20+ years before death,' the OR = 1.3 (95% CI: 0.7-2.4); for the third category of exposure defined as 'probably exposed most of working life and probably to high level in some year,' the OR = 1.4 (95% CI: 0.6-3.1); and for the fourth exposure category defined as 'probably exposed most of working life and probably exposed to high level 20+ years before death,' the OR = 2.3 (95% CI: 0.9-6.0).

West et al. (1993) investigated risk factors for nasopharyngeal cancer in the Philippines using a matched case-control study of 104 cases and 205 hospital and community controls. Personal interviews were conducted by a trained nurse who ascertained information on demographic factors including ethnicity and education, dietary consumption, occupational history, smoking, use of herbal medicine, betel nut, and anti-mosquito coils. An industrial

hygienist who was blinded to case-control status evaluated subject's occupational histories to estimate likely exposures to formaldehyde, solvents, dusts, exhausts, and pesticides. Conditional logistic regression was used to control for coexposures. Controlling for education, dust, processed meat and fresh fish consumption, mosquito coil usage, and use of herbal medicine, the RR of occupational formaldehyde exposure more than 25 years earlier was 4.0 (95% CI:L 1.3-12.3). The independent effect of dust was also statistically significant for exposure more than 35 years prior (OR = 4.4; 95% CI: 1.1-17.5) as was the independent effect of daily use of mosquito coils (OR = 5.9; 95% CI: 1.7-20.1). While the authors mentioned only that the compounds in the anti-mosquito coil smoke might be of further interest, they did identify those compounds. However, independent testing of 6 brands of East Asian mosquito coils evaluated the emission rates of carbonyl compounds in the mosquito smoke and reported that formaldehyde and acetaldehyde had the highest emission rates (Liu et al., 2003). Among the three experiments on each of the six brands, the range of formaldehyde concentrations was from 0.87 μg/m^3 (1 ppb) to 25 μg/m^3 (31 ppb).

Armstrong et al. (2000) studied 282 ethnically Chinese residents of Malaysia with recently diagnosed nasopharyngeal cancer (diagnosis from 1987−1992, with case identification occurring between 1990 and 1992). Cases were individually matched to 282 controls by age and sex; structured interviews collected residential and work history data. No association was seen between formaldehyde exposure and nasopharyngeal cancer (adjusted OR = 0.71 [95% CI: 0.34−1.43]), controlling for wood dust and industrial heat.

In a population-based case-control study of incident nasopharyngeal cancer cases identified through the U.S. SEER cancer registry, Vaughn et al. (2000) used work history data collected from interviews (see Table 4-3) to estimate each individual worker's formaldehyde exposure. Workers with more than 1.10 ppm-years of cumulative exposure were found to be at significantly higher risk of nasopharyngeal cancer, with an odds ratio (OR) of 3.0 (95% CI 1.3−6.6). Both duration of exposure and cumulative exposure were positively associated with increased risk of nasopharyngeal cancer (trend p = 0.014 and 0.033, respectively). The OR increased in magnitude as the probability of "Ever" having occupational exposure increased, from OR = 1.6 (95% CI 1.0−2.8) among the 61 cases whose exposure was judged to be "Possible, probable or definite", to 2.1 (5% CI 1.1, 4.2) among the 27 cases with "probable or definite" exposure, to OR = 13.3 (95% CI 2.5−70) among the 10 cases with "definite" exposure (p-trend < 0.001) (Vaughn et al., 2000).

The study design of the Hildesheim et al. (2001) study was a matched case-control study with controls individually matched to nasopharyngeal cancer cases on age, sex, and district/township of residence. Hildesheim et al. (2001) reported that exposure to formaldehyde

produced modest risk elevations: for duration of exposure, OR = 1.6 for 10 years or less and 1.2 for over 10 years of exposure; for cumulative exposure, OR = 1.3 for <25 years of exposure and 1.5 for 25+ years of exposure. Among those with EBV, the OR was 2.7 (95% CI: 1.2−6.2) for ever-exposed persons. The risk was higher among exposed persons whose work history was within the last 10 years (OR = 4.7 [95% CI: 1.1−20.0]) and for those followed 20+ years after exposure (OR = 2.8 [95% CI: 1.1−7.6]). The analysis of these data, however, was conducted using unconditional logistic regression. The authors stated that conditional logistic regression was not used "to avoid the loss of information from cases and controls without a matched pair." This loss of information was particularly acute among the EBV+ strata in which 360/375 cases were EBV+ while only 94/325 controls were EBV+. While the investigators did include age, sex and other potential confounders in the analytic models, this analytic methodology may not be sufficient to effectively control for these factors given the individual matching used in the study. Failure to condition on the matching factors can create bias that is unpredictable in direction or magnitude (Cox and Hinkley, 1974, p. 292 and Breslow and Day, 1980, p. 249). Had the study design specified frequency matching rather than individual matching, then the logistic regression analysis with the inclusion of the matching factors would have been appropriate; however, this apparent weakness in all the analyses by Hildesheim et al. (2001) raises considerable uncertainty regarding the results and less weight should be assigned to this study in the overall evaluation.

4.1.2.1.1.3. ***Summary of nasopharyngeal cancers.***

Because of the low incidence rate of nasopharyngeal cancer, cohort studies that rely on nonspecific exposure assessment techniques may not be expected to provide sufficiently precise data needed for evaluation of the contribution of formaldehyde to the etiology of this particular cancer type due to a lack of statistical power. Even the latest cohort study of the British industrial worker which evaluated 14,014 men (Coggon et al., 2003) only expected to observe two cases while the latest cohort study of the garment workers which evaluated 11,030 men and women (Pinkerton et al., 2004) only expected to observe one case. Cases counts depend upon both the number of people followed and the length of follow-up time as well as the baseline incidence rate of nasopharyngeal cancer. However, it is important to understand that the statistical power of these cohort studies largely depends upon the count of the number of observed and expected cases and not the number of people who were followed. The variance of an observed relative risk is a function of the inverse of the observed and expected case counts. Small case counts produce large statistical variances. The large variances in effect estimates results in wide confidence intervals that may be unstable and offer little insight into any exposure-response relationship other than to statistically rule out very strong adverse effects.

Thus EPA's evaluation of the epidemiologic data on formaldehyde and nasopharyngeal cancer focuses on the large NCI cohort study which evaluated the mortality among 25, 619 workers (Hauptmann et al., 2004) and the case-control studies with extensive work history data (e.g., lifetime job histories) and industrial hygiene assessment of exposure potential.

Hauptmann et al. (2004) reported that for mortality from all types of cancer combined, the workers experienced fewer deaths than would be expected in the general population (SMR = 0.90, 95% CI: 0.86-0.95). This typically reflects that fact that workers are healthier than the general population which includes nonworkers. In spite of this 'healthy worker effect,' the Hauptmann et al. (2004) analysis reported a doubling of nasopharyngeal cancer mortality risk in workers from 10 formaldehyde producing or using factories (SMR = 2.1, 95% CI: 1.05−4.21). Additional analyses demonstrated that relative risks increased with average exposure intensity (p-trend = 0.066), cumulative exposure (p-trend = 0.025), highest peak exposure (p-trend < 0.001) and duration of exposure to formaldehyde (p-trend = 0.147).

In addition to the evidence from the NCI cohort studies, modest additional evidence is found in the professional cohort studies of Hayes et al. (1990) and Hansen and Olsen (1995) however, the elevated risks overlapped with unity and the individual study results were not statistically significant. The rarity of the disease and difficulties in obtaining valid and reliable historical exposure estimates are substantial limitations of these cohort studies.

The case-control studies also provide a robust collection of data supporting a similar overall magnitude of association between formaldehyde exposure and nasopharyngeal cancer (i.e., relative risk estimates in the range of 1.5−3.0) (see Table 4-3). These studies were conducted in several different countries and work settings. Only one study (Armstrong et al., 2000) did not observe a risk consistent with these estimates.

Of particular note were three sets of effect estimates of unusual magnitude, especially when the definitions of exposure to formaldehyde were more specifically defined. Vaughan et al. (1986b) reported that the odds ratio for living in a mobile home for 1-9 years was 2.1 (95% CI: 0.7-6.6) compared to not living in a mobile home, while living in a mobile home for more than 10 years had an OR = 5.5 (95% CI: 1.6-19.4). Vaughan et al. (1986b) also reported results that could be considered as consistent with an exposure-response relationship. Compared to having no exposure to mobile homes, they reported OR = 1.7 (95% CI: 0.5-5.7) for working in a mobile home, OR = 2.8 (95% CI: 1.0-7.9) for living in a mobile home and OR = 6.7 (95% CI: 1.2-38.9) for both living in and working in a mobile home. While exposure to mobile homes is a crude proxy for formaldehyde exposure, the gradient in response and the magnitude of effect at the highest exposure level is striking.

Vaughan et al. (2000) also demonstrated a version on an exposure-response gradient. They reported a statistically significant trend of increasing risk of nasopharyngeal cancer with the probability of being exposed to formaldehyde. "Possible, probable or definite" probability of being exposed to formaldehyde having an OR = 1.6 (95% CI 1.0−2.8), with "probable or definite" exposure having an OR = 2.1 (5% CI 1.1, 4.2) and with "definite" exposure having an OR = 13.3 (95% CI 2.5−70) (p-trend < 0.001).

The study by West et al. (1993) was also remarkable for the exposure-response shown for formaldehyde and for anti-mosquito coils which have been shown to release high concentrations of formaldehyde. In multivariate analyses, compared to never having had occupational exposure to formaldehyde, having occupation exposure within the last 25 years had an OR = 1.2 (95% CI: 0.41-3.6) while having occupational exposure more than 25 years earlier had an OR = 4.0 (95% CI: 1.1-17.5). Compared to never using anti-mosquito coils, the OR for using them less than daily was 1.4 (95% CI: 0.64-2.8) while daily use of anti-mosquito coils had an OR = 5.9 (95% CI: 1.7-20.1).

Establishment of a concentration-response relationship is important evidence of causality. An attenuation of risk is often seen at the highest dose level in epidemiologic studies, however, it may possibly be due to exposure misclassification and of the use of inappropriate exposure models (Stayner 1985, 1988). Identification of concentration-response relationships in spite of potentially substantial effect measure attenuation provides even stronger evidence of causality. Findings from the large NCI cohort studies of nasopharyngeal cancer risk (Hauptmann et al., 2004) show a pattern of increased risk with increased formaldehyde exposures across multiple exposure metrics.

Several reanalyses of the NCI cohort have challenged the interpretation of these findings, although the reported excess in nasopharyngeal cancer mortality is also seen in these new analyses (Marsh et al., 2007a, b, 2002, 1996; Marsh and Youk, 2005). The major questions that have been raised by Marsh and coworkers highlight the observation that the nasopharyngeal cancer findings appear to depend on the results of 1 of the 10 plants that made up the NCI cohort. While it is possible for coexposures at that plant or among those workers to act as potential confounders or modifiers of the observed effect of formaldehyde on increased risk of nasopharyngeal cancer, there is no evidence of such a relationship as Hauptmann et al. (2004) reported that controlling for each of 11 coexposures did not meaningfully alter the formaldehyde association. While seven out of nine members of the cohort members at the Wallingford, Connecticut, plant were also exposed to particulates, the NCI investigators did observe an exposure-response relationship with formaldehyde among individuals with particulate exposures, thereby strengthening the causal interpretation of the formaldehyde relationship with an

increased risk of nasopharyngeal cancer (Hauptmann et al., 2004). The described association of a potential occupational relationship with silversmithing (brass plating, silver and other brass work) and nasopharyngeal cancer has little support in the medical literature. In addition, if silversmithing exposures are indeed independent risk factors for nasopharyngeal cancer, it would be expected that the rates of nasopharyngeal cancer in the surrounding counties with historical silver-and other metal-related exposures would be elevated. However they are not increased, as evidenced by the comparability of the increased rates of nasopharyngeal cancer among the plant workers based on the national and based on local county rates (Marsh et al., 2007a). The comparable rates indicate the counties' rates of nasopharyngeal cancer were very similar to the national rates. Given the limitations and the lack of comparability in the source of the work history data in the nested case-control study of Marsh et al. (2007a) (next of kin interviews for cases and self-interviews for controls) and the many post hoc re-examinations of alternative hypotheses to explain the original NCI findings, EPA believes the association seen with silversmithing does not represent an unmeasured confounder of the results of Hauptman et al. (2004). A more likely explanation for the increased risk seen in short-term workers at the Wallingford, CT plant is the specific work conditions of those jobs at that plant. It is also plausible that the observed association at the Wallingford plant reflects higher formaldehyde exposures than at other plants. Marsh and Youk (2005) reported that the exposure levels at Plant 2 were even higher than at the Wallingford plant; the fivefold increase in risk of nasopharyngeal cancer seen at this plant, although based on only a single observed case, supports the results seen in Wallingford, CT.

Two large cohort studies did not report associations between formaldehyde exposure and increased risk of nasopharyngeal cancer (Coggon et al. 2003; Pinkerton et al., 2004). These studies were extremely limited in terms of the observed and expected numbers of cases, with just three cases expected between them during their follow-up periods. Thus these studies did not have the statistical power to rule out effect sizes of the magnitudes reported from the studies of the NCI cohort. As such, their results provide little contribution to the weight of evidence regarding formaldehyde and nasopharyngeal cancer.

This evidence of a concentration-response relationship in the largest cohort study (Hauptman et al., 2004) is further supported by a meta-analysis of the relative risks for nasopharyngeal cancer by level or duration of exposure to formaldehyde which reported a concentration-response relationship ($p < 0.05$) with an effect estimate of RR = 2.1 for the highest exposure category based on five studies which reported sufficient information for an exposure-response evaluation (Blair et al., 1990). A subsequent meta-analysis by Partanen (1993) reabstracted the source data from the studies in the Blair et al. (1990) meta-analysis in an effort

to maximize the amount of relevant data from the source studies. The Partanen (1993) meta-analysis confirmed the finding of Blair et al. (1990) that the relative risk of nasopharyngeal cancer was increased among people exposed to 'substantial' levels or durations of formaldehyde exposure. Partanen (1993) computed confidence intervals for the Blair et al. (1990) results for the highest exposure category as RR = 2.06 (95% CI: 1.10-3.52); two other methods of computing effect estimates and confidence intervals yielding effect estimates of RR = 2.74 (95% CI: 1.36-5.55) and RR = 2.59 (95% CI: 1.29-5.36). More recent case-control studies also provide evidence of increasing risks with longer duration or other measures of exposure (Vaughan et al., 2000; West et al., 1993).

4.1.2.1.2. ***Nasal and paranasal (sinonasal) cancer.***

4.1.2.1.2.1. ***Cohort studies of nasal and paranasal cancers.***

IARC (1995) reported the results of several cohort studies of professional and industrial workers exposed to formaldehyde for the induction of nasal and paranasal cancers (Andjelkovich et al., 1995; Gardner et al., 1993; Hall et al., 1991; Hayes et al., 1990; Bertazzi et al., 1989; Edling et al., 1987; Blair et al., 1986; Stroup et al., 1986; Harrington and Oakes, 1984; Levine et al., 1984; Walrath and Fraumeni, 1984, 1983; Friedman and Ury, 1983). The likelihood of finding this rare tumor type in even long-term cohort study is low, and only a few studies reported any cases of nasal and paranasal cancer. No cases of this type of cancer were reported in any of the studies of professional workers examined by the IARC. Only two cases (2.2 expected) were reported in the NCI cohort of more than 25,000 workers by Blair et al. (1986) while the update by Hauptmann et al. (2004) identified three cases (2.5 expected). Marsh et al. examined a subset of the NCI cohort from the Wallingford, Connecticut plant and identified two cases compared to 0.5 in the 1984 follow-up (Marsh et al., 1996) and three cases against one expected in the 1998 follow-up (Marsh et al., 2002). The cohort study of 14,017 British chemical industry workers exposed to formaldehyde by Gardner et al. (1993) identified only one case (1.7 expected). Coggon et al. (2003) updated this cohort study with additional follow-up and reported only two cases compared to an expected number of 2.3 cases. The other large cohort studies of more than 11,000 garment workers by Stayner et al. (1988) and Pinkerton et al. (2004) did not identify any cases of sinonasal cancer. In contrast, a large population-based study using nationwide Danish Product Register data found an increased risk of sinonasal cancer (standardized proportional incidence ratio [SPIR]= 2.3 [95% CI: 1.3−4.0] based on 13 observed cases; 2,041 of 91,182 cancer patients had at least 10 years of continuous formaldehyde-related work experience before diagnosis (Hansen and Olsen, 1995).

4.1.2.1.2.2. *Case-control studies of nasal and paranasal cancers.*

Eight case-control studies were evaluated in the 1995 IARC monograph regarding the risk of cancers of nasal cavity and accessory sinuses from exposure to formaldehyde (Luce et al., 1993; Roush et al., 1987; Hayes et al., 1986; Olsen and Asnaes, 1986; Vaughan et al., 1986a, b; Brinton et al., 1984; Olsen et al., 1984). Study details of the epidemiologic studies of nasal and paranasal cancer are summarized in Table 4-4. Five studies were not limited to a specific cell type and of these, only Roush et al. (1987) and Olsen et al. (1984) found positive results. The remaining studies (Vaughan et al., 1986a, b; Brinton et al., 1984) did not find associations between exposure and sinonasal cancer.

Three other studies conducted analyses limited to a specific cell type (Luce et al., 1993; Hayes et al., 1986; Olsen and Asnaes, 1986) (see Table 4-4). Hayes et al (1986) conducted a case-control study of occupational exposure to formaldehyde and the risk of nasal and paranasal cancer in the Netherlands based on 116 cases and 259 controls. Analyses were done by histological type of tumor and controlled for history of tobacco use and occupational exposure to wood dust. Controlling for potential confounding by wood dust was important as wood dust was reported to be strongly associated with sinonasal cancer (Hayes et al., 1986). Two independent assessments of formaldehyde exposure were conducted with RRs of 3.0 (90% CI 1.3-6.4) and 1.9 (90% CI 1.0-3.6) seen for squamous cell carcinoma based on assessments A and B, respectively.

Olsen and Asnaes (1986) conducted a case-control of 287 histologically verified cancers of the nasal cavity and 179 cases of paranasal cancer in Denmark. Controlling for wood dust exposures, the investigators reported adjusted RR for formaldehyde exposure of 2.2 (95% CI: 0.9-5.8) for squamous cell carcinoma and RR = 2.3 (95% CI: 0.7-7.2) for adenocarcinoma. The results for squamous cell carcinoma did not appear to be influenced by coexposure to wood dust; however, for adenocarcinoma, among those who were never exposed to wood dust, the RR of formaldehyde exposure was 7.0 (95% CI: 1.1-43.9) and among those were ever exposed to wood dust the RR of formaldehyde exposure was 39.5 (95% CI: 22.0-70.8). Given the differences between the stratum-specific results, which indicate effect modification, and between the stratum-specific results and the adjusted results, it is the stratum-specific results that provide the most valid measures of effect. The analyses by Olsen and Asnaes (1986) did not control for smoking as that data was unavailable but tobacco use was not shown to be a confounder in the study by Hayes et al (1986) or the study by Luce et al. (2002).

The third study that identified cancers by cell type (Luce et al., 1993) did not find an association between formaldehyde exposure and squamous cell carcinoma after controlling for age, wood dust, glues, and adhesives. For adenocarcinoma, the investigators attempted to control for wood dust but found that almost all of the cases of were coexposure to formaldehyde

and wood dust. Among workers with lower exposures to wood dust, the OR for any exposure to formaldehyde was 8.1 (95% CI: 0.9-72.9). The OR for wood dust among those not exposed to formaldehyde was 130 (95% CI: 14.2-1191). Since the large but not statistically significant result for formaldehyde was not among those never exposed to wood dust but may have included workers 'probably' exposed or 'definitely exposed to low lifetime levels,' residual confounding by wood dust may have yielded the reported finding.

More recently, Luce et al. (2002) pooled data from 12 case-control studies. Pooled studies are different from meta-analyses in that they compile the original raw data at the level of the individual into a single pooled dataset while meta-analyses examine the summary results across studies. Luce et al. (2002) assessed the associations between sinonasal cancer and occupational exposures to formaldehyde after developing a common job exposure matrix specifically for this study which standardized the different classifications of exposures and potential confounders. Disease coding was also standardized to a single system to increase compatibility. Combined, these studies contributed 195 adenocarcinomas and 432 squamous cell carcinomas of the sinonasal passages compared with 3,136 controls. Analyses controlled for age and study and were conducted separately by gender. Potential confounders were evaluated and controlled for. Among men, the investigators were able to control for wood dust and leather dust when evaluating the effects of formaldehyde on adenocarcinoma. The authors reported a statistically significant increase in the risk of sinonasal adenocarcinoma in men (adjusted OR = 3.0 [95% CI: 1.5−5.7]) and in women (adjusted OR = 6.2 [95% CI: 2.0−19.7]) with a high probability of exposure to formaldehyde. Luce et al. (2002) checked for residual confounding by wood dust by regrouping wood dust exposure into 5 categories, using the cumulative level of wood dust as a continuous variable and using the highest lifetime level of exposure and reported that the results were 'not markedly changed' indicating no evidence of residual confounding. For squamous cell carcinomas, the ORs were more modest: OR = 1.2 in men and OR = 1.5 in women for a high probability of exposure to formaldehyde. In an analysis of 11 formaldehyde-exposed cases of sinonasal adenocarcinomas who were not exposed to wood dust, there was an elevated risk in men (OR = 1.9; 3 cases) and a significantly increased risk in women (OR = 11.1 [95% CI: 3.2−38.0]; 5 cases) with a high probability of exposure to formaldehyde. Limitations of these studies were the lack of information about the actual levels or intensity of exposure to formaldehyde, exposure to multiple occupational carcinogens, and the small number of cases in some subgroups. In spite of those limitations, which generally obfuscate the observation of a true underlying effect, the studies evaluated by Luce et al. (2002) identified effects of formaldehyde that were statistically significant predictors of sinonasal cancers and this association was not likely to be attributable to bias, chance or confounding.

Table 4-4. Case-control studies of formaldehyde exposure and nasal and paranasal (sinonasal) cancers

Reference	Study design	Exposure assessment	Results			
Studies without specification by cell types						
Brinton et al. (1984)	Case-control study of 160 patients with cancer of the nasal cavity and paranasal sinuses from four North Carolina and Virginia hospitals matched with 290 hospital controls with other conditions. Odds ratios adjusted for cigarette smoking, alcohol consumption, gender, and age.	Interview data on job history. Estimation of exposure based on industry type. Only two cases employed in industry associated with formaldehyde. There were no deaths in the high exposure category.	Overall male and female	RR	0.35	(95% CI: 0.1−1.8)
			Residence in mobile home	OR	0.6	(95% CI: 0.2−1.7)
			Years of exposure to particleboard			
			1 to 9	OR	1.8	(95% CI: 09−3.8)
			10 or more		1.5	(95% CI: 0.7−3.2)
Olsen et al. (1984)	Case-control study of 488 cases of nasal cancer linked to the Danish Cancer Registry during 1970−1982. Controls were individuals with cancer of the colon, rectum, breast, and prostate. Three controls per case were selected for the same distributions of age, sex, and year of diagnosis as cases.	Employment histories after 1964 from files maintained by Danish Cancer Registry estimated by industrial hygienists.	Men			
			Formaldehyde only			
			Ever exposed	RR	2.8	(95% CI: 1.8−4.3)
			Exposure to wood dust and formaldehyde	RR	3.1	(95% CI: 1.8−5.3)
			Ever exposed	RR	3.5	(95% CI: 2.2−5.6)
			1st exposure >10 years or more before diagnosis	RR	4.1	(95% CI: 0.2.3−7.3)
Vaughan et al. (1986a), Washington	Population-based, *n* = 53 incident cases (1980−1983) from a 13-county area (Washington State Cancer Surveillance System) and 552 matched controls from random digit dialing in same area, for occupational exposures. Adjusted for cigarette smoking,	Interview-based information on lifetime occupational exposure to formaldehyde with cases, next of kin, and controls. Exposure from available hygiene data, NIOSH and other data, and NCI job exposure linkage system. Exposure score	Intensity			
			Low	OR	0.8	(95% CI: 0.4−1.7)
			Medium/high		0.3	(95% CI: 0.0−1.3)
			No. years exposed			
			1−9	OR	0.7	(95% CI: 0.3−1.4)
			10 or more		0.4	(95% CI: 0.1−1.9)

Table 4-4. Case-control studies of formaldehyde exposure and nasal and paranasal (sinonasal) cancers (continued)

Reference	Study design	Exposure assessment	Results
Vaughan et al. (1986a), Washington (continued)	alcohol consumption, gender, and age.	based on sum of no. years spent per job weighted by estimated formaldehyde level.	Exposure score (no lag) 5−19 OR 0.5 (95% CI: 0.1−1.6) 20 or more 0.3 (95% CI: 0.0−2.3) Exposure score (15 year lag) 5−19 OR 1.0 (95% CI: 0.3−2.9) 20 or more 0.0 (95% CI: --)
Vaughan et al. (1986b), Washington	Same cases and controls as Vaughan et al. (1986a). Adjusted for ethnic origin and cigarette smoking.	Same as Vaughan et al. (1986a). Also included residential history in past 50 years, and use of particleboard or plywood.	Residence in mobile home OR 0.6 (95% CI: 0.2−1.7) Years of exposure to particleboard 1−9 OR 1.8 (95% CI: 0.9−3.8) 10 or more 1.5 (95% CI: 0.7−3.2)
Roush et al. (1987), Connecticut	Population-based case-control study of 198 male cases of sinonasal cancer from the Connecticut Tumor Registry who died of any cause in 1935−1975. Controls were 605 males dying in Connecticut during the same time period, randomly selected from state death certificates. Adjusted for age at death, year at death, and availability of occupational information.	Four exposure categories based on probability and duration: I, probably exposed most of working life; II, probably exposed most of working life and probably exposed 20+ years before death; III, probably exposed most of working life and probably to high level in some year; IV, probably exposed most of working life and probably exposed to high level 20+ years before death.	Sinonasal cancer Exposure level I OR[a] 0.8 (95% CI: 0.5−1.3) II 1.0 (95% CI: 0.5−1.8) III 1.0 (95% CI: 0.5−2.2) IV 1.5 (95% CI: 0.6−3.9)

Table 4-4. Case-control studies of formaldehyde exposure and nasal and paranasal (sinonasal) cancers (continued)

Reference	Study design	Exposure assessment	Results
Studies with specification by cell types			
Hayes et al. (1986)	Case-control study of 91 men with squamous cell carcinoma of the nasal cavity and paranasal sinuses, from clinical records of six medical institutions in the Netherlands. 195 controls from living and deceased males from municipal residence registries, from 1978−1981.	Industrial hygienists evaluated job histories according to probability of exposure based on job records.	**Squamous cell carcinoma** Industrial hygienist A Any exposure — RR — 3.0 (90% CI: 1.3−6.4) Moderate exposure — 2.7 (90% CI: 1.0−7.2) High exposure — 3.1 (90% CI: 0.9−10.0) Industrial hygienist B Any exposure — RR — 1.9 (90% CI: 1.0−3.6) Moderate exposure — 1.4 (90% CI: 0.5−3.4) High exposure — 2.4 (90% CI: 1.1−5.1)
Olsen and Asnaes (1986)	Case-control study of histologically confirmed cases of squamous cell carcinoma/lymphoepithelioma of the sinonasal cavities and paranasal cancers in 215 men and adenocarcinomas of the sinonasal cavities and paranasal cancers in 39 men matched with 2,465 controls with other cancers from the Danish Cancer Registry, 1970−1982.	Employment histories after 1964 from files maintained by Danish Cancer Registry estimated by industrial hygienists.	**Squamous cell carcinoma/lymphoepithelioma** Ever vs. never Formaldehyde only — RR — 2.0 (95% CI: 0.7−5.9) Formaldehyde + wood dust — RR — 1.6 (95% CI: 0.8−3.3) 10 or more years since first exposure Formaldehyde only — RR — 1.4 (95% CI: 0.3−6.4) Formaldehyde + wood dust — RR — 1.8 (95% CI: 0.7−4.4) **Adenocarcinoma** Ever vs. Never Formaldehyde only — RR — 7.0 (95% CI: 1.1−44) Formaldehyde + wood dust — RR — 40.0 (95% CI: 22−71)

Table 4-4. Case-control studies of formaldehyde exposure and nasal and paranasal (sinonasal) cancers (continued)

Reference	Study design	Exposure assessment	Results			
Olsen and Asnaes (1986) (continued)			10 or more years since first exposure			
			Formaldehyde only	RR	9.5	(95% CI: 1.6−58)
			Formaldehyde + wood dust	RR	44.0	(95% CI: 22−88)
Luce et al. (1993)	Case-control study of men with sinonasal cancer (histologically confirmed), 77 with adenocarcinoma, 59 with squamous cell carcinomas, and 25 tumors of other types, matched with 409 controls from 27 French hospitals and from lists of names supplied by patients.[b] All had medium to high exposure to wood dust. Adjusted for age and exposure to glues and adhesives. adjusted for age and study.	Industrial hygienist estimation based on job histories from personal interviews. Exposure classification based on probability, frequency, and concentration level. Frequency categories (% in normal week): 1 = <5%, 2 = 5−30%, 3 = >30%. Concentration categories: low (0.0-0.1 ppm), medium (0.1−1 ppm), high (>1 ppm). Exposure index = concentration × frequency. Cumulative level = sum of exposure indices. Average level = cumulative level/duration and ranged from 1 to 9. Nearly all cases had had wood dust exposure.	**Adenocarcinoma**			
			Possible exposure	OR	1.28	(95% CI: 0.16−10)
			Probable/definite exposure			
			Average level			
			≤2	OR	4.15	(95% CI: 0.96−18)
			>2		5.33	(95% CI: 1.28−22)
			Duration (years)			
			≤20	OR	1.03	(95% CI: 0.18−5.77)
			>20		6.86	(95% CI: 1.69−28)
			Cumulative level (years)			
			≤30	OR	1.13	(95% CI: 0.19−6.95)
			30−60		2.66	(95% CI: 0.38−19)
			>60		6.91	(95% CI: 1.69−28)
			Age 1st exposed (years)			
			≤15	OR	9.99	(95% CI: 1.85−54)
			16−20		4.12	(95% CI: 0.95−18)
			>20		2.74	(95% CI: 0.58−13)
			Date 1st exposed (years)			
			After 1954	OR	6.02	(95% CI: 1.18−31)
			Before 1954		4.26	(95% CI: 1.06−17)

Table 4-4. Case-control studies of formaldehyde exposure and nasal and paranasal (sinonasal) cancers (continued)

Reference	Study design	Exposure assessment	Results			
Luce et al. (1993) (continued)			**Other cell type carcinoma**			
			Possible exposure	OR	0.81	(95% CI: 0.15−4.36)
			Probable/definite exposure			
			Average level			
			≤2	OR	1.67	(95% CI: 0.51−5.42)
			>2		3.04	(95% CI: 0.95−9.7)
			Duration (years)			
			≤20	OR	2.82	(95% CI: 0.94−8.4)
			>20		1.62	(95% CI: 0.48−5.51)
			Cumulative level (years)			
			≤30	OR	2.18	(95% CI: 0.65−7.31)
			>30		2.21	(95% CI: 0.73−6.73)
			Age 1st exposed (years)			
			≤20	OR	2.03	(95% CI: 0.63−6.54)
			>20		2.36	(95% CI: 0.76−7.33)
			Date 1st exposed (years)			
			After 1954	OR	0.48	(95% CI: 0.05−4.35)
			Before 1954		3.27	(95% CI: 1.15−9.33)

Table 4-4. Case-control studies of formaldehyde exposure and nasal and paranasal (sinonasal) cancers (continued)

Reference	Study design	Exposure assessment	Results			
Luce et al. (2002)	Pooled analysis of 195 adenocarcinomas and 432 squamous cell carcinomas of the sinus/nasal cavity matched with 3,136 controls from 12 case-control studies. Adenocarcinoma results in men adjusted for age, study, and cumulative exposure to wood and leather dust. All other results adjusted for age and study.	Job exposure matrix based on interview data developed for pooled analysis. Industrial hygiene data used to develop indices of exposure. 11 formaldehyde cases reported no exposure to wood dust.	**Adenocarcinoma**			
			High probability of exposure			
			Men	OR[d]	3.0	(95% CI: 1.5−5.7)
			Women	OR[e]	6.2	(95% CI: 2.0−20)
			Squamous cell carcinoma			
			High probability of exposure			
			Men	OR[e]	1.2	(95% CI: 0.8−1.8)
			Women	OR[e]	1.5	(95% CI: 0.6−3.8)

4.1.2.1.2.3. ***Summary of nasal and paranasal cancers.***

Rare outcomes are difficult to study due to a lack of statistical power to identify risks. Most cohort studies observed no more than one case of sinonasal cancer and may not have had sufficient statistical power to show an association. The exception is the national population-based study linking the Danish cancer registry and industry data in which 13 cases were identified for a SPIR of 2.3 (95% CI 1.3−4.0) (Hansen and Olsen, 1995). The case-control studies of sinonasal cancer in which no excess risk was seen did not distinguish cancer type and thus may have aggregated a truly causal relationship with adenocarcinoma and a noncausal relationship with squamous cell carcinoma. Among all the studies of sinonasal cancer, the pooled case-control study of Luce et al. (2002) provides the strongest causal evidence of an association between formaldehyde exposure and increased risk of sinonasal adenocarcinoma. In summary, there appears to be increased risk of sinonasal cancer associated with formaldehyde exposure with or without exposure to wood dust (Luce et al., 2002). The effect appears to be stronger when the risk is stratified by cancer type with higher risks of adenocarcinoma compared with squamous cell carcinoma. Taken together with the nasopharyngeal cancer findings in the neighboring tissue, EPA concludes that there is evidence of a causal association of sinonasal cancer associated with exposure to formaldehyde.

4.1.2.1.3. *Cohort studies of lung cancer.*

Several industrial cohort studies (Andjelkovich et al., 1995; Stayner et al., 1988; Edling et al., 1987) reported no significant excess risks of lung cancer from exposure to formaldehyde. Bertazzi et al. (1986) studies a cohort of 1322 workers producing formaldehyde resins in Italy and reported an increased risk of lung cancer in the initial analysis with an SMR of 1.86 (95% CI:1.10-2.93) based on 18 cases; however, the reanalysis of this cohort with six additional years of follow-up (Bertazzi et al., 1989) showed no excess risk with 24 cases of lung cancer compared to 23.9 expected. No consistent association between formaldehyde exposure and lung cancer was found in several reports of the NCI 10-plant cohort study originally investigated by Blair et al. (1987, 1986). Hauptmann et al. (2004) give the most recent report on this cohort, which has been studied in part or in its entirety by several others (Marsh et al., 1994, 1992a, b; Sterling and Weinkam, 1994, 1989a, b, 1988; Robins et al., 1988; Liebling et al., 1984; Fayerweather et al., 1983; Wong, 1983; Marsh, 1982). Hauptmann et al. (2004) reported 641 cases of lung cancer against 660.8 expected for an SMR of 0.97 (95% CI: 0.90-1.05).

Gardner et al. (1993) found a modest association between lung cancer and formaldehyde exposure in the British chemical industry cohort study (n = 14,016) among men hired before 1965 with a SMR of 1.23 (95% CI: 1.10−1.36) based on national rates of expected deaths and a

SMR of 1.12 (95% CI: 1.00-1.24) based on local deaths rates. In workers hired after 1964, the corresponding SMRs were 1.14 (95% CI: 0.85-1.48) and 1.13 (95% CI: 0.85-1.47). No trends by level or duration of exposure were found (Gardner et al., 1993). Coggon et al. (2003) updated the Gardner et al. (1993) cohort study with additional follow-up through the end of 2000 and reported the effects of formaldehyde exposure on mortality from lung cancer among 14,014 workers. Coggon et al. (2003) reported an SMR of 1.22 (95% CI: 1.12-1.32) for lung cancer in the entire cohort. In the analysis of the combined data from the 6 factories, an excess risk of lung cancer was seen in the high-exposure category when compared with British national mortality rates (SMR = 1.58 [95% CI: 1.40−1.78]) and with local mortality rates (SMR = 1.28 [95% CI: 1.13−1.44]). Pinkerton et al. (2004) and Stayner et al. (1988) studied a cohort of 11,030 workers in three garment plants and found an SMR of 1.14 for lung cancer (95% CI: 0.81-1.56). Callas et al. (1996) reanalyzed the cumulative exposure of 279 lung cancer cases among white male workers from the NCI study, which comprised 80% of the NCI cohort (Blair et al., 1986). The analysis revealed RRs of 1.46, 1.27, and 1.38 for lung cancer in the cumulative exposure categories of 0.05 to 0.5 ppm-years, 0.51 to 5.5 ppm-years, and > 5.5 ppm-years, respectively. None of these RRs were statistically significant.

None of the cohort studies of workers in specific professions indicated excess risks of lung cancer. Of the professional studies reviewed, the RRs range from an extremely low SMR value of 0.2 in Hall et al. (1991), based on nine deaths, to an RR (proportional mortality ratio) of 1.1, based on 70 lung cancer deaths in Walrath and Fraumeni (1983). Matanoski (1991) reported a significant deficit in the risk of respiratory cancer (SMR = 0.56 [95% CI: 0.44−0.70]; 77 observed) in pathologists presumably exposed to formaldehyde based on U.S. mortality rates.

4.1.2.1.4. *Case-control studies of lung cancer.*

Several case-control studies of lung cancer (Partanen et al., 1990; Gerin et al., 1989; Bond et al., 1986; Coggon et al., 1984; Fayerweather et al., 1983; Anderson et al., 1982) showed no excess lung cancer risk associated with potential exposure to formaldehyde when analyzed by length of exposure, intensity, and potential exposure 5, 10, or 15 years before death or by combinations of these factors. By contrast, Coggon et al. (1984) reported an increased in risk of lung cancer among male patients with any potential exposure to formaldehyde based on occupations listed on death certificates (SMR = 1.5 [95% CI: 1.2−1.8]). De Stefani et al. (2005) conducted a case-control study of 338 adenocarcinomas of the lung in male patients admitted to four Montevideo hospitals in Uruguay from 1994 to 2000. Three agents (i.e., asbestos, silica dust, and formaldehyde) indicated excess risks of lung adenocarcinoma after adjusting for smoking history. The association seen with 21 or more years of exposure to formaldehyde was

OR = 3.0 (95% CI: 1.6−5.8), with an exposure-response trend seen across exposure levels (trend $p = 0.004$).

Summary of lung cancer. Evidence of a relationship between formaldehyde exposure and lung cancer is relatively weak and conflicting, with some studies showing modest increases (e.g., relative risks of 1.2, as seen in Coggon et al., 2003) while others show inverse associations between exposure and risk. In all studies of formaldehyde and lung cancer, smoking remains an important confounder and possibly an effect modifier. Residual confounding of smoking or other respiratory exposures (e.g., wood dust or chemical or particular exposures) must always be considered. A meta-analysis of workers exposed to formaldehyde by Bosetti et al. (2008) reported a summary effect estimate of RR = 1.06 (95% CI: 0.92-1.23) for industry workers and RR = 0.63 (95% CI: 0.47-0.84) for professionals. The meta-analysis by Blair et al. (1990) reported a RR = 0.9 for professional and nonoccupational exposures and RR = 1.1 among industrial workers. The meta-analysis by Partanen (1993) largely confirmed the results of Blair et al. (1990). Except for the findings of De Stefani et al. (2005), other studies of lung cancer and exposure to formaldehyde have not supported the finding by Coggon et al. (2003), including several well-done cohort studies that were specifically designed to evaluate lung cancer.

4.1.2.1.5. *Other respiratory tract cancers.*

Other cancers of the upper respiratory tract include buccal cavity, salivary gland, floor of the mouth, other mouth, larynx, pharynx (including oropharynx and hypopharynx)

4.1.2.1.5.1. ***Cohort studies of other respiratory tract cancers.***

In studies of industrial worker cohorts where buccal/pharyngeal cancer was examined (Andjelkovich et al., 1995; Stayner et al., 1988; Blair et al., 1986), only one (Stayner et al., 1988) reported an excess risk of death from this tumor (SMR = 3.4, based on four deaths in a cohort of 6,741 white women). Of six cohort studies of buccal/pharyngeal cancer in studies of professionals reviewed by IARC (Hayes et al., 1990; Logue et al., 1986; Stroup et al., 1986; Levine et al., 1984; Walrath and Fraumeni, 1984, 1983), no evidence of a risk associated with exposure to formaldehyde was reported.

Hansen and Olsen (1995) reported a 10% increase in the risk of cancer of the buccal cavity and pharynx (SPIR = 1.1, 95% CI 0.7, 1.7) in their proportional incidence study of Danish workers. Marsh et al. (1996) reported no significant excess risk of buccal cavity cancer cases (SMR = 1.31) based on U.S. mortality rates and no excess based on State mortality rates (SMR = 1.0). For oropharyngeal cancer, the SMR was 1.84 (based on two cases), the SMR for

hypopharyngeal cancer was 1.41 (based on one case), and the SMR for laryngeal cancer was 1.47 (based on six cases). The latter risks were elevated even when SMRs were derived from Connecticut mortality rates.

A nested case-control study, based on the 22 pharyngeal cancer (including the 7 nasopharyngeal cancers) deaths in the Wallingford, Connecticut, plant cohort was also reported in Marsh et al. (2002). Each of the pharyngeal cancer deaths was matched on race, sex, age, and year of birth to four controls from the cohort. Data on smoking status and date of hire were collected and controlled for in the analyses of the pharyngeal cancers but apparently not for nasopharyngeal cancer due to sparse data. Twenty of the 22 cases were exposed to formaldehyde, yielding an odds ratio (OR) of 3.04 (95% CI: 0.36-145.58) after adjustment for smoking and year of hire. There was little or no association of pharyngeal cancer incidence in these workers with either average or cumulative exposure, based on the exposure estimates in this study. There was a suggested trend of increasing OR with increasing duration of exposure for any formaldehyde exposure as well as for formaldehyde exposure >0.2 ppm. The relatively flat dose-response curve in the nested case-control study contradicts the positive dose-response curves reported (particularly for nasopharyngeal cancers) in the same study, based on SMRs derived from county and U.S. death rates in the cohort analysis.

Hauptmann et al. (2004) combined upper respiratory tract cancers (cancers of the salivary gland, mouth, nasopharynx, nasal cavity, and larynx; n = 11 observed deaths). For average intensity of exposure, the RR was 1.47, 1.69, and 2.21 for the low (> 0−0.5 ppm), medium (0.5 to <1.0 ppm) and high (≥ 1.0 ppm) exposure categories, respectively, (trend p = 0.122). Confidence intervals were not provided for these RR; however, a footnote states that for the RR = 2.21 for the highest exposure category (≥ 1.0 ppm), the 95% confidence interval does not include 1.00. For peak exposure, the RR was 1.32, 1.24, and 1.65 for the low (> 0− <2.0 ppm), medium (2.0− <4.0 ppm) and high (≥4.0 ppm) exposure categories, respectively (trend p = 0.142). For cumulative exposure, the RR was 1.24, 1.92 and 0.86 for the low (> 0−1.5 ppm-yrs), medium (1.5− <5.5 ppm-yrs) and high (≥5.5 ppm-yrs) exposure categories (trend p = 0.0.765). Increased relative risks of cancer of the buccal cavity (which included salivary glands, the floor of the mouth, other mouth and the nasopharynx) were reported for the medium and high exposure categories with 95% confidence intervals that did not include 1.00 for the medium exposure category. Hauptmann et al. (2004) concluded that in spite of the small number of deaths from these rare cancers of the upper respiratory tract, the positive associations of increased cancer risk with increased formaldehyde exposure were consistent with the carcinogenicity of formaldehyde at these sites of first contact.

4.1.2.1.5.2. ***Case-control studies of other respiratory tract cancers.***

Three case-control studies (Merletti et al., 1991; Vaughan et al., 1986a, b) did not find an association between oral, oropharyngeal, and hypopharyngeal cancers and formaldehyde exposure. However, Merletti et al. (1991) found an elevated OR of 1.8 associated with probable or definite exposure to formaldehyde in a study of 86 patients with oral or oropharyngeal cancer matched with 373 controls. There was no increased risk of laryngeal cancer associated with formaldehyde in a case-control study (Wortley et al., 1992) of 235 patients with laryngeal cancer and 547 controls. The OR in that study was 1.0 (adjusted for age, smoking, drinking, and level of education). IARC (1995) concluded that there was little evidence of an increased risk of laryngeal cancer.

Three additional studies, published since the IARC (1995) report of other respiratory cancers (e.g., oral cavity, oropharynx, and/or larynx), are available (Shangina et al., 2006; Laforest et al., 2000; Gustavsson et al., 1998). Gustavsson et al. (1998) conducted a case-control study of 545 cases of squamous cell carcinoma of the oral cavity, oropharynx, hypopharynx, larynx, and esophagus and frequency-matched by age and region with 641 controls. Regression analyses among 545 male cases showed elevated but nonsignificant risks of squamous cell carcinoma of the oral cavity (OR = 1.28), esophagus (OR = 1.90), and larynx (OR = 1.45) associated with formaldehyde exposure. However, several of the carcinoma types were statistically significantly associated with exposure to welding fumes, polyaromatic hydrocarbons, asbestos, and metal dust. Laforest et al. (2000) examined 201 patients with squamous cell carcinoma of hypopharynx and 296 patients with squamous cell carcinoma of larynx, who were matched to 296 controls with cancers of other sites in 15 French hospitals. Adjusting for potential confounders, the OR of hypopharyngeal cancer in patients with a high probability of exposure to formaldehyde was 3.78 (95% CI: 1.50−9.49). The ORs were significantly increased with both exposure durations and high cumulative level of exposure. Shangina et al. (2006) conducted a multicentered case-control study of 34 cases of hypopharyngeal cancer, 316 cases of laryngeal cancer, and 728 hospital-based controls. The study was based on Romania, Poland, Russia and Slovakia. Data was collected through a structured interview and included a detailed work history including information on all jobs held for at least one year and more detailed modules for specific jobs. These data were evaluated by local industrial hygienists, chemists, and physicians, blinded to disease status. Exposure classifications were developed for 73 agents; frequency was estimated as the proportion of time a worker was exposed. Linear trends were examined for duration in years, weighted duration in hours, and cumulative exposure. The OR for formaldehyde exposure and laryngeal cancer was 1.68 (95% CI: 0.85−3.31). Trends over increasing exposure were found for duration of exposure in years (p = 0.06) and for cumulative

exposures (p = 0.07). The investigators reported an OR of 3.12 (95% CI: 1.23−7.91) for the highest cumulative exposure group (>22,700 mg/m^3-hours) compared with the unexposed group.

4.1.2.1.5.3. ***Summary of other respiratory tract cancers.***

The evidence for a compound-specific effect on the risk of buccal/pharynx, oral cavity, oropharynx, hypopharynx, and laryngeal cancers as a result of exposure to formaldehyde is slight when examining each rare endpoint on its own. The study by Hauptmann et al. (2004) did show an increased risk of cancers of the buccal cavity and the upper respiratory tract as a group that had confidence interval that did not include 1.00. Further evidence comes from the results of the study by Laforest et al. (2000) showing nearly four-fold increased risk of hypopharyngeal cancer and, to a lesser extent, that by Shangina et al. (2006) who demonstrated an exposure-response relationship for duration of formaldehyde exposure and risk of laryngeal cancer. However, taken together with the causal evidence of an association between formaldehyde and nasopharyngeal and sinonasal cancers in neighboring tissues of the upper respiratory tract and sites of first contact, these sporadic results in humans may indicate a broader pattern of carcinogenicity within the upper respiratory tract.

4.1.2.1.5.4. ***Summary of respiratory tract cancers.***

The epidemiologic studies of nasopharyngeal cancer provide strong support for a causal role of formaldehyde exposure to the etiology of this rare cancer. The Hauptmann et al. (2004) analysis of the large NCI cohort study of 10 worksites in the United States reported a doubling of nasopharyngeal cancer mortality risk in workers (SMR = 2.1, 95% CI: 1.05−4.21). Hauptmann et al. demonstrated that relative risks increased with average exposure intensity (p-trend = 0.066), cumulative exposure (p-trend = 0.025), highest peak exposure (p-trend < 0.001) and duration of exposure to formaldehyde (p-trend = 0.147). The case-control studies also provide a robust collection of data supporting a similar association between formaldehyde exposure and nasopharyngeal cancer (i.e., relative risk estimates in the range of 1.5−3.0). The association between formaldehyde and nasopharyngeal cancer persisted when adjusted for the effect of potential confounders. Data from several of these studies have identified a concentration-response relationship (Hauptmann et al., 2004; Marsh et al., 2002; Vaughan et al., 2000; West et al., 1993), adding weight to the causal evidence of an association between formaldehyde exposure and cancer. The studies of the single Wallingford plant by Marsh et al. (2002, 1996, 1994) and Marsh and Youk (2005) also revealed a dose-response trend, although the absolute exposure level estimates were much lower according to Marsh et al. (2002).

Sinonasal cancer is also a rare cancer, and most cohort studies have had insufficient statistical power to show an association. The exception is the national population-based study linking the Danish Cancer Registry and industry data in which 13 cases were identified for a SPIR of 2.3 (95% CI 1.3−4.0) (Hansen and Olsen, 1995). The case-control studies of sinonasal cancer in which no excess risk was seen, did not distinguish cancer type and thus may have aggregated a truly causal relationship with adenocarcinoma with a noncausal relationship with squamous cell carcinoma. Among all the studies of sinonasal cancer, the pooled analysis by Luce et al. (2002) provides the strongest evidence of a relationship between formaldehyde and sinonasal cancer, particularly adenocarcinoma, which did not appear to be confounded by concurrent exposure to wood dust. The effect appears to be stronger when the risk is stratified by cancer type with higher risks of adenocarcinoma compared with squamous cell carcinoma. Taken together with the nasopharyngeal cancer findings in the neighboring tissue, EPA concludes that there is evidence of a causal association of sinonasal cancer associated with exposure to formaldehyde.

Evidence of a relationship between formaldehyde exposure and lung cancer is relatively weak and conflicting, with some studies showing modest increases (e.g., relative risks of 1.2, as seen in Coggon et al., 2003) while others show inverse associations between exposure and risk.

The evidence for a compound-specific effect on the risk of buccal/pharyngeal, oral, oropharyngeal, hypopharyngeal, and laryngeal cancers as a result of exposure to formaldehyde is slight when examining each rare endpoint on its own. The study by Hauptmann et al. (2004) did show an increased risk of cancers of the buccal cavity and the upper respiratory tract as a group that had confidence interval that did not include 1.00. Further evidence comes from the results of the study by Laforest et al. (2000) showing nearly four-fold increased risk of hypopharyngeal cancer and, to a lesser extent, that by Shangina et al. (2006) who demonstrated an exposure-response relationship for duration of formaldehyde exposure and risk of laryngeal cancer. However, taken together with the causal evidence of an association between formaldehyde and nasopharyngeal cancer and sinonasal cancer in neighboring tissues of the upper respiratory tract and sites of first contact, these sporadic results in humans may indicate a broader pattern of carcinogenicity within the upper respiratory tract.

4.1.2.2. *Nonrespiratory Tract Cancer*

4.1.2.2.1. *Lymphohematopoietic (LHP) cancers.*

Cancers of the hematopoietic system include lymphosarcoma, reticulosarcoma, Hodgkin's disease, non-Hodgkin's disease, multiple myeloma, and all types of leukemia, including lymphoid and myeloid. Virtually all of the studies of LHP cancers and formaldehyde are cohort studies and are divided into two groups: professional and industrial. Several of the

studies of professional groups were reviewed in an IARC (1995) monograph and are briefly discussed in the next section regarding their findings on cancer of the LHP system. One case-control study of non-Hodgkin's lymphoma is discussed at the end of this section.

4.1.2.2.1.1. ***Professional cohort studies.***

Several cohort studies have been undertaken by professional groups (i.e., anatomists, pathologists, embalmers, and funeral directors) because their careers are likely to bring them into contact with formaldehyde. Some studies have reported an increase in the risk of myelogenous leukemia and other LHP cancers (see Table 4-5). A few of the increased risks were statistically significant. None of the studies of professionals have used personal exposure measurements of formaldehyde or other chemicals, making specificity for any single exposure difficult to determine.

Harrington and Shannon (1975) conducted a cohort mortality study of 2,079 British pathologists (1955−1973) and 12,944 British medical laboratory technicians (1963−1973). When compared with death rates for England and Wales, the all-cause SMR for the pathologists was 0.60 versus 0.67 for the laboratory technicians. There was a significant increase in the risk of lymphatic and hematopoietic neoplasia (SMR 2.0; 8 observed with 3.3 expected; $p < 0.01$) among male pathologists. However, the SMR for technicians was only 0.6 (3 observed). The low SMRs suggest that these professionals have a healthier profile compared with the British population. No actual exposure estimates are available.

Harrington and Oakes (1984) expanded the above study to include 2,307 male and 413 female pathologists. Mortality was only examined from 1973 until 1980; deaths that occurred before 1974 were not included in the update. The SMR for leukemia was 0.91 in men and 9.26 (based on one case) in women. Although the earlier LHP cancer deaths were not included in this analysis, the investigators say in their conclusion that their previous suggestion of an increase in certain lymphatic neoplasia was not confirmed in the present study because of small numbers. The exceptionally low SMRs suggest that this group of professionals enjoyed a healthier lifestyle compared with the British population as a whole. Just as in the earlier studies of these professionals, no exposure estimates are available.

Hall et al. (1991) expanded the above study by including the newest members of the Royal College of Pathologists. The cohort totaled 4,512 individuals, although only 3,069 males and 803 females were included in the analysis. The reasons for this discrepancy were not specified, although the authors mentioned that an unknown number of expected deaths for Northern Irish and female Scottish pathologists were not calculated, 32 pathologists were lost in follow-up, and cause of death was unknown for 9 individuals. Follow-up was extended from

Table 4-5. Epidemiologic studies of formaldehyde and pharyngeal cancer (includes nasopharyngeal cancer)

Reference	Study design	Exposure assessment	Results, statistical significance (number observed deaths for cohort study)				
Marsh et al. (2002)	Retrospective cohort mortality study of 7,328 workers hired up to 1984 and followed until 1998 in one plant from Blair et al. (1986, 1987) and Hauptmann et al. (2004). Mortality was compared with death rates in two Connecticut counties and U.S. A nested case-control analysis was also conducted with 4 controls matched on age, year of birth, race, and sex randomly selected from cohort. Conditional logistic model was used for nested case-control analysis.	Worker-specific exposures from job exposure matrix were based on available sporadic sampling data from 1965−1987, job descriptions, and verbal job descriptions by plant personnel and industrial hygienists. Exposures were then ranked on a 7-point scale. An exposure range was assigned to each rank. 17% of jobs validated with company monitoring data, remaining 83% based on professional judgment. Pre-1965 levels of formaldehyde were assumed to be the same as post-1965 levels.	Cohort study				
			Overall				
			U.S.	SMR	2.63	(95% CI: 1.65−3.98)	(22)
			County	SMR	2.23	(95% CI: 1.40−3.38)	(22)
			Short-term worker (<1 year)				
				SMR	2.35	(95% CI: 1.22−4.11)	(12)
			Long-term worker (1 or more years)				
				SMR	2.10	(95% CI: 1.01−3.86)	(10)
			Cumulative exp. (ppm-years) county				
			Unexposed	SMR	1.24	(95% CI: 0.15−4.49)	(2)
			>0 to <0.004	SMR	3.31	(95% CI: 1.22−7.21)	(6)
			0.004−0.219	SMR	2.06	(95% CI: 0.83−4.24)	(7)
			0.22+	SMR	2.30	(95% CI: 0.92−4.73)	(7)
			Average Exposure (ppm) county				
			Unexposed	SMR	1.24	(95% CI: 0.15−4.49)	(2)
			>0 to <0.03	SMR	2.02	(95% CI: 0.74−4.40)	(6)
			0.03−0.159	SMR	3.82	(95% CI: 1.54−7.88)	(7)
			0.16+	SMR	2.03	(95% CI: 0.82−4.19)	(7)
			Exposure to ≤0.2 ppm	SMR	1.72	(95% CI: 0.74−3.39)	(8)
			Exposure to >0.2 ppm	SMR	2.68	(95% CI: 1.46−4.49)	(14)
			Exposure to ≤0.7 ppm	SMR	2.12	(95% CI: 1.21−3.45)	(16)
			Nested case-control analysis				
			Cumulative exp. (ppm-years)				
			<0.004	OR	1.00		(8)
			0.004−0.219	OR	0.71	(95% CI: 0.20−2.43)	(7)
			0.22+	OR	0.79	(95% CI: 0.18−3.20)	(7)

Table 4-5. Epidemiologic studies of formaldehyde and pharyngeal cancer (includes nasopharyngeal cancer) (continued)

Reference	Study design	Exposure assessment	Results, statistical significance (number observed deaths for cohort study)				
Marsh et al. (2002) (continued)			Average exposure (ppm)				
			<0.03	OR	1.00		(8)
			0.03−0.159	OR	1.71	(95% CI: 0.47−6.10)	(7)
			0.16+	OR	0.99	(95% CI: 0.27−3.55)	(7)
			Exposure to >0.2 ppm	OR	1.35	(95% CI: 0.45−4.25)	(14)
			Exposure to >0.7 ppm	OR	1.60	(95% CI: 0.15−9.77)	(6)
Coggon et al. (2003)	Cohort mortality study of 14,014 chemical workers employed in 6 British factories.	Based on data abstracted from company records. Each job was categorized as having background, low, moderate, high, or unknown levels of formaldehyde.	Overall	SMR	1.55	(95% CI: 0.87−2.56)	(15)
			High exposure	SMR	1.91	(95% CI: 0.70−4.17)	(6)
Shangina et al. (2006)	Multicentered, hospital-based case-control study in four European countries; men only. Cancer cases: 34 hypopharyngeal; 316 laryngeal. Controls: 728 hospital patients with various conditions.	Exposures determined by local industrial hygienists, chemists, and physicians. Coding was established and standardized. Categories were developed for 73 agents; frequency was estimated as the proportion of time a worker was exposed. Linear trends were examined for duration in years, weighted duration in hours, and cumulative exposure.	<u>Laryngeal cancer:</u>				
			Formaldehyde				
			Ever vs. never	OR	1.68	(95% CI: 0.85−3.31)	
			Highest cumulative ($>22{,}700$ mg/m^3-hours) vs. lowest	OR	3.12	(95% CI: 1.23−7.91)	
			Tests of trends:				
			Years exposed	$p = 0.06$			
			Cumulative exposure	$p = 0.07$			

1980 to 1986. Mortality was enumerated from 1974 to 1987, a period of time that differed from both of the earlier studies described above. There were statistically not significant excess risks for lymphatic and hematopoietic cancer (SMR 1.44; 10 observed) and leukemia (SMR 1.52; 4 observed) for both sexes combined, based on mortality rates in England and Wales. Separately, there was 1 female death in the lymphatic and hematopoietic cancer category (0.57 expected). The most striking observation in this study is that, despite the low cancer mortality (SMR 0.45 for all cancer; 53 observed but 118.19 expected), there was still an excess (but not statistically significant) risk of hematopoietic cancers. This finding of an extremely low risk for all cancers suggests that population death rates may not be appropriate as a referent group—for example, the SMRs for lung cancer (0.19) and nonneoplastic respiratory diseases (0.23) were significantly decreased, suggesting a lower prevalence of smoking among the pathologists compared with the general population of England and Wales. However, the finding of a possibly increased risk of LHP cancers should be analyzed further by selecting a more appropriate reference population (another professional group without exposure to formaldehyde) or by utilizing internal comparisons.

Walrath and Fraumeni (1983) conducted a proportionate mortality study of all embalmers and funeral directors licensed in the state of New York between 1902 and 1980 who were known to have died between 1925 and 1980. While the investigators called their study a proportionate mortality ratio (PMR) study, the methods described the comparison population with which cancer deaths as the general population and not other deaths in the study population. The authors computed their expected number of cause-specific deaths based on the US male population stratified by 5-year age and calendar time periods. As such, their PMR meets the modern definition of the more methodologically appropriate standardized mortality ration (SMR). The authors did also compute what they called the proportionate cancer mortality ratio (PCMR) which is not standardized and may overestimate effects. The investigators requested death certificates for 1,678 persons but received only 1,263 (75%). The investigators restricted their analysis to 1,132 males. The distribution of the causes of death was compared with the age-, race-, and calendar-year-specific proportions of deaths for each cause among the male U.S. population. Duration of exposure was approximated by time since first license. While the methodology could not be applied in all calculations because of data gaps, excess risks were found for lymphatic and hematopoietic cancers, with a SMR of 1.2 (observed 25), and for leukemia, with a SMR of 1.32 and proportionate cancer mortality ratio (PCMR) of 1.19 (12 observed). The SMRs were not affected when the estimates were stratified by latency (<35 years or 35 years since first license) or by age at first license. Because the cause of death could not be determined for nearly 25% of the study group, the risk estimates could be

underestimated. When the mortality from leukemia was further examined, the investigators identified six of the 12 leukemias as myeloid and five as multiple myeloma. Walrath and Fraumeni (1983) reported that 4.1 myeloid leukemias were expected and therefore a true SMR for myeloid leukemia of can be calculated as SMR = 1.46 (0.21-2.72).

Using what was actually the standardized mortality ratio methods (although referring to it as the proportionate mortality method), Walrath and Fraumeni (1984) studied 1,007 deceased white male embalmers, members of the California Bureau of Funeral Directing and Embalming, whose deaths occurred between 1925 and 1980. The decedents had to have been licensed to practice between 1916 and 1978. For lymphatic and hematopoietic cancer, the PMR (actually an SMR) was 1.22 (19 deaths observed). For leukemia alone, the PMR was 1.75 and significant (12 deaths observed, $p < 0.05$). Among embalmers licensed for 20 years or longer, the risk of leukemia increased and was also significant (PMR (actually an SMR) 2.21; 8 observed; $p < 0.05$).

Levine et al. (1984) conducted a cohort mortality study of 1,477 male Ontario undertakers first licensed between 1928 and 1957 and followed until the end of 1977. Out of 359 subjects who had died, there were 8 deaths from lymphatic and hematopoietic cancers compared with 6.5 expected. Additionally, there were 4 deaths from leukemia versus 2.5 expected. Because death rates were not available for Ontario before 1950, person-years and deaths before 1950 could not be counted. No actual exposure estimates are available for these undertakers.

Stroup et al. (1986) conducted an historic cohort mortality study of 2,317 men who were members of the American Association of Anatomists between 1888 and 1969. The investigators derived SMRs from the U.S. white male population and used members of the American Psychiatric Association (APA) as a comparison group. Vital status was ascertained between 1925 and 1979. Women were excluded from analysis because of the small numbers. Only 738 deaths were observed versus 1,133.9 expected, based on U.S. death rates (SMR 0.65), possibly indicating a sizable HWE. However, a slight increase in the risk of lymphatic and hematopoietic cancers (SMR 1.2; 18 observed) and the risk of leukemia (SMR 1.5; 10 observed) was evident. A significant increase in the risk of brain cancer (SMR 2.7; 10 observed; $p < 0.05$) was also reported. When the leukemia analysis was restricted to the myeloid type, the SMR increased to 8.8, based on five deaths ($p < 0.05$). The analysis using the APA group was restricted to deaths that occurred between 1900 and 1969. This restriction removed five leukemia deaths and person-years from the analysis because they likely died after 1969. Because of this, there were only 3 leukemia deaths versus 3.6 expected, based on APA death rates. The investigators concluded that the etiological agent had not been definitively identified, mentioning

that a wide range of solvents, stains, and preservatives, including formaldehyde, are used to prepare biological specimens.

Logue et al. (1986) conducted a cohort study of male radiologists and pathologists registered with the Radiation Registry of Physicians and the College of American Pathologists (CAP) between 1962 and 1977. Although the main focus was on determining mortality in radiologists from exposure to ionizing radiation, mortality was also ascertained for pathologists alone. To derive SMRs, expected deaths were the sum of the products of person-years times death rates for both cohorts during the follow-up period in white males only. However, there were no exposure measurements, and the SMRs were not adjusted for calendar time. Of 5,585 members of the CAP, 496 had died by December 31, 1977. Although the SMR was 0.48 for pathologists for cancer of the lymphatic and hematopoietic system, for the more specific category of leukemia and aleukemia the SMR was 1.06 (neither was significant). For radiologists, the SMRs were 0.78 and 1.55, respectively, also not significant. Cause of death could not be determined for 8% of the deaths. Although age-adjusted rates for leukemia were also calculated for each cohort, they were only used for comparison between the two separate professional groups.

Hayes et al. (1990) conducted a cohort study of 3,649 deceased white and 397 deceased nonwhite U.S. male embalmers and funeral directors who had died between 1975 and 1985, using records from local licensing boards, state funeral directors' associations in 32 states and the District of Columbia, the National Funeral Directors' Association, and state offices of vital statistics (n = 894). Expected deaths by cause were derived from 5-year age- and calendar-year-specific proportions of deaths among appropriate race groups from the U.S. population. No measured exposure data were available. A PCMR would be derived by excluding noncancer causes of death. Statistically significant excesses in hematopoietic and lymphatic cancers were found in white (PMR (actually SMR)1.31 [95% CI: 1.06−1.59]; 100 observed) and nonwhite (PMR (actually SMR) 2.41 [95% CI: 1.35−3.97]; 15 observed) embalmers and funeral directors. The combined PMR (actually SMR) was 1.39 (95% CI: 1.15−1.63). The excess risk for all men was higher for myeloid leukemia (ML) (PMR (actually SMR) 1.57 [95% CI: 1.01−2.34]; 24 observed) and for other unspecified leukemias (PMR (actually SMR) 2.28 [95% CI: 1.39−3.52]; 20 observed) in white males.

Matanoski (1991) conducted a study of 6,111 male pathologists for NIOSH. Members of the cohorts were part of an earlier unpublished study. Twenty-nine thousand psychiatrists were used as a comparison group. Both samples were selected from the membership rolls of professional associations. A total of 3,787 pathologists died between 1940 and 1978. Women were excluded from the analysis. Of the population of psychiatrists, 4,788 died by 1980. U.S.

age- and calendar-time-specific death rates from 1925 were used to develop SMRs. Separate SMRs were based on psychiatrists' death rates. The risk of hematopoietic cancer (excluding Hodgkin's disease) was elevated (SMR 1.25; 57 observed) based on U.S. white males. For leukemia, the SMR was 1.35 (31 observed). The SMR for leukemia among psychiatrists was 0.83 (35 observed). Compared with leukemia in psychiatrists, the SMR for pathologists was 1.68 (95% CI: 1.14−2.38). The SMR for other lymphatic cancers was 1.53 (16 observed) and for LHP cancer 1.22 (64 observed). Comparing the pathologists' death rates to those of psychiatrists could be thought to have greater validity than if death rates for the U.S. population as a whole had been used, because of shared socioeconomic circumstances and access to medical care between the two professional groups. Differences in access to health care might have been greater for subjects in the earlier part of the study, because improved diagnosis and medical care for LHP cancers became more broadly available later in the study period. By using SMRs based on U.S. death rates, which include those who do not have adequate access to medical care, the difference between expected and observed deaths would be reduced. This is less likely to occur when one professional group is compared with another professional group, assuming psychiatrists and pathologists have equal access to care.

Hauptmann et al. (2009) conducted a nested case-control study of lymphohematopoietic, brain and nasopharyngeal cancers that included embalmers and funeral directors from previous cohort mortality studies (Walrath and Fraumeni 1983, 1984; Hayes et al. 1990). Death certificates for 6,808 embalmers and funeral directors who had died between January 1, 1960 and January 1, 1986 were coded for underlying and contributory causes of death. There were 168 deaths attributed to lymphohematopoietic cancers, with 99 of lymphoid origin and 48 of nonlymphoid origin, including 34 myeloid leukemias. Cases were matched to control subjects randomly selected from cohort members, died of other causes, excluding cancers of the buccal cavity and pharynx, respiratory system, and eye, brain and other parts of the nervous system. Controls (N = 265) were matched on data source, sex, and dates of birth and death.

Extensive interviews with the next of kin and coworkers of the cases and controls provided detailed information on the funeral homes and work practices of the study subjects. The authors noted that since the funeral industry is often a family business, these study subjects' next of kin were believed to be unusually knowledgeable of funeral home work practices. The work history component of these interviews provided data on the frequency and duration of embalmings for jobs held at least five years, as well as information on ventilation of the premises and the frequency of spills. These data were linked to data from an exposure-reconstruction experiment which sought to replicate standard funeral home practices while measuring exposures to formaldehyde. These exposure data were not specific to any of the workplaces, but do provide

a general idea of exposures during embalming. Specific work practices, facilities, and ventilation systems may vary widely between funeral homes potentially impacting actual exposure levels for any location (Stewart et al., 1992). An exposure model was constructed to retrospectively estimate study subjects' exposure. Multiple exposure metrics were estimated, including lifetime 8-hour time weighted average (TWA), cumulative, and peak exposures to formaldehyde.

Odds ratios were estimated for all lymphohematopoietic cancers combined, cancers of lymphoid as well as nonlymphoid origin, and specifically for myeloid leukemia using the following exposure metrics: ever embalming, number of embalmings, number of years of working in embalming, and four quantitative estimates of exposure to formaldehyde (see Table 4-5). Exposure to formaldehyde as estimated as "ever embalming" was not associated with an increased risk for all lymphohematopoietic cancers combined (OR = 1.4, 95% CI: 0.8−2.6). The ORs for lymphohematopoietic cancers of nonlymphoid origin which includes Hodgkin disease as well as other specific subtype were generally elevated but there did not appear to be any clear patterns of association. However there was an increased risk for lymphohematopoietic cancers of nonlymphoid origin (OR = 3.0, 95% CI: 1.0-9.5). An exposure-response trend showed that more years of working in embalming was associated with an increase in risk of lymphohematopoietic cancers of nonlymphoid origin ($p < 0.05$); the other exposure metrics did not demonstrate significant exposure-response relationships. Statistically significant increases were observed for lymphohematopoietic cancers of nonlymphoid origin for the highest exposure category for durations of working in embalming jobs (OR = 3.7, 95% CI:1.1-12.2), for the number of embalmings (OR = 3.9, 95% CI: 1.2−12.8), for cumulative exposure (OR = 4.0, 95% CI: 1.2−13.2), as well as for the cumulative mid-level exposures (OR = 4.2, 95% CI: 1.2−14.3) and high-level exposures (OR = 3.4, 95% CI: 1.0−11.8) for 8-hour TWA intensity of exposure, and for the highest exposure category for peak exposure (OR = 3.8, 95% CI: 1.1−12.7).

The highest reported increases in risk for ever embalming were for myeloid leukemia (OR = 11.2, 95% CI: 1.3-95.6). Duration of employment in jobs with embalming demonstrated an exposure-response relationship with increased risk of myeloid leukemia ($p = 0.02$). The number of embalming was also significantly associated with increased risk of myeloid leukemia in the middle (OR = 12.7, 95%: 1.4-116.7) and highest exposure levels (OR = 12.7, 95% CI: 1.6-119.7). Cumulative exposure was associated with increased risk of myeloid leukemia, with the highest category of exposure showing OR = 13.2 (95% CI: 1.5-115.4).

All three categories of average formaldehyde intensity while embalming were very strongly and significantly associated with the risk of myeloid leukemia mortality (OR = 11.1,

14.8, and 9.5), and the test for an exposure-response trend was of borderline statistical significance (p = 0.058). The risk of myeloid leukemia mortality was also very strongly and significantly associated with 8-hour TWA exposure for mid-level exposures (OR = 13.6, 95% CI: 1.5-125.8) and high-level exposures (OR = 12.0, 95% CI: 1.3-107.4), as well as for peak exposure in two of three categories (0-7 ppm OR = 15.2, 95% CI: 1.6-141.6; 7-9.3 ppm OR = 8.0, 95% CI: 0.9-74.0; and >9.3 ppm OR = 13.0, 95% CI: 1.4-116.9). For peak exposures, there was also a statistically significant exposure-response tend (p = 0.036).

The study by Hauptmann et al. (2009) stands out among the studies of embalmers and professionals in the funeral industry based on the strength of the quantitative exposure data and the demonstration of exposure-response relationships which provide causal evidence of an association between formaldehyde exposure and increased risk of myeloid leukemia. The results also show an association between the broader categories of lymphohematopoietic cancers of nonlymphoid origin which includes myeloid leukemia. These results were internally consistent and demonstrated statistically significant associations that were unlikely the result of chance. As this nested case-control study was based on the cohorts of Hayes et al. (1990) and those of Walrath and Fraumeni (1983, 1984), the potential for selection bias is considered to be low. Further, the controls in Hauptmann et al. (2009) were carefully selected to avoid individuals who died of any causes that were thought to even possibly be related to formaldehyde exposure. Confounding is also unlikely to be an alternative explanation for the observed results as there were clear and convincing exposure-responses and the magnitude of the effect estimates were extremely large.

4.1.2.2.1.2. ***Industry worker cohort studies.***

This section discusses updated industrial worker studies that show associations between LHP cancer and formaldehyde. The studies by Marsh et al. (1994), Blair et al. (1986), and Acheson et al. (1984) and the later update by Gardner et al. (1993) provide estimates of exposure to formaldehyde. The remaining studies generally rely either on duration of exposure (number of years in the job) as a surrogate (Pinkerton et al., 2004) or provide no exposure assessment.

Blair et al. (1986) reported on 4,396 deaths from all causes in the 10 formaldehyde-associated factories that made up the NCI cohort of 26,561 workers employed before January 1, 1966. There was little evidence of an association with LHP system cancer (SMR 0.91; 56 observed) in exposed white men, who dominated the cohort. Marsh et al. (1994), in an early study of the Wallingford plant, which is also part of the Hauptmann et al. (2004, 2003) and Blair et al. (1986) studies, found SMRs of 0.89 and 0.91, based on U.S. and county death rates,

respectively (25 observed deaths). The authors did not further discuss this cancer site until after Hauptmann et al. (2003) was published.

Hauptmann et al. (2003) updated the cohort mortality study of Blair et al. (1986) that consisted of predominantly the same (25,619) workers from 10 plants. The primary focus of this analysis was cancer of the LHP system, including leukemia. The description and demographics of the current study are the same as those reported by Blair et al. (1986) and Stewart et al. (1986). In this update, follow-up was extended through December 31, 1994. The additional 15 years of follow-up increased the number of deaths from 4,349 to 8,486. However, in the course of updating this cohort with follow-up through 2004, Beane Freeman et al. (2009) (including Hauptmann) discovered that the matching of death certificates in Hauptmann et al. (2003) had inexplicatively missed 1,006 deaths. Beane Freeman et al. (2009) reanalyzed the cohort with follow-up through 1994 in order to replicate the analyses in Hauptmann et al. (2003) and provided those results in supplementary tables. Nonetheless, the focus of the most recent re-analysis of the NCI cohort was on follow-up completed through 2004.

While the results of the earlier Hauptmann et al. (2003) analysis are called into question based on the missing deaths, the authors concluded that formaldehyde may cause leukemia, particularly ML, in humans. However, because results from other studies were inconsistent, they suggested caution in drawing definite conclusions as they could not provide a biological basis for the significant excess risk of LHP. The authors pointed out several studies that indicate changes that are consistent with chromosomal changes in formaldehyde-exposed persons, such as increased frequencies of (MN (He et al., 1998; Kitaeva et al., 1996; Suruda et al., 1993), sister chromatid exchanges (SCEs) (Shaham et al., 2002, 1997; Yager et al., 1986), chromosomal aberrations (CAs) (He et al., 1998; Bauchinger and Schmid, 1985), and DNA-protein cross-links (DPXs) (Shaham et al., 1997, 1996) in peripheral lymphocytes of humans exposed to formaldehyde.

In the latest update to the large NCI cohort, Beane Freeman et al. (2009) extended the follow-up through 2004. This cohort is based on workers from 10 manufacturers of formaldehyde, formaldehyde resins, molding compounds, plastic products, film or plywood who were first employed prior to 1966. The median follow-up time for workers was 42 years, representing 998,106 person-years of follow-up among 25,619 workers (Beane Freeman et al., 2009). Exposure to formaldehyde was estimated for each individual job category from work histories, with calendar-time and plant-specific estimates based on assessments of job titles and tasks associated with those jobs using plant visits by industrial hygienists and monitoring data (Blair et al. 1986; Stewart et al. 1986; Blair and Stewart 1990). Exposures were categorized by peak exposure, average intensity of exposure and cumulative exposure. Peak exposure

categories were defined as nonexposed, low (0.1−1.9 ppm), medium (2.0−3.9 ppm), and high (4.0 ppm or greater). Average intensity categories of exposure were defined as nonexposed, low (0.1−0.4 ppm), medium (0.5 to <0.9 ppm), and high (≥1.0 ppm). Cumulative exposure was defined as nonexposed, low (0.1−1.4 ppm-years), medium (1.5−5.4 ppm-years), and high (≥5.5 ppm-years). Duration of exposure was defined as 0, 0.1−4.9 years, 5.0−14.9 years, and ≥15 years. The presence of formaldehyde-containing particulates and other potential chemical coexposures in the plants were indentified. Information on potential formaldehyde exposures after 1980 was unavailable.

Among those workers who were exposed, the median estimated time-weighted average exposure to formaldehyde was 0.3 ppm (Range: 0.01-4.3 ppm). There were 4359 workers (17%) who were classified as never exposed. Standardized mortality ratios (SMRs) were calculated using sex-, race, age-, and calendar-year-specific U.S. mortality rates. Relative risks based on internal comparisons within the cohort were estimated using Poisson regression, controlling for calendar year, age (5-year categories), sex, race, and pay category.

A total of 319 deaths from all lymphohematopoietic cancers were identified. Among them, there were 286 ever-exposed and 33 never-exposed workers. SMR analyses based on external comparison of populations showed that mortality from lymphohematopoietic cancers was not elevated in either the ever-exposed (SMR = 0.94, 95% CI: 0.84-1.06) or never-exposed workers (SMR = 0.86, 95% CI: 0.61-1.21). The ratio of the exposed to unexposed SMR was 1.09 (95% CI: 0.76-1.57)[5]. Table 4-6 shows the ratios of the SMRs in the exposed workers to the SMRs in the unexposed workers. An elevated risk for Hodgkin lymphoma among exposed workers was observed (SMR = 1.42, 95% CI: 0.96-2.10) which was twice the SMR for the unexposed workers but not significantly different from the null. The SMR for lymphatic leukemia was only 1.15 among exposed workers but this was more than four times higher than the SMR for lymphatic leukemia among unexposed worker although not significantly so. Mortality from other subtypes of lymphohematopoietic cancers among the exposed workers did not show increased mortality rates compared with the U.S. population. It is important to note that workers are generally healthier than the general population and, as such, are expected to have lower baseline risk for cancer and mortality. This healthy worker effect effectively biases many external rate and risk comparisons to yield SMR values below unity. Internal analyses can provide a more appropriate comparison, as exposed and unexposed workers are likely to have background risks of cancer and mortality that are more similar.

[5] $\text{Variance}(SMR_{Exposed}/SMR_{Unexposed}) \approx [1/\text{Observed}_{Exposed} + 1/\text{Observed}_{Unexposed}]$

Table 4-6. Comparison of SMRs in the exposed workers to the SMRs in the unexposed workers from NCI cohort reported by Beane Freeman et al. 2009.

Cause of Death	Nonexposed		Exposed		Ratio of SMRs	95% CI[a]
	N	SMR	*N*	SMR		
All LHP	33	0.86	286	0.94	1.09	0.76-1.57
NHL	12	0.86	94	0.85	0.99	0.54-1.80
Hodgkin Disease	2	0.7	25	1.42	2.03	0.48-8.56
Multiple Myeloma	11	1.78	48	0.94	0.53	0.27-1.02
All Leukemia	7	0.48	116	1.02	2.13	0.99-4.56
Lymphatic Leukemia	1	0.26	36	1.15	4.42	0.61-32.26
Myeloid Leukemia	4	0.65	44	0.9	1.38	0.50-3.85

[a]Variance($SMR_{Exposed}/SMR_{Unexposed}$) ≈ [1/$Observed_{Exposed}$ + 1/$Observed_{Unexposed}$].

Internal analyses of exposed workers indicated that peak exposures in the highest exposure category were associated with a significant increase in all lymphohematopoietic deaths comparing death rates among workers with peaks of ≥4 ppm to those with >0 to 2.0 ppm (RR = 1.37, 95% CI: 1.03-1.81). Across the three categories of peak exposure (i.e., exposure >0 ppm), there was also a statistically significant exposure-response trend ($p = 0.02$). The exposure-response trend including the never exposed workers was also statistically significant ($p = 0.04$). No association was observed for all lymphohematopoietic cancers for average intensity or cumulative exposure.

Among the specific subtypes of lymphohematopoietic cancer mortality, Hodgkin lymphoma and multiple myeloma were both shown to be at increased risk associated with peak exposure concentrations. Peak exposures in the highest exposure category were associated with a significant increase in Hodgkin lymphoma deaths comparing death rates among workers with peaks of ≥ 4 ppm to those with > 0 to 2.0 ppm (RR = 3.96, 95% CI: 1.31-12.02). Across the three categories of peak exposure, there was a statistically significant exposure-response trend ($p = 0.01$). The exposure-response trend including the never-exposed workers was also statistically significant ($p = 0.004$).

Peak exposures in the highest exposure category were associated with a significant increase in multiple myeloma deaths comparing death rates among workers with peaks of ≥ 4 ppm to those with > 0 to 2.0 ppm (RR = 2.04, 95% CI: 1.01-4.12). Across the three categories of peak exposure, there was some evidence of an exposure-response trend ($p = 0.08$); however,

there was no evidence of an exposure-response trend including the never-exposed workers. The association of multiple myeloma with formaldehyde exposure was also shown throughout the cohort experience (see Figure 4-3). Regarding leukemia mortality, there was indication of a modest exposure-response trend of peak exposure concentrations ($p = 0.12$); likewise for myeloid leukemia specifically ($p = 0.13$). At the highest exposure category of peak exposure (peaks ≥ 4 ppm vs. > 0 to 2.0 ppm), the RR = 1.42 (95% CI: 0.92-2.18) for leukemia and RR = 1.78 (95% CI: 0.87-3.64) for myeloid leukemia.

Beane Freeman et al. (2009) further showed plots presenting the RR from the internal analyses for each endpoint and for each year of follow-up. The association of Hodgkin lymphoma with formaldehyde exposure is not only seen for the complete 2004 follow-up, but throughout the cohort experience (see Figure 4-3). These plots show that during the 1970's, the relative risk of Hodgkin lymphoma with peak exposure was greater than 10 and diminished to approximately RR = 8 in the 1980's and remained at about RR = 4 through the end of follow-up in 2004. Similarly, the relative risks for myeloid leukemia were greater than 10 in the early 1970's and diminished with extended follow-up. Beane Freeman et al. (2009) stated that "this pattern could reflect the increased precision of the relative risk estimates with accrual of additional person-years and myeloid leukemia or could reflect a relatively short induction-incubation time for myeloid leukemia because by time since first exposure and first high peak both indicate highest risks within the first 25 years."

The exposure metric based on the average intensity of formaldehyde exposure was associated with increased risks of Hodgkin lymphoma mortality but not with other specific subtypes of lymphohematopoietic cancer mortality. Average exposures in the highest exposure category were associated with an increase in Hodgkin lymphoma deaths (RR = 2.48, 95% CI: 0.84-7.32), comparing death rates among workers with peaks of ≥ 4 ppm to those with > 0 to 2.0 ppm. Across the three categories of average intensity, there was a statistically significant exposure-response trend ($p = 0.05$). The exposure-response trend including the never exposed workers was also statistically significant ($p = 0.03$). As with peak exposure, there is a consistent trend across the years of follow-up for RR for myeloid leukemia and multiple myeloma of the mid and high exposed individuals be elevated, although only nearing significance at a few points (see Figure 4-4).

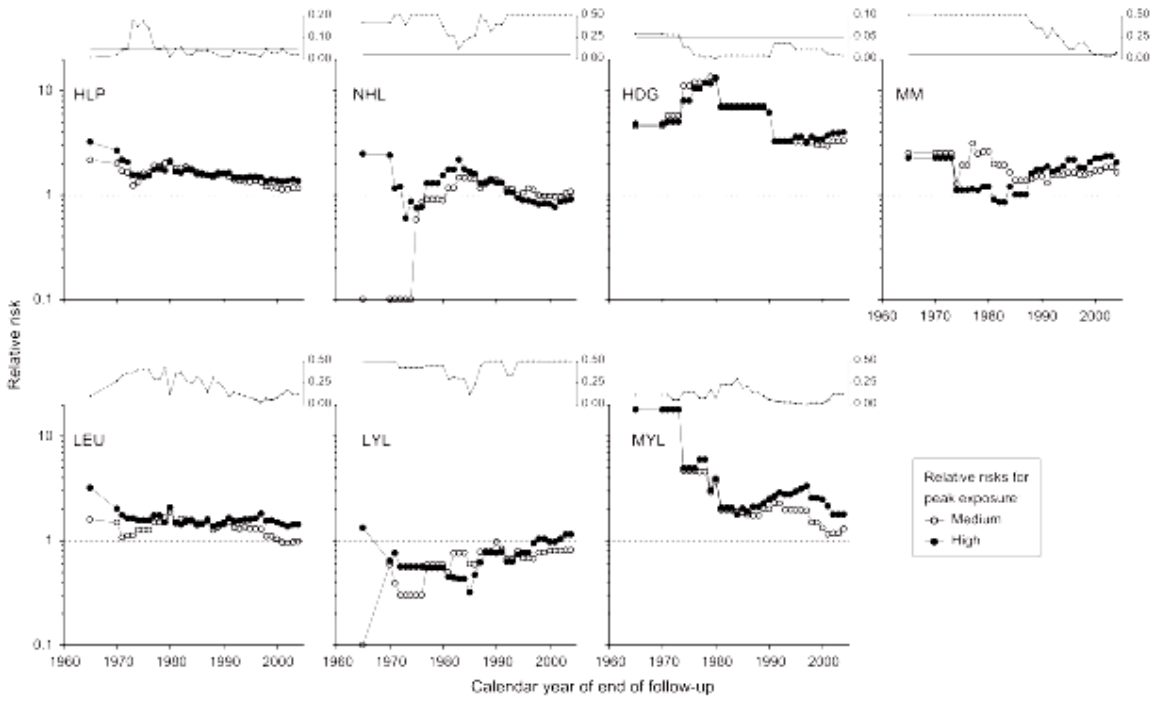

Figure 4-3. Association between peak formaldehyde exposure and the risk of lymphohematopoietic malignancy.

Relative risks for medium-peak (2.0 to <4.0 ppm) and high-peak (≥4.0 ppm) formaldehyde exposure categories compared with the low exposed category (>0 to <2.0 ppm) and *P* values for trend tests among the exposed person-years for lymphohematopoietic malignancies are shown by year of end of follow-up, 1965-2004. Values plotted at 0.1 represent RR = 0 due to no cases in the exposure category values plotted at 20 represent RR = infinity due to no cases in the referent category. The **small graphs** above the relative risk plots represent the exposure-response trend *P* values based on two-sided likelihood ratio tests (1 df) of zero slope for continuous formaldehyde exposure among exposed person-years only. The **points** represent the relative risk estimates based on the cumulative number of cases and person-years accrued from the start of the study to that point in time and for 2004 are equivalent to the relative risk estimates presented in Table 2 (Beane Freeman et al., 2009). HLP = lymphohematopoietic malignancies, NHL = non-Hodgkin lymphoma, HDG = Hodgkin lymphoma, MM = multiple myeloma, LEU = leukemia, LYL = lymphatic leukemia, MYL = myeloid leukemia, RR = relative risk.

Source: Beane Freeman et al. (2009)

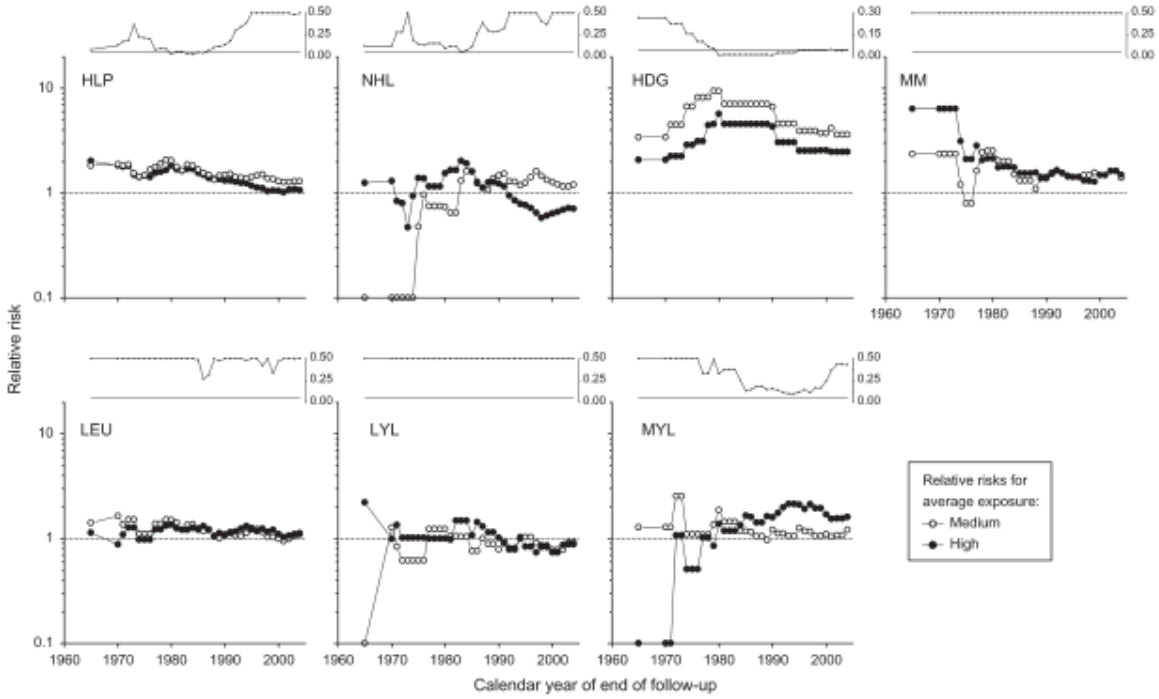

Figure 4-4. Association between average intensity of formaldehyde exposure and the risk of lymphohematopoietic malignancy.

Relative risks for medium (0.5−0.9 ppm) and high (≥ 1.0 ppm) average-intensity formaldehyde exposure categories compared with the low exposed category (0.1−0.4 ppm) and *P* values for trend tests among the exposed person-years for lymphohematopoietic malignancies by year of end of follow-up, 1965−2004. Values plotted at 0.1 represent RR •• = due to no cases in the exposure category. The **small graphs** above the relative risk plots represent the exposure—response trend *P* values based on two-sided likelihood ratio tests (1 *df*) of zero slope for continuous formaldehyde exposure among exposed person-years only. The **points** represent the relative risk estimates based on the cumulative number of cases and person-years accrued from the start of the study to that point in time and for 2004 are equivalent to the relative risk estimates presented in Table 3, (Beane Freeman et al., 2009). HLP = lymphohematopoietic malignancies, NHL = non-Hodgkin lymphoma, HDG = Hodgkin lymphoma, MM = multiple myeloma, LEU =
leukemia, LYL = lymphatic leukemia, MYL = myeloid leukemia; RR = relative risk.

Source: Beane Freeman et al. (2009)

The third exposure metric that was assessed was cumulative exposure. There was no clear trend toward increasing risk with increasing cumulative exposure to formaldehyde for all lymphohematopoietic deaths. Among the subtype-specific causes of deaths, Hodgkin lymphoma showed some evidence of an exposure-response association among the exposed workers ($p = 0.08$) and among all workers ($p = 0.06$) which was consistent with the more clearly demonstrated and stronger statistical findings of increased risk of Hodgkin lymphoma with higher peak and average intensity. Leukemia also showed some evidence of an exposure-response association among the exposed workers ($p = 0.12$) and among all workers ($p = 0.08$) which was consistent with the findings of increased risk of leukemia with higher peak; however there did not appear to be an association of with average intensity.

The strongest causal evidence of an association between exposure to formaldehyde and lymphohematopoietic cancer mortality was demonstrated by the exposure-response gradient for peak exposure and mortality from Hodgkin lymphoma and leukemia and the nonlinear increase in RR for multiple myeloma. The plots showing a consistent increased in the relative risk over time are further supportive of a causal association. The results for Hodgkin lymphoma are supported by the demonstration of an exposure-response relationship for increased average intensity of formaldehyde exposures and a consistent pattern of increasing risk with increasing cumulative exposure to formaldehyde. The results for leukemia are supported by the exposure-response relationship for cumulative exposure to formaldehyde.

This well-conducted study (Beane Freeman et al., 2009) showed adverse effects of exposure to formaldehyde based on extensive and laborious reconstruction of individuals' exposures (Blair et al. 1986; Stewart et al. 1986; Blair and Stewart 1990). The internal analyses reflect control for potential selection bias akin to the healthy worker effect, and the Poisson regression results controlled for numerous potential confounders. Potential confounding was evaluated for exposure to 11 concomitant occupational substances (ever/never), as well as working as a chemist or lab technician (for several years). In a follow-up analysis, although Beane Freeman et al. (2009) excluded 586 individuals with possible exposure to benzene, a known leukemogen, and the results did not change the relative risk for myeloid or lymphatic leukemia in the highest peak exposure category (RR = 1.77; 95% CI = 0.85 to 3.69 and RR = 1.16; 95% CI = 0.54 to 2.48, respectively) or any other cancer (data not shown). Exposure lags ranging from 2 to 20 years were considered to account for latency; all exposures were subsequently calculated using a 2-year lag interval for the analyses of lymphohematopoietic malignancies (Beane Freeman et al. 2009, Hauptmann et al. 2003) and a 15-year lag interval for the analyses of solid cancers (Hauptmann et al. 2004). The authors did note that smoking information was unavailable for most of the cohort. However, smoking was not considered to be

a source of confounding in internal analyses since analysis of a sample of workers revealed no major differences in smoking prevalence by cumulative formaldehyde exposure. Further, an earlier analysis of the association between formaldehyde exposure and lung cancer in the same cohort (Blair et al., 1990b) identified a small subset of workers with information on smoking from medical records and reported that the prevalence of smoking did not appear to be strongly associated with exposure to formaldehyde.

Beane Freeman et al. (2009) concluded that the pattern of attenuating lymphohematopoietic risk over time was "consistent with a causal association within the relatively short induction-incubation periods, characteristic of leukemogenesis." To understand the implication of this conclusion, it should be noted that if the etiologically-relevant window of time for formaldehyde-induced lymphohematopoietic cancer mortality is relatively short, then extending a follow-up of the cohort well beyond the upper bound of that window will not allow for the additional inclusion of more formaldehyde-induced lymphohematopoietic cancer deaths. Rather, it allows for the inclusion of unrelated lymphohematopoietic cancer mortality which serves to dilute any true effect of formaldehyde exposure. The median follow-up time in the Beane Freeman et al. (2009) was 42 years and the analyses reported that exposure lags between 2 and 25 years were tested for fit, with an 18-year lag having the best fit. Since exposures were not considered to have continued beyond 1980 and the cohort mortality was complete through 2004, the mortality in the later years of follow-up was unlikely to be related to formaldehyde as the deaths may have been outside of any causal exposure window. It should be noted that the analyses present in Beane Freeman et al. (2009) were based on a 2-year lag, as there was not strong enough support for assuming either a longer or a shorter lag.

Two sensitivity analyses evaluated the assumption of no formaldehyde exposure after 1980. If exposure was considered to continue at 1980 levels until age 65 years or death, risk patterns for all lymphohematopoietic cancers were reported to be similar to those observed in the primary analysis. If the cohort follow-up was censored two years after the last job for the 2,810 individuals who were still exposed in 1979 and alive two years later, the association for myeloid leukemia with peak and average intensity of exposure was stronger than that observed in the primary analyses. The investigators did not report the results of the sensitivity analyses for each subtype but did report that the patterns for the other subtypes were unchanged.

Table 4-7. Epidemiologic studies of formaldehyde and lymphohematopoietic cancers

Reference	Study design	Exposure assessment	Results, statistical significance (number of observed deaths for cohort study)				
Harrington and Shannon (1975)	Cohort mortality study of 2,079 pathologists and 12,944 laboratory technicians from the Royal College of Pathologists and the Pathological Society of Great Britain from 1955−1973. The comparison population came from national mortality data.	Presumed exposure to formaldehyde tissue fixative.	<u>Pathologists</u>				
			All cause mortality	SMR	0.60	*NR*	(156)
			LHP cancers	SMR	2.0	$p < 0.01$	(8)
			Hodgkin's disease	SMR	1.4	*NR*	(1)
			Leukemia	SMR	0.6	*NR*	(1)
			<u>Technicians</u>				
			All cause mortality	SMR	0.67	*NR*	(154)
			LHP cancers	SMR	0.5	*NR*	(3)
			Hodgkin's disease	SMR	−	*NR*	(0)
			Leukemia	SMR	0.5	*NR*	(1)
Harrington and Oakes (1984)	Cohort mortality study of 2,720 pathologists from the Royal College of Pathologists and the Pathological Society of Great Britain from 1974−1980. Vital status obtained from the census, a national health registry, and other sources. SMRs developed from the English, Scottish, Irish, and Welsh populations.	Presumed exposure to formaldehyde tissue fixative.	All causes				
			Men	SMR	0.56	(90% CI: 0.48−0.66)	(110)
			Women	SMR	0.99	(90% CI: 0.62−1.50)	(16)
			Leukemia				
			Men	SMR	0.91	(90% CI: 0.05−4.29)	(1)
			Women	SMR	9.26	(90% CI: 0.47−43.9)	(1)
			Other LHP cancers				
			Men	SMR	0.53	(90% CI: 0.03−2.54)	(1)
			Women	SMR	−	−	(0)

Table 4-7. Epidemiologic studies of formaldehyde and lymphohematopoietic cancers (continued)

Reference	Study design	Exposure assessment	Results, statistical significance (number of observed deaths for cohort study)				
Hall et al. (1991)	Cohort mortality study of 4,512 pathologists from the Royal College of Pathologists and the Pathological Society of Great Britain from 1974−1987. Vital status obtained from the census, a national health registry, and other sources. SMRs developed from the English and Welsh populations.	Presumed exposure to formaldehyde tissue fixative.	All cause mortality				
			Men	SMR	0.43	(95% CI: 0.37−0.50)	(176)
			Women	SMR	0.65	(95% CI: 0.38−1.03)	(18)
			Hodgkin's disease	SMR	1.21	(95% CI: 0.03−6.71)	(1)
			All cancers	SMR	1.44	(95% CI: 0.69−2.63)	(10)
			Leukemia	SMR	1.52	(95% CI: 0.41−3.89)	(4)
Levine et al. (1984)	Cohort mortality study of 1,477 male Ontario undertakers first licensed 1928−1957, followed from 1950 to 1977. SMRs developed from Ontario mortality rates.	Presumed exposure to formaldehyde tissue fixative.	All LHP cancers	SMR	1.24	*NR*	(8)
			Leukemia	SMR	1.60	*NR*	(4)
Stroup et al. (1986)	Cohort mortality study of 2,317 white male members of the American Association of Anatomists from 1888 to 1969 who died 1925−1979. SMRs developed using U.S. population mortality rates.	Presumed exposure to formaldehyde tissue fixative.	All cause mortality	SMR	0.65	(95% CI: 0.60−0.70)	(738)
			All LHP cancers	SMR	1.2	(95% CI: 0.7−2.0)	(18)
			Lymphosarcoma and reticulosarcoma	SMR	0.7	(95% CI: 0.1−2.5)	(2)
			Hodgkin's disease	SMR	−	−	(0)
			Leukemia	SMR	1.5	(95% CI: 0.7−2.7)	(10)
			Other lymphatic	SMR	2.0	(95% CI: 0.7−4.4)	(6)

Table 4-7. Epidemiologic studies of formaldehyde and lymphohematopoietic cancers (continued)

Reference	Study design	Exposure assessment	Results, statistical significance (number of observed deaths for cohort study)				
Logue et al. (1986)	Cohort mortality study of 5,585 pathologists who were members of the College of American Pathologists, 1962−1972, followed for mortality through 1977. SMRs developed from U.S. population mortality rates.	Presumed exposure to formaldehyde tissue fixative.	LHP cancer other than leukemia	SMR	0.48	*NR*	(NR)
			Leukemia	SMR	1.06	*NR*	(NR)
Matanoski (1991)	Cohort mortality study of 6,111 male pathologists from membership rolls of the American Medical Association 1912−1950. Mortality was followed through 1978. SMRs developed from U.S. population white male mortality rates.	Presumed exposure to formaldehyde tissue fixative.	All cancer	SMR	0.78	(95% CI: 0.71−0.85)	(508)
			All LHP cancers	SMR	1.25	(95% CI: 0.95−1.62)	(57)
			Lymphosarcoma and reticulosarcoma	SMR	1.31	(95% CI: 0.66−2.35)	(11)
			Hodgkin's disease	SMR	0.36	(95% CI: 0.04−1.31)	(2)
			Leukemia	SMR	1.35	(95% CI: 0.92−1.92)	(31)
			Other lymphatic	SMR	1.54	(95% CI: 0.82−2.63)	(13)
Beane Freeman et al. (2009) Previous reports: Hauptmann et al. (2003) Blair et al. (1986)	Retrospective cohort mortality study of 25,619 workers employed at 10 formaldehyde plants in the U.S. followed from either the plant start-up or first employment through 2004. SMRs calculated using sex-, age-, race-, and calendar-year-specific U.S. mortality rates. RRs estimated using Poisson regression stratified by calendar year, age, sex, and race; adjusted for pay category.	Exposure estimates based on job titles, tasks, visits to plants by study industrial hygienists, and monitoring data through 1980. Peak exposure defined as short-term excursions exceeding the 8-hour TWA formaldehyde intensity and knowledge of job tasks. Exposures to 11 other compounds were	All LHP cancers				
			Exposed	SMR	0.94	(95% CI: 0.84−1.06)	(286)
			Unexposed	SMR	0.86	(95% CI: 0.61−1.52)	(33)
			Peak exposure (ppm)				
			0	RR	1.07	(95% CI: 0.70−1.62)	(33)
			0.1−1.9	RR	1.00	*Reference value*	(103)
			2.0 to <4.0	RR	1.17	(95% CI: 0.86−1.59)	(75)
			4.0 or greater	RR	1.37	(95% CI: 1.03−1.81)	(108)
			Trend p = 0.02				
			Average exposure (ppm)				

Table 4-7. Epidemiologic studies of formaldehyde and lymphohematopoietic cancers (continued)

Reference	Study design	Exposure assessment	Results, statistical significance (number of observed deaths for cohort study)				
Beane Freeman et al. (2009) (continued)		identified. Workers contributed pre-exposure person-time to nonexposed category. Poisson regression models used a 2-year lag to account for tumor latency.	0	RR	0.99	(95% CI: 0.66−1.48)	(33)
			0.1−0.4	RR	1.00	*Reference value*	(164)
			0.5 to <1.0	RR	1.29	(95% CI: 0.97−1.73)	(67)
			1.0 or greater	RR	1.07	(95% CI: 0.78−1.47)	(55)
			Trend p > 0.50				
			Cumulative exposure(ppm-years)				
			0	RR	0.89	(95% CI: 0.59−1.34)	(33)
			0.1−1.4	RR	1.00	*Reference value*	(168)
			1.5 to 5.4	RR	0.77	(95% CI: 0.56−1.07)	(49)
			5.5 or greater	RR	1.07	(95% CI: 0.80−1.42)	(69)
			Trend p = 0.25				
			Leukemia				
			Peak exposure (ppm)				
			0	RR	0.59	(95% CI: 0.25−1.36)	(7)
			0.1−1.9	RR	1.00	*Reference value*	(41)
			2.0 to <4.0	RR	0.98	(95% CI: 0.60−1.62)	(27)
			4.0 or greater	RR	1.42	(95% CI: 0.92−2.18)	(48)
			Trend p = 0.012				
			Average exposure (ppm)				
			0 ppm	RR	0.54	(95% CI: 0.24−1.22)	(7)
			0.1−0.4	RR	1.00	*Reference value*	(67)
			0.5 to <1.0	RR	1.13	(95% CI: 0.71−1.79)	(25)
			1.0 or greater	RR	1.10	(95% CI: 0.68−1.78)	(24)

Table 4-7. Epidemiologic studies of formaldehyde and lymphohematopoietic cancers (continued)

Reference	Study design	Exposure assessment	Results, statistical significance (number of observed deaths for cohort study)
Beane Freeman et al. (2009) (continued)			*Trend p > 0.50* Cumulative exposure (ppm-years) 0 RR 0.53 (95% CI: 0.23−1.21) (7) 0.1−1.4 RR 1.00 *Reference value* (63) 1.5−5.4 RR 0.96 (95% CI: 0.60−1.56) (24) 5.5 or greater RR 1.11 (95% CI: 0.70−1.74) (29) *Trend p = 0.12* Hodgkin Lymphoma Peak exposure (ppm) 0 RR 0.67 (95% CI: 0.12−3.60) (2) 0.1−1.9 RR 1.00 *Reference value* (6) 2.0 to <4.0 RR 3.30 (95% CI: 1.04−10.50) (8) 4.0 or greater RR 3.96 (95% CI: 1.31−12.02) (11) *Trend p = 0.01* Average exposure (ppm) 0 RR 0.46 (95% CI: 0.05−3.93) (2) 0.1−0.4 RR 1.00 *Reference value* (10) 0.5 to <1.0 RR 3.62 (95% CI: 1.41−9.31) (9) 1.0 or greater RR 2.48 (95% CI: 0.84−7.32) (6) *Trend p = 0.05* Cumulative (ppm-years) 0 RR 0.42 (95% CI: 0.09−2.05) (2) 0.1−1.4 RR 1.00 *Reference value* (14)

Table 4-7. Epidemiologic studies of formaldehyde and lymphohematopoietic cancers (continued)

Reference	Study design	Exposure assessment	Results, statistical significance (number of observed deaths for cohort study)				
Beane Freeman et al. (2009) (continued)			1.5−5.4	RR	1.71	(95% CI: 0.66−4.38)	(7)
			5.5 or greater	RR	1.30	(95% CI: 0.40−4.19)	(4)
			Trend p = 0.08				
			Myeloid Leukemia				
			Peak exposure (ppm)				
			0	RR	0.82	(95% CI: 0.25−2.67)	(4)
			0.1 to 1.9	RR	1.00	*Reference value*	(14)
			2.0 to <4.0	RR	1.30	(95% CI: 0.58−2.92)	(11)
			4.0 or greater	RR	1.78	(95% CI: 0.87−3.64)	(19)
			Trend p = 0.13				
			Average exposure (ppm)				
			0	RR	0.70	(95% CI: 0.23−2.16)	(4)
			0.1 to 0.4	RR	1.00	*Reference value*	(24)
			0.5 to <1.0	RR	1.21	(95% CI: 0.56−2.62)	(9)
			1.0 or greater	RR	1.61	(95% CI: 0.76−3.39)	(11)
			p = 0.43				
			Cumulative (ppm-years)				
			0	RR	0.61	(95% CI: 0.20−1.91)	(4)
			0.1-1.4	RR	1.00	*Reference value*	(26)
			1.5-5.4	RR	0.82	(95% CI: 0.36−1.83)	(8)
			5.5 or greater	RR	1.02	(95% CI: 0.48−2.16)	(10)
			Trend p > 0.50				

Table 4-7. Epidemiologic studies of formaldehyde and lymphohematopoietic cancers (continued)

Reference	Study design	Exposure assessment	Results, statistical significance (number of observed deaths for cohort study)
Hauptmann et al. (2009) Previous reports: Hayes et al. (1990) Walrath and Fraumeni (1983) Walrath and Fraumeni (1984) Related re-analyses: Marsh et al. (2007a) Marsh et al. (2007b) Marsh and Youk (2005) Marsh et al. (1996)	Nested case-control study within cohort mortality study of 6,808 deaths from 1960 to 1986. Identified from registries of the National Funeral Director Association, licensing boards and state funeral directors' associations, NY State Bureau of Funeral Directors and CA Funeral Directors and Embalmers. Odds ratios calculated using unconditional logistic regression	Occupational history obtained by interviews with next of kin and coworkers using detail questionnaires. Exposure was assessed by linking questionnaire responses to an exposure assessment experiment. Exposure levels (peak, intensity and cumulative) were assigned to each individual using a predictive model based on the exposure data.	All LHP cancers Embalming Never — OR 1.0 — *Reference value* — (24) Ever — OR 1.4 — (95% CI: 0.8−2.6) — (144) Duration of working in jobs with embalming (years) 0 — OR 1.0 — *Reference value* — (24) > 0 to 20 — OR 0.8 — (95% CI: 0.4−1.8) — (28) > 20 to 34 — OR 1.5 — (95% CI: 0.8−2.8) — (50) > 34 — OR 1.8 — (95% CI: 1.0−3.4) — (66) *Trend p = 0.058* Number of embalmings 0 — OR 1.0 — *Reference value* — (24) > 0 to 1422 — OR 0.9 — (95% CI: 0.6−1.8) — (29) > 1422 to 3068 — OR 1.9 — (95% CI: 1.0−3.6) — (62) > 3068 — OR 1.5 — (95% CI: 0.8−2.9) — (53) *Trend p = 0.477* Cumulative exposure (ppm-hours) 0 — OR 1.0 — *Reference value* — (24) > 0 to 4058 — OR 1.3 — (95% CI: 0.6−2.5) — (40) > 4058 to 9253 — OR 1.4 — (95% CI: 0.8−2.8) — (49) > 9253 — OR 1.6 — (95% CI: 0.8−3.0) — (55) *Trend p = 0.422*

Table 4-7. Epidemiologic studies of formaldehyde and lymphohematopoietic cancers (continued)

Reference	Study design	Exposure assessment	Results, statistical significance (number of observed deaths for cohort study)				
Hauptmann et al. (2009) (continued)			Average formaldehyde intensity while embalming (ppm)				
			0	OR	1.0	*Reference value*	(24)
			> 0 to 1.4	OR	1.6	(95% CI: 0.9−3.2)	(53)
			> 1.4 to 1.9	OR	1.4	(95% CI: 0.7−2.7)	(47)
			> 1.9	OR	1.3	(95% CI: 0.7−2.5)	(44)
			Trend p = 0.591				
			Time-weighted average exposure over 8 hours (ppm)				
			0	OR	1.0	*Reference value*	(24)
			> 0 to 0.10	OR	1.3	(95% CI: 0.7−2.6)	(47)
			> 0.1 to 0.18	OR	1.6	(95% CI: 0.8−3.1)	(52)
			> 0.18	OR	1.4	(95% CI: 0.7−2.8)	(45)
			Trend p = 0.635				
			Peak formaldehyde exposure (ppm)				
			0	OR	1.0	*Reference value*	(24)
			> 0 to 7.0	OR	1.6	(95% CI: 0.8−3.2)	(48)
			> 7.0 to 9.3	OR	1.6	(95% CI: 0.9−3.1)	(55)
			> 9.3	OR	1.2	(95% CI: 0.6−2.3)	(41)
			Trend p = 0.555				
			Myeloid leukemia				
			Embalming				
			Never	OR	1.0	*Reference value*	(1)
			Ever	OR	11.2	(95% CI: 1.3−95.6)	(33)

Table 4-7. Epidemiologic studies of formaldehyde and lymphohematopoietic cancers (continued)

Reference	Study design	Exposure assessment	Results, statistical significance (number of observed deaths for cohort study)
Hauptmann et al. (2009) (continued)			Duration of working in jobs with embalming (years) 0 OR 1.0 *Reference value* (1) > 0 to 20 OR 5.0 (95% CI: 0.5−51.6) (6) > 20 to 34 OR 12.9 (95% CI: 1.4−117.1) (13) > 34 OR 13.6 (95% CI: 1.6−119.7) (14) *Trend p = 0.02* Number of embalmings 0 OR 1.0 *Reference value* (1) > 0 to 1422 OR 7.6 (95% CI: 0.8−73.5) (7) > 1422 to 3068 OR 12.7 (95% CI: 1.4−116.7) (12) > 3068 OR 12.7 (95% CI: 1.4−112.8) (14) *Trend p = 0.314* Cumulative exposure (ppm-hours) 0 OR 1.0 *Reference value* (1) > 0 to 4058 OR 10.2 (95% CI: 1.1−95.6) (9) > 4058 to 9253 OR 9.4 (95% CI: 1.0−85.7) (10) > 9253 OR 13.2 (95% CI: 1.5−115.4) (14) *Trend p = 0.192* Average formaldehyde intensity while embalming (ppm) 0 OR 1.0 *Reference value* (1) > 0 to 1.4 OR 11.1 (95% CI: 1.2−106.3) (10) > 1.4 to 1.9 OR 14.8 (95% CI: 1.6−136.9) (13) > 1.9 OR 9.5 (95% CI: 1.1−86.0) (10) *Trend p = 0.058*

Table 4-7. Epidemiologic studies of formaldehyde and lymphohematopoietic cancers (continued)

Reference	Study design	Exposure assessment	Results, statistical significance (number of observed deaths for cohort study)
Hauptmann et al. (2009) (continued)			Time-weighted average exposure over 8 hours (ppm) 0 OR 1.0 *Reference value* (1) > 0 to 0.10 OR 8.4 (95% CI: 0.8−79.3) (8) > 0.1 to 0.18 OR 13.6 (95% CI: 1.5−125.8) (13) > 0.18 OR 12.0 (95% CI: 1.3−107.4) (12) *Trend p = 0.396* Peak formaldehyde exposure (ppm) 0 OR 1.0 *Reference value* (1) > 0 to 7.0 OR 15.2 (95% CI: 1.6−141.6) (12) > 7.0 to 9.3 OR 8.0 (95% CI: 0.9−74.0) (9) > 9.3 OR 13.0 (95% CI: 1.4−116.9) (12) *Trend p = 0.036*
Wang et al. (2009)	Population-based case-control study of incident cases of non-Hodgkin lymphoma diagnoses 1996-2000.	Exposures classified using job exposure matrix based on occupational and industry data obtained from personal interviews.	Non-Hodgkin lymphoma Formaldehyde Never OR 1.0 *Reference value* (398) Ever OR 1.3 (95% CI: 1.0−1.7) (203) Average exposure intensity Never OR 1.0 *Reference value* (398) Low OR 1.4 (95% CI: 1.0−1.8) (129) Medium-High OR 1.2 (95% CI: 0.8−1.7) (74) *Trend p = 0.21*

Table 4-7. Epidemiologic studies of formaldehyde and lymphohematopoietic cancers (continued)

Reference	Study design	Exposure assessment	Results, statistical significance (number of observed deaths for cohort study)				
Wang et al. (2009) (continued)			Average exposure probability				
			Never	OR	1.0	*Reference value*	(398)
			Low	OR	1.3	(95% CI: 1.0−1.7)	(129)
			Medium-High	OR	1.4	(95% CI: 0.9−2.3)	(74)
			Trend p > 0.50				
			Both average exposure intensity and average exposure probability				
			Low Intensity and Low Probability	OR	1.4	(95% CI: 1.1−1.9)	(115)
			Med-High Intensity and Low Probability	OR	1.0	(95% CI: 0.7−1.6)	(50)
			Med-High Intensity and Med-High Prob.	OR	1.1	(95% CI: 0.5−2.4)	(14)
			Med-High Intensity and Med-High Prob.	OR	1.6	(95% CI: 0.9−3.1)	(24)
			Leukemia				
			Peak exposure (ppm)				
			0	RR	0.59	(95% CI: 0.25−1.36)	(7)
			0.1−1.9	RR	1.00	*Reference value*	(41)
			2.0 to <4.0	RR	0.98	(95% CI: 0.60−1.62)	(27)
			4.0 or greater	RR	1.42	(95% CI: 0.92−2.18)	(48)
			Trend p = 0.012				
			Average exposure (ppm)				
			0 ppm	RR	0.54	(95% CI: 0.24−1.22)	(7)
			0.1−0.4	RR	1.00	*Reference value*	(67)

Table 4-7. Epidemiologic studies of formaldehyde and lymphohematopoietic cancers (continued)

Reference	Study design	Exposure assessment	Results, statistical significance (number of observed deaths for cohort study)				
Wang et al. (2009) (continued)			0.5 to <1.0	RR	1.13	(95% CI: 0.71−1.79)	(25)
			1.0 or greater	RR	1.10	(95% CI: 0.68−1.78)	(24)
			Trend p > 0.50				
			Cumulative exposure (ppm-years)				
			0	RR	0.53	(95% CI: 0.23−1.21)	(7)
			0.1−1.4	RR	1.00	*Reference value*	(63)
			1.5−5.4	RR	0.96	(95% CI: 0.60−1.56)	(24)
			5.5 or greater	RR	1.11	(95% CI: 0.70−1.74)	(29)
			Trend p = 0.12				
			Hodgkin Lymphoma				
			Peak exposure (ppm)				
			0	RR	0.67	(95% CI: 0.12−3.60)	(2)
			0.1−1.9	RR	1.00	*Reference value*	(6)
			2.0 to <4.0	RR	3.30	(95% CI: 1.04−10.50)	(8)
			4.0 or greater	RR	3.96	(95% CI: 1.31−12.02)	(11)
			Trend p = 0.01				
			Average exposure (ppm)				
			0	RR	0.46	(95% CI: 0.05−3.93)	(2)
			0.1−0.4	RR	1.00	*Reference value*	(10)
			0.5 to <1.0	RR	3.62	(95% CI: 1.41−9.31)	(9)
			1.0 or greater	RR	2.48	(95% CI: 0.84−7.32)	(6)
			Trend p = 0.05				

Table 4-7. Epidemiologic studies of formaldehyde and lymphohematopoietic cancers (continued)

Reference	Study design	Exposure assessment	Results, statistical significance (number of observed deaths for cohort study)				
Wang et al. (2009) (continued)			Cumulative (ppm-years)				
			0	RR	0.42	(95% CI: 0.09−2.05)	(2)
			0.1−1.4	RR	1.00	*Reference value*	(14)
			1.5−5.4	RR	1.71	(95% CI: 0.66−4.38)	(7)
			5.5 or greater	RR	1.30	(95% CI: 0.40−4.19)	(4)
			Trend p = 0.08				
			Myeloid Leukemia				
			Peak exposure (ppm)				
			0	RR	0.82	(95% CI: 0.25−2.67)	(4)
			0.1 to 1.9	RR	1.00	*Reference value*	(14)
			2.0 to <4.0	RR	1.30	(95% CI: 0.58−2.92)	(11)
			4.0 or greater	RR	1.78	(95% CI: 0.87−3.64)	(19)
			Trend p = 0.13				
			Average exposure (ppm)				
			0	RR	0.70	(95% CI: 0.23−2.16)	(4)
			0.1 to 0.4	RR	1.00	*Reference value*	(24)
			0.5 to <1.0	RR	1.21	(95% CI: 0.56−2.62)	(9)
			1.0 or greater	RR	1.61	(95% CI: 0.76−3.39)	(11)
			p = 0.43				
			Cumulative (ppm-years)				
			0	RR	0.61	(95% CI: 0.20−1.91)	(4)
			0.1-1.4	RR	1.00	*Reference value*	(26)
			1.5-5.4	RR	0.82	(95% CI: 0.36−1.83)	(8)
			5.5 or greater	RR	1.02	(95% CI: 0.48−2.16)	(10)
			Trend p > 0.50				

Table 4-7. Epidemiologic studies of formaldehyde and lymphohematopoietic cancers (continued)

Reference	Study design	Exposure assessment	Results, statistical significance (number of observed deaths for cohort study)				
Pinkerton et al. (2004) Update of Stayner et al. (1988)	Cohort mortality study of 11,098 workers in 3 garment plants exposed ≥3 months after formaldehyde was introduced. Women comprised 81.7% of the cohort. Vital status was followed through 1998. SMRs were calculated by using sex-, age-, race-, and calendar-year-specific U.S. mortality rates. Multiple cause SMRs were derived from all contributing causes from death certificates.	Data for 549 randomly selected employees in 5 departments in 1981 and 1984 used to estimate overall exposure levels. Levels presumed to be 0.09−0.20 ppm.	All LHP cancers	SMR	0.97	(95% CI: 0.74−1.26)	(59)
			Lymphosarcoma and reticulosarcoma	SMR	0.85	(95% CI: 0.28−1.99)	(5)
			Hodgkin's disease	SMR	0.55	(95% CI: 0.07−1.98)	(2)
			Other lymphatic	SMR	0.97	(95% CI: 0.64−1.40)	(28)
			Leukemia	SMR	1.09	(95% CI: 0.70−1.62)	(24)
			Mortality since 1960				
			Lymphocytic leukemia	SMR	0.60	(95% CI: 0.12−1.75)	(3)
			ML	SMR	1.44	(95% CI: 0.80−2.37)	(15)
			10+ years of exposure	SMR	2.19	NS	(8)
			20+ years since 1st exposure	SMR	1.91	$p > 0.05$	(13)
			Multiple cause leukemia				
			10+ years of exposure and 20+ years since 1st exposure	SMR	1.92	(95% CI: 1.08−3.17)	(15)
			Multiple cause ML				
			20+ years since 1st exposure	SMR	2.02	(95% CI: 1.13−3.34)	(15)
			10+ years of exposure and 20+ years since 1st exposure	SMR	2.55	(95% CI: 1.10−5.03)	(8)

Table 4-7. Epidemiologic studies of formaldehyde and lymphohematopoietic cancers (continued)

Reference	Study design	Exposure assessment	Results, statistical significance (number of observed deaths for cohort study)				
Coggon et al. (2003) Update of Gardner et al. (1993)	Cohort mortality study of 14,014 men employed in 6 factories of the chemical industry in Great Britain from periods during which formaldehyde was produced. Cohort mortality followed from 1941 through 2000. SMRs based on English and Welsh age- and calendar-year-specific mortality rates.	Exposure assessment based on data abstracted from company records. Jobs categorized as background, low, moderate, high, or unknown levels.	Non-Hodgkin's lymphoma				
			Overall	SMR	0.98	(95% CI: 0.67−1.39)	(31)
			High exposure	SMR	0.89	(95% CI: 0.41−1.70)	(9)
			Leukemia				
			Overall	SMR	0.91	(95% CI: 0.62−1.29)	(31)
			High exposure	SMR	0.71	(95% CI: 0.31−1.39)	(8)
			Multiple myeloma				
			Overall	SMR	0.86	(95% CI: 0.48−1.41)	(15)
			High exposure	SMR	1.18	(95% CI: 0.48−2.44)	(7)
Andjelkovich et al. (1995)	Cohort mortality study of 3,929 automotive industry iron foundry workers exposed from 1960−1987 and followed through 1989. SMRs calculated using sex-, age-, race-, and calendar-year-specific U.S. mortality rates.	Exposure assessment based on review of work histories by an industrial hygienist.	All LHP cancers	SMR	0.59	(95% CI: 0.23−1.21)	(7)
			Leukemia	SMR	0.43	(95% CI: 0.05−1.57)	(2)
Bertazzi et al. (1986)	Cohort mortality study of 1,330 male workers in an Italian resin plant. Subjects were employed any time between 1959 and 1980 for at least 30 days. Vital status followed through 1986. SMRs calculated using sex-, age-, race-, and calendar-year-specific national and local mortality rates.	Exposure assessment based on reconstruction of work history. Exposure levels were 0.16 to 3.1 ppm	All LHP cancers	SMR	2.01		(5)

Table 4-7. Epidemiologic studies of formaldehyde and lymphohematopoietic cancers (continued)

Reference	Study design	Exposure assessment	Results, statistical significance (number of observed deaths for cohort study)				
Edling et al. (1987)	Cohort mortality and incidence study of 521 Swedish workers in an abrasive production plant with at least 5 years of employment between 1955 and 1983, followed through 1983.	Exposure level of 0.1−1 mg/m^3.	Lymphoma	SMR	2.0	(95% CI: 0.2−7.2)	(2)
			Multiple myeloma	SMR	4.0	(95% CI: 0.5−14)	(2)
Dell and Teta (1995)	Cohort mortality study of 5,932 male employees of a New Jersey plastics manufacturing, research and development facility that produced phenol-formaldehyde resins.	Examination of work histories to identify jobs where formaldehyde was involved.	All LHP cancers				
			Hourly workers	SMR	0.93		(28)
			Salaried workers	SMR	1.69		(23)
			Leukemia				
			Hourly workers	SMR	0.98		(12)
			Salaried workers	SMR	1.98		(11)
Walrath and Fraumeni (1983)	Cohort study of 1,132 white male embalmers licensed to practice between 1902 and 1980 in New York who died between 1925 and 1980 identified from registration files. Deaths were compared with age-, race-, and calendar-year-expected numbers of deaths from the U.S. population.	No direct measurements. Presumed exposure to formaldehyde tissue fixative.	All LHP cancers	SMR[b]	1.15		(21)
			Lymphosarcoma and reticulosarcoma	SMR[b]	1.08		(4)
			Hodgkin's disease	SMR[b]	1.0		(2)
			Other lymphatic lymphoma	SMR[b]	1.18		(5)
			Leukemia	SMR[b]	1.32		(10)

Table 4-7. Epidemiologic studies of formaldehyde and lymphohematopoietic cancers (continued)

Reference	Study design	Exposure assessment	Results, statistical significance (number of observed deaths for cohort study)				
Walrath and Fraumeni (1984)	Cohort study of 1,007 white male embalmers from the California Bureau of Funeral Directing and Embalming who died between 1925 and 1980. Deaths were compared with age- and calendar-year-expected numbers of deaths from the U.S. population.	No direct measurements. Presumed exposure to formaldehyde tissue fixative.	All LHP cancers	SMR[b]	1.22		(19)
			Lymphosarcoma and reticulosarcoma	SMR[b]	0.97		(3)
			Hodgkin's disease	SMR[b]	–		(0)
			Other lymphatic lymphoma	SMR[b]	1.33		(4)
			Leukemia	SMR[b]	1.75	$p < 0.05$	(12)
			Licensed <20 years	SMR[b]	1.24		(4)
			Licensed ≥20 years	SMR[b]	2.21	$p < 0.05$	(8)
Hayes et al. (1990)	Proportionate mortality cohort study of 3,649 deceased white and 397 deceased nonwhite U.S. male embalmers and funeral directors, derived from licensing boards and funeral director associations in the 32 states and the District of Columbia. Occupation was confirmed on death certificate. Deaths were compared with age- and calendar-year-expected numbers of deaths from the U.S. population.	No direct measurements. Presumed exposure to formaldehyde tissue fixative.	All LHP cancers	SMR[b]	1.39	(95% CI: 1.15−1.67)	(115)
			Race				
			White	SMR[b]	1.31	(95% CI: 1.06−1.59)	(100)
			Nonwhite	SMR[b]	2.41	(95% CI: 1.35−3.97)	(15)
			Occupation on death certificate				
			Embalmer	SMR[b]	1.23	(95% CI: 0.78−1.85)	(23)
			Funeral director	SMR[b]	1.56	(95% CI: 1.23−1.94)	(78)
			Other	SMR[b]	1.30	(95% CI: 0.67−2.28)	(12)
			Age at death				
			<60	SMR[b]	1.35	(95% CI: 0.88−1.98)	(26)
			60−74	SMR[b]	1.72	(95% CI: 1.33−2.19)	(66)
			≥75	SMR[b]	1.16	(95% CI: 0.74−1.74)	(23)

Table 4-7. Epidemiologic studies of formaldehyde and lymphohematopoietic cancers (continued)

Reference	Study design	Exposure assessment	Results, statistical significance (number of observed deaths for cohort study)				
Hayes et al. (1990) (continued)			Hodgkin's disease	SMR[b]	0.72	(95% CI: 0.15−2.10)	(3)
			Non-Hodgkin's lymphoma	SMR[b]	1.26	(95% CI: 0.87−1.76)	(34)
			Lymphosarcoma and reticulosarcoma	SMR[b]	1.12	(95% CI: 0.58−1.96)	(12)
			Multiple myeloma	SMR[b]	1.37	(95% CI: 0.84−2.12)	(20)
			Other lymphatic lymphoma	SMR[b]	1.35	(95% CI: 0.85−2.01)	(22)
			Lymphatic leukemia	SMR[b]	0.74	(95% CI: 0.29−1.53)	(7)
			ML	SMR[b]	1.57	(95% CI: 1.01−2.34)	(24)
			Other leukemia	SMR[b]	2.28	(95% CI: 1.39−3.52)	(20)
Blair et al. (1993)	Population-based case-control study of 622 white men with LHP cancers. Cancers selected from Iowa and Minnesota cancer surveillance networks diagnosed between 10/80 and 9/82. 1,245 matched controls for living cases selected by random digit dialing if younger than age 65 and from Medicare records if 65 or older. Study focused on agricultural exposures.	Personal interviews of subjects or next of kin included job histories, agricultural exposures, and chemical exposures. Job titles used to create job exposure matrix. Industrial hygienist estimated probability and intensity of exposures to large numbers of substances.	Non-Hodgkin's lymphoma (formaldehyde exposure)	OR[a]	1.2	(95% CI: 0.9−1.7)	
			Funeral service worker	OR[a]	2.1	(95% CI: 0.5−7.9)	(6)

[a]Adjusted for age, state, smoking, family history of malignant proliferative disease, agricultural exposure to pesticides, use of dye, and direct/surrogate response to interview.

[b]Walrath and Fraumeni (1983, 1984). These studies are referred to by the authors as proportionate mortality studies and report proportional mortality ratios which are known to be potentially biased. However, review of the actual methods described clear shows that the expected numbers of cause-specific deaths were based on a standardized general population and therefore the reported PMRs are more accurately called SMRs.

Stayner et al. (1988) conducted a cohort study of 11,030 workers (82% female) followed from 1955 or the beginning date of exposure through 1982 in three garment factories. Personnel records from three garment manufacturing facilities, one in Pennsylvania and two in Georgia, were used to assemble a cohort of workers who attained a minimum of 3 months of exposure after the introduction of formaldehyde into these facilities. Formaldehyde resins were used to treat fabrics, beginning in 1955 and 1959. Although formaldehyde levels were available on a subset of the employees from monitoring data available from surveys completed in 1981 and 1984, they were not used in this analysis. Instead, the results were stratified by duration and latency. SMRs were based on U.S. population mortality rates. Based on six cases, the SMRs for leukemia were 2.43 and 3.81 among workers with 20 or more years since first exposure or at least 10 years of exposure, respectively. In their conclusions, the authors suggested that, although the numbers of deaths from LHP cancers were small, the risks were related to duration and latency.

Pinkerton et al. (2004) updated the Stayner et al. (1988) study by adding 16 years of follow-up. No new exposure information was added. The mean TWA exposure in 1981−1984 for the three plants was 0.15 ppm. No additional information regarding earlier industrial hygiene data was available, although the authors stated that the levels of exposure to formaldehyde were greater in the years before 1980. Stayner et al. (1988) cited independent studies of exposure levels in similar garment factories in the 1960s that seemed to indicate that the formaldehyde levels during that period ranged from 0.9 to 2.7 ppm (Blejer and Miller, 1966) in one garment manufacturing area. Another report (Shipkovitz, 1966) of 10-minute personal exposure samples indicated a range from 0.3 to 2.7 ppm in eight garment plants. In another study (Ahmad and Whitson, 1973), the levels ranged from 2 to 10 ppm. Goldstein (1973) calculated that concentrations in the cutting rooms of garment plants dropped from 10 ppm in 1968 to less than 2 ppm in 1973 because of an improvement in the resin treating process. The authors assumed that exposure ceased in 1981 and 1983. This produced an underestimate of exposure based on duration of employment for about 11% of the cohort who were still actively employed after those dates. Stayner et al. (1988) speculated that the risks of cancer of the buccal cavity, leukemia, and other LHP neoplasia may have been due to exposure to the highest potential formaldehyde levels in the industry between 1955 and 1962, because the resin used to treat permanent press fabrics still contained a relatively large amount of formaldehyde.

The SMRs were derived from age-, race-, and calendar-time-adjusted U.S. mortality rates. The analysis was repeated using Georgia or Pennsylvania mortality rates. In addition to the primary analysis of the underlying cause of death, the analysis used all causes listed on the death certificates to evaluate multiple cause mortality. As a referent for this, the analysis relied

on multiple cause death rates available since 1960 from the National Death Index maintained by the U.S. Centers for Disease Control and Prevention (CDC).

Altogether, 608 cancer deaths were observed (Pinkerton et al. 2004). The SMR for all cancer was 0.89 (95% CI: 0.82−0.97). The overall SMR for leukemia was 1.09 (24 deaths) and 1.44 (15 deaths) for ML. After 10 years of exposure, the risk for ML was 2.19. Exposure prior to 1963 was associated with a risk of 1.61. Among garment workers followed for 20 or more years from initial exposure, the SMR was significantly elevated for ML (1.91; $p < 0.05$; 13 deaths), as was the SMR for multiple cause leukemia (1.92 [95% CI: 1.08−3.17]; 15 deaths) in the subgroup with 10 or more years of exposure to formaldehyde and who were followed for 20 or more years after first exposure. The multiple cause mortality for ML for this subgroup of workers was also significant (SMR 2.55 [95% CI: 1.10−5.03]; 8 deaths).

The study by Stayner et al. (1988) has only limited power to detect excess risks of rare cancers, such as NPC and nasal cancer (13 and 16%, respectively). Limitations to the interpretations of the findings include a lack of any monitoring data before 1981, particularly during the critical time period 1955 to 1962, and lack of personal exposure estimates for any members of the cohort. The possibility exists that misclassification may still be present because the intensity of exposure to formaldehyde decreased as improvements were made in the resin systems used to treat fabrics (e.g., a person who worked 5 years beginning in 1955 might have been subject to greater exposure than a person who worked 5 years beginning in 1993). However, workers from the 1950s and 1990s were both placed in the same category of having worked fewer than 10 years. The median duration of exposure was 3.3 years. Work histories were not updated in the follow-up study; however, the low or background exposure levels that probably existed after 1981 were not likely to contribute substantially to the risk of cancer. The use of mortality data to estimate risk, when the case fatality rate was less than 100% for most cancer sites evaluated, could potentially produce an underestimate of the actual risk. Despite these limitations, this study provides additional evidence of an association between leukemia, especially ML, and formaldehyde in comparison with the general population.

Gardner et al. (1993) reported that the risk of leukemia was not statistically significant (SMR 0.9) based on 15 deaths among workers employed before 1965. Only four leukemia deaths were observed after 1964 through 1989, producing an SMR of 0.9.

When Coggon et al. (2003) updated the above cohort study of 14,014 men first employed before 1965 in six factories by adding 11 additional years of follow-up (ending December 31, 2000), no increase in the risk of leukemia or related cancers of the hematopoietic system was reported, either in the entire cohort (SMR 0.91; 31 observed) or in the group with the highest formaldehyde exposure (>2 ppm) (SMR 0.71; 8 observed). Similar results were obtained for

Hodgkin's disease, non-Hodgkin's lymphoma, and multiple myeloma. No other cancers of the hematopoietic system were evaluated, and no additional analyses were performed to assess a possible leukemia risk. However, the main finding from this study was a marked association of lung cancer with formaldehyde (discussed in the lung cancer section). This study's main focus was respiratory disease, lung cancer, and stomach cancer, not LHP cancers. For cancers of the LHP system, there was neither latency evaluation nor internal comparisons. The HWE is also potentially a problem.

Andjelkovich et al. (1995) studied a cohort of 3,929 male iron foundry workers potentially exposed to formaldehyde between January 1, 1960, and December 31, 1989, in which 127 cancer deaths had occurred during the observation period. An industrial hygienist, after reviewing work histories, categorized formaldehyde exposure into four levels corresponding to the approximate midpoint of the ranges: none, low (0.05 ppm), medium (0.55 ppm), and high (1.5 ppm) for exposure to formaldehyde. Boundaries of these exposure categories were not given. The authors warned that the assignment of exposure levels was not perfect because "subjective judgment had to be applied in many instances." SMRs were based on U.S. male mortality rates, but actual ranges were not specified. The authors also compared the exposed to 2,032 nonexposed workers from the same company. The population-based SMR for hematopoietic cancer in the exposed population was 0.59 (based on seven observed deaths). For leukemia the SMR was 0.43, based on two deaths. There were no other analyses for leukemia or LHP cancers in this study. Because of the uncertainty about workers' true formaldehyde exposure, there was no analysis by level of exposure, duration, or latency. There were also very few LHP cancers in the cohort. Thus, these results neither support nor refute an association of formaldehyde exposure with LHP cancers. The main focus of this paper was on lung cancer risk.

Bertazzi et al. (1989, 1986), in a cohort mortality study, followed 1,330 male workers from 1959 through 1986 at a formaldehyde resin plant in Italy. The workers had to have been employed for at least 30 days at the plant sometime between 1959 and 1980 to be included in the study. Their mortality was compared with national and local rates adjusted for age and calendar time period. No individual exposure estimates were available, but mean levels were estimated to be between 0.2 and 3.8 mg/m^3 (0.16 and 3.1 ppm) during the period 1974−1979. The authors found an SMR of 2.01 (five deaths observed) for cancer of the lymphatic and hematopoietic system. The study's limitations included incomplete work histories, small numbers of deaths, and a follow-up period that may not have been sufficient to allow for a latency period for the development of LHP cancers. As before, the results neither support nor refute an association of formaldehyde exposure with LHP cancers.

Edling et al. (1987) reported on the incidence of disease in a cohort of 521 blue collar Swedish workers in plants where abrasives bound with formaldehyde resins were manufactured. Formaldehyde levels ranged from 0.1 to 1.0 mg/m^3 (0.08−0.8 ppm). The workers in the cohort were employed between 1955 and 1983, and incidence rates were calculated from 1958 through 1981. There were only 24 total cancer cases (28.5 expected) of which 2 (1.0 expected) were lymphomas and 2 (0.5 expected) were multiple myelomas. Expected cases were determined through the Swedish National Cancer Register. No other LHP cancers were observed. This study lacked the power to detect any significant associations between LHP cancer and exposure to formaldehyde.

Dell and Teta (1995) conducted a cohort mortality study of 5,932 male employees of a New Jersey plastics manufacturing, research, and development facility that produced phenol-formaldehyde resins. The workers, who had been employed during the period 1946−1967, were followed-up for an average of 32 years. SMRs were based on U.S. and New Jersey mortality rates. Hourly workers (n = 3,853) were analyzed separately from the 2,079 salaried employees. Although no excess risk was evident for hematopoietic cancer in hourly workers (SMR 0.93; 28 observed), there was an SMR of 1.69 (95% CI: 1.07−2.53; 23 observed) among salaried workers. This association was further narrowed to mainly research and development workers (eight leukemia deaths observed with three expected, for an SMR of 2.67). No common exposure was found when work history records were examined. The decedents were mostly associated with process development in two research pilot plants, where chemical engineers, lab technicians, and plant operators executed small-scale product development. Although notebooks referred to benzene and toluene solvents, no definite connection was made with formaldehyde or any of the solvents. No ambient air measurements of formaldehyde were available. The findings cannot be assumed to be due to formaldehyde exposure because of the presence of other potential leukemogens.

Blair et al. (1993) conducted a study that evaluated the risk of non-Hodgkin's lymphoma from exposure to formaldehyde. This was a population-based, case-control, interview-based study of 1,867 white males of whom 622 cases had the disease and 1,245 were controls. Subjects had lived in Iowa and Minnesota between 1980 and 1983. This study was exploratory and designed to find associations with any environmental exposures and non-Hodgkin's lymphoma. Subjects or next of kin were interviewed to determine what exposures the cases and controls may have received based on agricultural exposures, work histories, medical conditions, and family history. Extra effort was made to collect information about occupation, industrial exposures, and other selected exposures. The analysis revealed an OR of 1.2 for exposure to formaldehyde. Similar associations were found for metals and other substances in the study.

This study, because it did not select cases and controls from a population with possible formaldehyde exposure, could not detect specific relationships between formaldehyde and non-Hodgkin's lymphoma.

Wang et al. (2009) assessed the effect of formaldehyde exposure on the risk of non-Hodgkin lymphoma in a population-based case-control study among women in Connecticut. Incident cases (N = 601) were frequency matched to 717 controls by age. A standardized questionnaire was used to gather information on lifetime occupational history and other risk factors. Exposure to organic solvents and formaldehyde for each job was assessed by linking study participant's occupational data to a job-exposure matrix created by industrial hygienists at the National Cancer Institute (Dosemeci et al., 1994; Gomez et al., 1994). Semiquantitative exposure metrics included average exposure intensity and average exposure probability which were evaluated individually and together. Analyses use unconditional logistic regression and controlled for age, family history of hematopoietic cancers, alcohol consumption and race. For the low average exposure intensity category the OR = 1.4 (95% CI: 1.0-1.8), while for the Medium-High category the OR = 1.2 (95% CI: 0.8-1.7). For the Low average exposure probability category the OR = 1.3 (95% CI: 1.0-1.7), while for the Medium-High category the OR = 1.4 (95% CI: 0.9-2.3). When both factors were considered jointly, the Low intensity and Low probability exposure category had OR = 1.4 (95% CI: 1.1-1.9); Medium-High intensity and Low probability exposure category had OR = 1.0 (95% CI: 0.7-1.6); Low intensity and Medium-High probability exposure category had OR = 1.1 (95% CI: 0.5-2.4); Medium-High and Medium-High probability exposure category had OR = 1.6 (95% CI: 0.9-3.1). The investigators also examined the risk of non-Hodgkin lymphoma among major subtypes. The risk of follicular lymphoma and chronic lymphocytic leukemia/Small lymphocytic lymphoma was slightly elevated but the risk of diffuse large B-cell lymphoma was OR = 1.9 (95% CI: 1.3-2.6) for ever having been exposed to formaldehyde. For Low average intensity exposure, the risk was OR = 2.1 (95% CI: 1.3-2.6) while for Medium-High average intensity exposure, the risk was OR = 1.5 (95% CI: 0.9-2.4). Even so, an exposure-response relationship was demonstrated using the continuous parameterization of average intensity rather that the categorical (p = 0.03). Likewise, an exposure-response relationship was demonstrated using the continuous parameterization of average probability of exposure rather that the categorical (p = 0.01).

The findings of Wang et al. (2009) provide some support for an association between formaldehyde exposure and non-Hodgkin lymphoma. It should be noted that a population-based case-control study, where incidence rather than mortality defines the case—may be more appropriate for cancers with relatively low mortality (i.e., CCL, large B-cell lymphoma, Small lymphocytic lymphoma).

4.1.2.2.1.3. ***Meta-analyses of epidemiological studies for lymphohematopoietic malignancies.***

Several meta-analyses have reported risks for all lymphohematopoietic cancers and leukemia (Collins and Lineker, 2004; Bosetti et al., 2008; Zhang et al., 2009). The meta-analysis conducted by Collins and Lineker (2004) was based on 18 studies. Fixed-effects models were used to obtain summary relative risk values and 95% confidence intervals, and random effects models were used to evaluate heterogeneity across studies. The summary RR across all studies was 1.1 (95% CI = 1.0 to 1.2) for leukemia. The effect estimates varied by type of study, country of study population, type of industry, year of publication, and study size. The cohort studies had a summary RR = 1.0 (95% CI: 0.9-1.2) while the summary estimate for the case-control studies was RR = 2.4 (95% CI: 0.9-6.5). For industrial type jobs the RR was 0.9 (95% CI: 0.8-1.0) while for embalmers the RR was 1.6 (95% CI: 1.2-2.0) and for pathologists and anatomists the RR was 1.4 95% CI: 1.0-1.9).

Bosetti et al. (2008) reviewed cohort studies of industry workers and health professional using fixed-effect and random-effect models depending on the degree of heterogeneity among the individual cohorts. They did not indentify sufficient heterogeneity among the studies and used the fixed effect models to estimate the relative risk of lymphatic and hematopoietic cancers among four studies of industrial workers exposed to formaldehyde with RR = 0.85 (95% CI: 0.74-0.96) and among eight studies of health professionals with RR = 1.31 (95% CI: 1.16-1.47). Likewise for leukemia, they used the fixed effect model to the estimate the RR among four studies of industry workers of lymphatic and hematopoietic cancers among workers exposed to formaldehyde with RR = 0.90 (95% CI: 0.75-1.07) and among eight studies of health professional with RR = 1.39 (95% CI: 1.15-1.68).

Zhang et al. (2009) reviewed many of the same studies of lymphohematopoietic cancers and all leukemia that were included in the Bosetti et al. (2008) meta-analysis using a different methodology that focused on the highest exposure groups in each study in order to increase the statistical power of the meta-analysis by minimizing bias from type II error. This method of focusing on the highest exposure groups also reduces the likelihood that any individual study results were confounded or otherwise biased (Greenland, 1998). The authors noted that in the presence of a true causal association, "combining workers with very low exposures with workers with high exposures into one overall exposed group can dilute relative risk estimates towards the null." The authors also preferentially selected the results for myeloid leukemia over all leukemia when these data were reported. This methodology is appropriate if the underlying hypothesis is that formaldehyde causes myeloid leukemia which has the ancillary effect of increasing the risk of all leukemias. By increasing the specificity of the endpoint definition, this meta-analytic method does not create any bias but rather increases the statistical power of the study to detect an

association with formaldehyde that is driven by myeloid leukemia. If the any association between formaldehyde exposure and all leukemia were driven by nonmyeloid leukemias, then this method would have less statistical power.

Zhang et al. (2009) also used fixed-effects and random-effect models depending on the degree of heterogeneity among the individual cohorts. Zhang et al. (2009) found the heterogeneity among the studies sufficiently high to warrant use of the random-effects model and reported a summary RR = 1.25 (95% CI: 1.09-1.43) for the 19 studies with data on all types of lymphohematopoietic cancers combined. For the 15 studies reporting data on all leukemias, the summary RR = 1.54 (95% CI: 1.18-2.00). For the six studies of myeloid leukemia, the summary RR = 1.90 (95% CI: 1.31-2.76). The authors concluded that the primary reason that their meta-analytic results differed from those previously reported, was attributable to their differential use of the results from the studies by Hauptmann et al. (2004, Stroup et al. (1986) and Pinkerton et al. (2004) for which they used either the relative risks for myeloid leukemia or the highest exposure category in each study.

A sensitivity analysis showed that had Zhang et al. (2009) replaced the results of those three studies with the results used in Bosetti et al (2008) meta-analyses, the summary relative risk for leukemia dropped from 1.54 (95% CI: 1.18-2.00) to RR = 1.10 (95% CI: 0.93-1.31). The authors state that the primary reason for the different results is the use of myeloid-specific data from Hauptmann et al. (2003), Stroup et al. (1986) and Pinkerton et al. (2004). Bosetti et al. (2008) use the SMR results from Hauptmann et al. (2003) while Zhang et al. (2009) use the highest peak exposure results for myeloid leukemia. The use of the result from the internal comparison of Hauptmann et al. (2003) appears to be more appropriate. However, those data from Hauptmann et al. (2003) were missing 1,006 deaths and were reanalyzed by Beane Freeman et al. (2009) and were judged to be similar. The correct RR for the highest exposure level of peak exposure should have been 2.79 rather than the value of 3.46 used by Zhang et al. (2009). Likewise, the value of the SMR used by Bosetti et al. (2008) was 0.93 rather than SMR = 0.85 which they used. In a similar fashion, the Bosetti et al. (2008) meta-analysis used the overall SMR of 1.19 from Stroup et al. (1986) while Zhang et al. (2009) used the myeloid leukemia SMR for longest duration which was 2.19. Zhang et al. (2009) also used the SMR from Coggon et al. (2003) for the highest exposure group which was 0.71 while Bosetti et al. (2008) used the overall SMR of 0.91. The Zhang et al. (2009) also excluded five studies that were included by Collins and Lineker (2004). Had the Zhang et al. (2009) analysis included these five studies, their summary relative risk would have been RR = 1.38 (95% CI: 1.15-1.65).

Zhang et al. (2009) also report results of the meta-analysis of formaldehyde and other lymphohematopoietic cancers. The RR for Hodgkin lymphoma was 1.23 (95% CI: 0.67-2.29)

and was based on 8 studies. The RR for non-Hodgkin lymphoma was 1.08 (95% CI: 0.86-1.35) and was based on 11 studies while the RR for multiple myeloma was 1.31 (95% CI: 1.02-1.67).

The criteria for study inclusion and exclusion applied by Zhang et al. (2009) appear to be appropriate and the methodology for using myeloid-specific results where possible also appears to be appropriate. This study found statistically significant increases in risk of all lymphohematopoietic cancers with RR = 1.25(95% CI: 1.12-1.39), all leukemia with RR = 1.54 (95% CI: 1.24-1.91), myeloid leukemia with RR = 1.90 (95% CI: 1.41-2.55) and multiple myeloma with RR = 1.31 (95% CI: 1.02-1.67).

Of the three meta-analyses, the study by Zhang et al. (2009) appears to be the most rigorous methodologically. One difference between them is that while Zhang et al. (2009) combined across industry, Collins and Lineker (2003) and Bosetti et al. (2008) stratified by industry which may have reduced the statistical power of those analyses. Nonetheless, both Collins and Lineker (2003) and Bosetti et al. (2008) both reported statistically significant increases in risk of leukemia mortality among the nonindustrial workers. And Bosetti et al. (2008) also reported statistically significant increases in risk of all lymphohematopoietic cancers mortality among the nonindustrial workers.

4.1.2.2.1.4. ***Summary of lymphohematopoietic cancers.***

All LHP Malignancies

The majority of studies of all LHP malignancies (as a group) have been based on comparison of the risk of cancer incidence or mortality in the studied population compared to the risk in a general population. These external analyses rely on the assumption that cancer incidence or mortality rates are expected to be similar between the general population and the study population in the absence of exposure. However, the 'healthy worker effect' is well known to bias rates in workers downwards compared to general populations, and there may be differences in the magnitude of this selection bias by industry or profession.

Positive associations between formaldehyde exposure and LHP cancers have been reported based on external comparison groups for chemical workers (Wong, 1983; Bertazzi et al., 1986), embalmers (Walrath and Fraumeni, 1983, 1984; Hayes et al., 1990), anatomists and pathologists (Harrington and Shannon, 1975; Hall et al., 1991; Levine at al., 1984; Stroup et al., 1986; Matanoski et al., 1989). However, associations were not reported in external comparison group analyses for garment workers, iron-foundry workers, and a large US industrial cohort (Pinkerton et al., 2004; Andjelkovich et al., 1995; Beane Freeman et al., 2009; Marsh et al., 1996), although associations were observed in some of these studies when exposure-response relationships were considered.

Several published meta-analyses are available which more formally assess the strength of association between formaldehyde exposure and mortality from all LHP cancers. Bosetti et al. (2008) reviewed cohort studies of industry workers and health professionals (i.e., embalmers, anatomists, and pathologists). They used fixed effect models to estimate the relative risk of lymphatic and hematopoietic cancers among four studies of industrial workers exposed to formaldehyde with RR = 0.85 (95% CI: 0.74-0.96) and among eight studies of health professionals with RR = 1.31 (95% CI: 1.16-1.47). A subsequent meta-analysis by Zhang et al. (2009) reports a summary relative risk (RR) of 1.25 (95% CI 1.09-1.43) for both professional and industry workers for all LHP cancers (ICD 9 codes 200-209). These researchers identified 19 cohort study analyses, including cohort study updates. Zhang et al. (2009) used the reported RR from the highest exposure category to increase statistical power and reduce uncertainty regarding confounding or other bias. These two summary evaluations of the weight of evidence of a causal association between exposure to formaldehyde and increased risk of mortality from all LHP malignancies both showed statistically significant association. Therefore it is judged that the collective evidence shows a causal association for all LHP as a group.

While most of the studies reporting SMRs in the meta-analyses were limited in their exposure assessment to work in an industry known or thought to be exposed to formaldehyde, some of the studies did conduct detailed exposure assessment and were able to compute internal comparison of risk within the group of studied workers. These internal analyses are less likely to be biased by the selection pressures for hiring and retaining healthy workers and represent stronger methodologies.

The two individual studies with the relatively strongest exposure assessment reported results that are consistent with the results of the meta-analyses (Hauptmann et al., 2009; Beane Freeman et al. 2009). Hauptmann et al. (2009) conducted extensive interviews with the next of kin and coworkers of the cases and controls provided detailed information on the funeral homes and work practices of the study subjects. The authors noted that since the funeral industry is often a family business, these study subjects' next of kin were believed to be unusually knowledgeable of funeral home work practices. The work history component of these interviews provided data on the frequency and duration of embalmings for jobs held at least five years, as well as information on ventilation of the premises and the frequency of spills. These data were linked to data from an exposure-reconstruction experiment which sought to replicate standard funeral home practices while measuring exposures to formaldehyde.

While the results of Hauptmann et al. (2009) are not conclusive of an association on a stand-alone basis, they do show consistently elevated odds ratios for all LHP associated with ever embalming, duration of working in embalming jobs, number of embalmings and cumulative

formaldehyde exposure. Of the multiple OR results presented for all LHP, 17 of 19 were elevated but only two were statistically significantly elevated. For the middle category of duration, the OR = 1.5 (95% CI: 0.8-2.8) and for the highest category of duration, the OR = 1.8 (95% CI: 1.0-3.4) with a borderline significant test for trend ($p = 0.058$). For the middle category of number of embalming, the OR = 1.9 (95% CI: 1.0-3.6). Based on a binomial distribution assuming that half the ORs would be elevated and half below unity, the probability of 17 OR being elevated out of 19 is $p = 0.0003$. While only two of the OR results were statistically significant elevated, the overall findings were consistent with the results of the meta-analyses. Hauptmann et al. (2009) show elevated risks of all LHP of a somewhat higher magnitude than the summary effects in the meta-analyses that are, however, less statistically precise as they are based on a single study rather than on multiple studies.

The individual study with the most detailed and objectively ascertained exposure assessment was that of Beane Freeman et al. (2009). Exposure to formaldehyde was estimated for each individual job category from work histories, with calendar-time and plant-specific estimates based on assessments of job titles and tasks associated with those jobs using plant visits by industrial hygienists and monitoring data (Blair et al. 1986; Stewart et al. 1986; Blair and Stewart 1990). Exposures were categorized by peak exposure, average intensity of exposure and cumulative exposure. The median follow-up time for workers was 42 years, representing 998,106 person-years of follow-up among 25,619 workers (Beane Freeman et al., 2009).

Internal analyses of exposed workers indicated that peak exposures in the highest exposure category were associated with a significant increase in all lymphohematopoietic deaths comparing death rates among workers with peaks of ≥ 4 ppm to those with > 0 to 2.0 ppm (RR = 1.37, 95% CI: 1.03-1.81). Across the three categories of peak exposure (i.e., exposure >0 ppm), there was also a statistically significant exposure-response trend ($p = 0.02$). The exposure-response trend including the never exposed workers was also statistically significant ($p = 0.04$). However, no association was observed for all lymphohematopoietic cancers for average intensity or cumulative exposure.

The results of Beane Freeman et al. (2009) confirm those of the meta-analyses showing statistically significant increased risk at the highest category of peak exposure that was associated with all LHP mortality. These results do not appear to be confounded by any known coexposures as the investigators reported that controlling for duration of exposure to 11 potentially confounding coexposures did not meaningfully change results. As these results are based on a large and well-followed occupational cohort, these results are unlikely to be influenced by selection bias. The estimated magnitude of the identified association (OR = 1.37)

was approximately equivalent to the summary RR of Zhang et al. (2009) which was 1.25 and the summary RR = 1.31 for health professionals analyzed by Bosetti et al. (2008).

Given the consistency and strength of the positive associations for all LHP cancer mortality in professional cohorts (embalmers, anatomists and pathologists) taken together with the strong positive results of the NCI cohort, human epidemiologic evidence are sufficient to conclude that there is a causal association between formaldehyde exposure and mortality from all LHP malignancies (as a group).

All Leukemia

Like the studies of all LHP (as a group), the majority of studies of all leukemia malignancies (as a group) have been based on comparison of the risk of cancer incidence or mortality in the studies population compared to the risk in a general population and may be influenced by the healthy worker effect which effective depresses effect estimates. In spite of this potential bias which may mask a true effect of exposure, an association between formaldehyde exposure and leukemia mortality is supported by cohort analyses of embalmers, pathologists and anatomists (Hayes et al., 1990; Walrath and Fraumeni, 1983; Walrath and Fraumeni 1984; Hall et al., 1991; Levine et al., 1984; Stroup et al., 1986; Matanoski et al., 1989). Formaldehyde exposure and formaldehyde-related occupation have also been shown to be associated with leukemia diagnosis in a case-control study (RR = 5.79 (95% CI 1.44-23.25), but not formaldehyde exposure alone (RR = 0.96; 95% CI 0.54-1.71) (Stellman et al., 1998).

However, SMR analyses of the large industrial cohorts do not indicate a similar association (Coggon et al., 2000; Beane Freeman et al., 2009, Pinkerton et al, 2004). Although the SMR analysis provided for the NCI cohort does not indicate a positive association for all leukemia using an external reference group (Beane Freeman et al., 2009), the SMR for exposed versus unexposed workers within the cohort suggests all leukemia is elevated 2.13-fold with this type of internal comparison (95% CI 0.99-4.56).

Several meta-analyses have been conducted for formaldehyde exposure and all leukemia (as a group) which indicate a positive association. Collins and Lineker (2004) reported an overall RR for 18 available studies of 1.1 (95% CI: 1.0-1.2), suggesting an association of leukemia with formaldehyde exposure. This association was stronger for both pathologists/anatomists (1.4; 95% CI: 1.0-1.9) and embalmers (RR = 1.6; 95% CI: 1.2-2.0) than for industrial workers (RR = 0.9; 95% CI: 0.8-1.0). Study design also impacted the apparent strength of association, with stronger associations seen in case-control studies (RR = 2.4; 95% CI: 0.9-6.5) versus cohort studies (RR = 1.0; 95% CI: 0.9-1.2). Bosetti et al. (2008) reported an association between formaldehyde exposure and leukemia mortality with a pooled RR of 1.39

(95% CI 1.15-1.68) for 8 groups of professional workers. In the same analysis, the pooled RR for the 4 industrial cohorts was 0.90 (95% CI: 0.75-1.07). Zhang et al. (2009) reported a pooled RR of 1.54 (95% CI: 1.18-2.00) for all cohorts identified in their meta-analysis, although this pooled RR should be considered with the knowledge that when the source studies specifically identified results for myeloid leukemia those results and not the all leukemia results were included (Zhang et al., 2009). As discussed earlier, this methodology is appropriate if the underlying hypothesis is that formaldehyde causes myeloid leukemia which has the ancillary effect of increasing the risk of all leukemias. By increasing the specificity of the endpoint definition, this meta-analytic method does not create any bias but rather increases the statistical power of the study to detect an association with formaldehyde that is driven by myeloid leukemia. If the association between formaldehyde exposure and all leukemia as a group were driven by nonmyeloid leukemias, then this method would have less statistical power.

Of two individual studies with the relatively strongest exposure assessment (Hauptmann et al., 2009; Beane Freeman et al. 2009), Hauptmann et al. (2009) did not report specifically on leukemia (ICD-8: 205-207) but rather on lymphohematopoietic cancers of nonlymphoid origin (ICD-8: 205, 206, 208 or 209) and on myeloid leukemia specifically (ICD-8: 205). Statistically significant increased risk were reported for the larger grouping for 'ever embalming' with RR = 3.0 (95% CI: 1.0-9.5), for the highest category of duration with RR = 3.7 (95% CI: 1.1−12.2), for the highest category of number of embalmings with RR = 3.9 (95% CI: 1.2-12.8) and for the highest category of cumulative exposure with RR = 4.0 (95% CI: 1.2-13.2). However, none of the associated linear tests for tend were significant. Statistically significant increased risks were also reported for the highest category TWA formaldehyde intensity with RR = 3.4 (95% CI: 1.0-11.8) and the highest category of peak formaldehyde exposure with RR = 3.8 (1.1-12.7), both without showing a linear trend. These results were considered to be consistent with the meta-analytic findings of an association between formaldehyde exposure and all leukemia as a group.

While not the same as all leukemia as a group, Hauptmann et al. (2009) did look specifically at myeloid leukemia and reported extremely strong associations of each exposure metrics with the majority of RRs greater than 10 and statistically significant as detailed in the following subsection devoted to myeloid leukemia specifically. These myeloid leukemia results are strongly supportive of a causal association with formaldehyde and therefore are, in turn, supportive of a causal relationship with all leukemia as a group. The study by Beane Freeman et al. (2009) did look specifically at all leukemia as a group (ICD-8: 204-207) and reported an elevated RR = 1.42 (95% CI:0.92-2.18) for the highest category of peak exposure that was not statistically significant. Two trend tests were reported for peak exposures with the trend within

only the exposure groups having a $p = 0.12$ and the trend across all exposure groups including the unexposed having a significant trend ($p = 0.02$). Results from the analyses of average formaldehyde intensity did not show an association while results from the analyses of cumulative exposure did show some indication of a trend both within the exposed workers ($p = 0.12$) and across all the workers ($p = 0.08$).

While the epidemiologic evidence for a causal association between formaldehyde and all leukemia as a group is not at strong as for all LHP as a group, the repeated identification of an association in multiple meta-analyses taken together with the clear causal association between myeloid leukemia demonstrated by Hauptmann et al. (2009) and the consistent evidence reported by Beane Freeman et al. (2009) are sufficient to conclude that there is a causal association between formaldehyde exposure and mortality from all leukemia as a group.

Myeloid Leukemia

The associations between myeloid leukemia and formaldehyde exposure are strong and consistent. Of the six studies which formally assess myeloid leukemia mortality, five are positive, including cohorts of both professional and industrial workers (Hauptmann et al., 2009; Pinkerton at al., 2003; Hayes et al., 1990; Stroup et al., 1986; Walrath and Fraumeni, 1984, Walrath and Fraumeni, 1983; but not Beane Freeman et al., 2009).

Walrath and Fraumeni (1983) studied embalmers in NY and reported 6 deaths from myeloid leukemia compared to 4.1 expected based on the US male population stratified by age and calendar time which is equivalent to a SMR = 1.46 (exact 95% CI: 0.54-3.19). Walrath and Fraumeni (1984) studied embalmers in California and reported 6 deaths from myeloid leukemia compared to 4.0 expected based on the U.S. male population stratified by age and calendar time which is equivalent to a SMR = 1.50 (exact 95% CI: 0.55-3.26). While these studies were called proportionate mortality studies by the authors, the reported PMRs are more actually SMRs and should be interpreted as such.

Stroup et al. (1986) conducted an historic cohort mortality study of men who were members of the American Association of Anatomists between 1888 and 1969. Only 738 deaths were observed versus 1,133.9 expected, based on U.S. death rates (SMR 0.65), possibly indicating a sizable HWE. However, a slight increase in the risk of lymphatic and hematopoietic cancers (SMR 1.2; 18 observed) and the risk of leukemia (SMR 1.5; 10 observed) was evident. When the leukemia analysis was restricted to the myeloid type, the SMR increased to 8.8, based on five deaths ($p < 0.05$).

Hayes et al. (1990) conducted a cohort study of deceased U.S. male embalmers and funeral directors who had died between 1975 and 1985, using records from local licensing

boards, state funeral directors' associations in 32 states and the District of Columbia, the National Funeral Directors' Association, and state offices of vital statistics (n = 894). Expected deaths by cause were derived from 5-year age- and calendar-year-specific proportions of deaths among appropriate race groups from the U.S. population. No measured exposure data were available. Statistically significant excesses in hematopoietic and lymphatic cancers were found in embalmers and funeral directors. The PMR (actually SMR) was 1.39 (95% CI: 1.15−1.63). The excess risk in all males was higher for myeloid leukemia (ML) (PMR (actually SMR) 1.57 [95% CI: 1.01−2.34]; 24 observed).

The Pinkerton et al. (2004) occupational cohort study of garment workers also reported SMRs derived from age-, race-, and calendar-time-adjusted U.S. mortality rates. The overall SMR for leukemia was 1.09 (24 deaths) and 1.44 (95% CI: 0.80-2.37; 15 deaths) for ML. After 10 years of exposure, the risk for ML was 2.19 (exact 95% CI: 0.95-4.32). Exposure prior to 1963 was associated with a risk of 1.61 (exact 95% CI: 0.80-2.88). Among garment workers followed for 20 or more years from initial exposure, the SMR was significantly elevated for ML (1.91 [exact 95% CI: 1.02-3.26]; 13 deaths), as was the SMR for multiple cause leukemia (1.92 [95% CI: 1.08−3.17]; 15 deaths) in the subgroup with 10 or more years of exposure to formaldehyde and who were followed for 20 or more years after first exposure. The multiple cause mortality for ML for this subgroup of workers was also significant (SMR 2.55 [95% CI: 1.10−5.03]; 8 deaths).

The meta-analysis by Zhang et al. (2009) evaluated the studies of formaldehyde exposure and myeloid leukemia available at the time including Hauptmann et al. (2003), Pinkerton at al., 2003; Hayes et al., 1990; Stroup et al., 1986; Walrath and Fraumeni, 1984, Walrath and Fraumeni, 1983. While the findings of Hauptmann et al. (2003) on the NCI cohort have been recently updates by those of Beane Freeman et al. (2009) who updated the cohort, the Zhang et al. (2009) analysis provide the only formal meta-analysis specific to myeloid leukemia. Zhang et al., 2009 reported a statistically significant summary RR of 1.90 (95% CI: 1.31-2.76).

As previously mentioned, the two individual studies with the relatively strongest exposure assessment reported results that are also consistent with the results of the meta-analyses (Hauptmann et al., 2009; Beane Freeman et al. 2009). Because the results of Beane Freeman et al. (2009) are based on an update of the Hauptmann et al. (2003) study which was included in the meta-analysis by Zhang et al. (2009) it is not surprising that they are consistent with those of the meta-analyses showing statistically significant increased risk at the highest category of peak exposure that was associated with all LHP mortality. However, Beane Freeman et al. (2009) did not report statistically significant associations between formaldehyde exposure and mortality from myeloid leukemia. At the highest exposure category of peak exposure (peaks ≥4 ppm vs.

>0 to 2.0 ppm), the RR = 1.42 (95% CI: 0.92-2.18) for myeloid leukemia. Two test for trend were report for myeloid leukemia which provide some support for a concentration-response relationship with peak exposure but neither was statistically significant ($p = 0.13$ and $p = 0.07$).

Hauptmann et al. (2009) reported increases in risk for ever embalming with myeloid leukemia (OR = 11.2, 95% CI: 1.3-95.6). Duration of employment in jobs with embalming demonstrated an exposure-response relationship with increased risk of myeloid leukemia ($p = 0.02$). The number of embalming was also significantly associated with increased risk of myeloid leukemia in the middle (OR = 12.7, 95%: 1.4-116.7) and highest exposure levels (OR = 12.7, 95% CI: 1.6-119.7). Cumulative exposure was associated with increased risk of myeloid leukemia, with the highest category of exposure showing OR = 13.2 (95% CI: 1.5−115.4).

Hauptmann et al. (2009) showed that all three categories of average formaldehyde intensity while embalming were very strongly and significantly associated with the risk of myeloid leukemia mortality (OR = 11.1, 14.8, and 9.5), and the test for an exposure-response trend was of borderline statistical significance ($p = 0.058$). The risk of myeloid leukemia mortality was also very strongly and significantly associated with 8-hour TWA exposure for mid-level exposures (OR = 13.6, 95% CI: 1.5-125.8) and high-level exposures (OR = 12.0, 95% CI: 1.3-107.4), as well as for peak exposure in two of three categories (0-7 ppm OR = 15.2, 95% CI: 1.6-141.6; 7-9.3 ppm OR = 8.0, 95% CI: 0.9-74.0; and >9.3 ppm OR = 13.0, 95% CI: 1.4-116.9). For peak exposures, there was also a statistically significant exposure-response tend ($p = 0.036$).

The study by Hauptmann et al. (2009) stands out among the studies of embalmers and professionals in the funeral industry based on the strength of the quantitative exposure data and the demonstration of exposure-response relationships which provide causal evidence of an association between formaldehyde exposure and increased risk of myeloid leukemia. These results were internally consistent and demonstrated statistically significant associations that were unlikely the result of chance. As this nested case-control study was based on the cohorts of Hayes et al. (1990) and those of Walrath and Fraumeni (1983, 1984), the potential for selection bias is considered to be low. Further, the controls in Hauptmann et al. (2009) were carefully selected to avoid individuals who died of any causes that were thought to even possibly be related to formaldehyde exposure. Confounding is also unlikely to be an alternative explanation for the observed results as there were clear and convincing exposure-responses and the magnitude of the effect estimates were extremely large.

Given the consistency of the positive associations for formaldehyde with myeloid leukemia cancer mortality across five of the six studies (Hauptmann et al., 2009; 2009; Pinkerton

at al., 2003; Hayes et al., 1990; Stroup et al., 1986; Walrath and Fraumeni, 1984, Walrath and Fraumeni, 1983; but not Beane Freeman et al., 2009), the statistically significant meta analysis by Zhang et al. (2009) and the convincing results from Hauptmann et al. (2009), the human epidemiologic evidence is sufficient to conclude that there is a causal association between formaldehyde exposure and mortality from myeloid leukemia.

Hodgkin Lymphoma

The only meta-analysis to specifically address Hodgkin lymphoma was conducted by Zhang et al. (2009) and included eight studies (Anjelkovich et al., 1995; Coggon et al., 2003; Harrington and Shannon, 1975; Hauptmann et al., 2003; Hayes et al., 1990; Pinkerton et al., 2004; Walrath and Fraumeni, 1983, 1984; and Wong, 1983). Zhang et al. (2009) reported a summary RR = 1.23 (95% CI 0.67-2.29). This elevated, but nonstatistically significant finding is consistent with the large variance on reported results among the individual studies as well as the wide confidence intervals of the results which were based on small numbers of cases—even from the large cohort studies. Six of the eight studies observed three or fewer deaths from Hodgkin lymphoma. Coggon et al. (2003) reported 6 deaths from Hodgkin lymphoma against 8.5 expected for an SMT = 0.70 (95% CI: 0.26-1.53) and Hauptmann et al. (2003) reported 21 observed deaths with 20 deaths among the exposed workers who has an SMR = 1.26 (95% CI: 0.81-1.95). However, the Beane Freeman et al. (2009) update of the Hauptmann et al. (2003) study had the largest number of observed cases (n = 27) and was not included in the Zhang et al. (2009) meta-analysis. In fact, the Beane Freeman et al. (2009) study describes more deaths from Hodgkin lymphoma than all the other studies in Zhang et al. (2009) combined. Excluding the Hauptmann et al. (2003) results from the list of studies in the meta-analysis leaves 19 cases.

There is evidence for an exposure-response relationship for Hodgkin lymphoma in the NCI industrial cohort among exposed workers (Beane Freeman et al., 2009). Clear exposure response relationships for Hodgkin lymphoma are defined with all three metrics of exposure, peak average intensity and cumulative exposure (p = 0.01, p = 0.05 and p = 0.08 respectively for mortality through 2004). These associations have been evident from first follow-up through the current publication, and statistically significant for the majority of the follow-up period demonstrating that this is a strong and consistent finding in the NCI cohort (see Figures 4-3 and 4-4) (Beane Freeman et al., 2009).

As the majority of the studies reported specific data on Hodgkin lymphoma report on just three or fewer cases, the best epidemiologic evidence is obtained from the most recent evaluation of the NCI cohort by Beane Freeman et al. (2009). This cohort study, on its own, reported on more deaths from Hodgkin lymphoma that the remainder of the epidemiologic literature.

Hodgkin lymphoma was both shown to be at increased risk associated with peak exposure concentrations. Peak exposures in the highest exposure category were associated with a significant increase in Hodgkin lymphoma deaths comparing death rates among workers with peaks of ≥ 4 ppm to those with > 0 to 2.0 ppm (RR = 3.96, 95% CI: 1.31-12.02). Across the three categories of peak exposure, there was a statistically significant exposure-response trend (p = 0.01). The exposure-response trend including the never-exposed workers was also statistically significant (p = 0.004). The RR was also elevated for average intensity of formaldehyde exposure with RR = 2.48 (95% CI: 0.84-7.32) and there were significant tests for trend among only the exposed workers (p = 0.05) and all workers (p = 0.03). Similarly, there were nearly significant tests for trend with cumulative exposure among only the exposed workers (p = 0.08) and all workers (p = 0.06).

The majority of the studies reporting on Hodgkin lymphoma did not have sufficient statistical power describe any potential association with formaldehyde as the numbers of observed and expect cases were small and the resulting effects estimates were imprecise. As the Beane Freeman et al. (2009) study reported on the largest number of cases and was the individual study with the most detailed and objectively ascertained exposure assessment and demonstrated significant exposure-response gradients, it is judged that this epidemiologic evidence is supportive of a causal association between formaldehyde and Hodgkin lymphoma.

Non-Hodgkin Lymphoma

The only meta-analysis to specifically address non Hodgkin lymphoma was conducted by Zhang et al. (2009) and included eleven studies. Zhang et al. (2009) reported a summary RR = 1.08 (95% CI 0.86-1.35). Hauptmann et al. (2009) did not specifically report on non-Hodgkin lymphoma. Beane Freeman et al. (2009) did report on 106 deaths from non-Hodgkin lymphoma but did not identify any significant association in their categorical analyses or in their tests for trend for either peak exposure, average intensity of exposure or for cumulative exposure.

Wang et al. (2009) assessed the effect of formaldehyde exposure on the risk of non-Hodgkin lymphoma in a population-based case-control study. Semiquantitative exposure metrics included average exposure intensity and average exposure probability which were evaluated individually and together. Analyses use unconditional logistic regression and controlled for age, family history of hematopoietic cancers, alcohol consumption, and race. For the low average exposure intensity category the OR = 1.4 (95% CI: 1.0-1.8), while for the Medium-High category the OR = 1.2 (95% CI: 0.8-1.7). For the Low average exposure probability category the OR = 1.3 (95% CI: 1.0-1.7), while for the Medium-High category the

OR = 1.4 (95% CI: 0.9-2.3). The investigators also examined the risk of non-Hodgkin lymphoma among major subtypes. The risk of follicular lymphoma and chronic lymphocytic leukemia/Small lymphocytic lymphoma was slightly elevated but the risk of diffuse large B-cell lymphoma was OR = 1.9 (95% CI: 1.3-2.6) for ever having been exposed to formaldehyde. For Low average intensity exposure, the risk was OR = 2.1 (95% CI: 1.3-2.6) while for Medium-High average intensity exposure, the risk was OR = 1.5 (95% CI: 0.9-2.4). Even so, an exposure-response relationship was demonstrated using the continuous parameterization of average intensity rather that the categorical ($p = 0.03$). Likewise, an exposure-response relationship was demonstrated using the continuous parameterization of average probability of exposure rather that the categorical ($p = 0.01$).

The findings of Wang et al. (2009) provide some support for an association between formaldehyde exposure and non-Hodgkin lymphoma. It should be noted that a population-based case-control study, where incidence rather than mortality defines the case—may be more appropriate for cancers with relatively low mortality (i.e., CCL, large B-cell lymphoma, Small lymphocytic lymphoma).

Aside from the semiquantitative study by Wang et al (2009), non-Hodgkin lymphoma does not appear to be associated with formaldehyde exposure. There is not sufficient evidence of a causal association between formaldehyde exposure and non-Hodgkin lymphoma.

Multiple Myeloma

The only meta-analysis to specifically address Hodgkin lymphoma was conducted by Zhang et al. (2009) and included nine studies (Boffetta et al., 1989; Coggon et al., 2003; Dell and Teta, 1995; Edling et al. 1987; Hauptmann et al. 2003; Hayes et al. 1990; Heineman et al. 1982; Pottern et al. 1992; Stellman et al. 1998). Zhang et al. (2009) reported a summary RR = 1.31 (95% CI 1.02-1.67). This statistically significant finding is consistent with the findings of Beane Freeman et al. (2009) who reported that peak exposures in the highest exposure category were associated with a significant increase in multiple myeloma deaths comparing death rates among workers with peaks of ≥ 4 ppm to those with > 0 to 2.0 ppm (RR = 2.04, 95% CI: 1.01-4.12). Across the three categories of peak exposure, there was also some evidence of an exposure-response trend ($p = 0.08$); however, there was no evidence of an exposure-response trend including the never-exposed workers. The association of multiple myeloma with formaldehyde exposure was also shown throughout the cohort experience (see Figure 4-3 and 4-4) which adds strength to this finding.

The epidemiologic evidence for a causal association between formaldehyde and all multiple myeloma as described by the statistically significant increased risk identified in the

meta-analysis of Zhang et al. (2009) and the most recently updated analysis of the NCI cohort by Beane Freeman et al. (2009) are considered to be supportive of a causal association between formaldehyde exposure and mortality from multiple myeloma.

4.1.2.2.2. *Brain and CNS cancer.*

Several studies of professional groups discussed earlier investigated brain and other CNS cancers among those exposed to formaldehyde on the job. Several of these studies found that exposure increased risk two to three times among exposed professionals (Hall et al., 1991; Stroup et al., 1986; Walrath and Fraumeni, 1984), while others found modest or no increase in risk (Hayes et al., 1990; Levine et al., 1984; Walrath and Fraumeni, 1983).

None of the industrial cohort worker mortality studies of exposure to formaldehyde found a clear relationship between formaldehyde exposure and risk of brain or CNS cancer (Pinkerton et al., 2004; Coggon et al., 2003; Andjelkovich et al., 1995; Gardner et al., 1993; Stayner et al., 1988; Blair et al., 1987, 1986). To date, no case-control studies of brain and CNS cancer have been completed. In the Hauptmann et al. (2004) study, the authors reported that no clear association was seen for cancer of the brain and CNS and exposure to formaldehyde.

4.1.2.2.3. *Pancreatic and other cancers.*

Two studies (Kernan et al., 1999; Dell and Teta, 1995) have found increases in the risk of pancreatic cancer in association with possible exposure to formaldehyde. Collins et al. (2001a) conducted a meta-analysis of fourteen studies (Kernan et al., 1999; Andjelkovich et al., 1995; Hansen and Olsen, 1995; Gardner et al., 1993; Hall et al., 1991; Matanoski, 1991; Hayes et al., 1990; Gerin et al., 1989; Stayner et al., 1988; Blair et al., 1986; Stroup et al., 1986; Levine et al., 1984; Walrath and Fraumeni, 1984, 1983) and found a small increase in risk (RR = 1.1 [95% CI: 1.0−1.3]).

Other sites that have been examined are stomach cancer (Coggon et al., 2003) (SMR = 1.47; $p < 0.05$), intraocular melanoma (Holly et al., 1996) (OR = 2.9 [95% CI: 1.2−7.0]), and thyroid cancer among women (Wong et al., 2006) (OR = 8.33 [95% CI: 1.16−60.0]; 2 cases). However, without further substantiation, it is difficult to infer causation based on these isolated results alone.

4.1.2.3. *Summary: Carcinogenic Hazard in Humans*

The weight of the epidemiologic evidence at this time supports a link between formaldehyde exposure and NPC in humans. This conclusion is based on the longitudinal cohort study of Hauptmann et al. (2004) as well as the case-control studies of NPC and formaldehyde

exposure completed by Vaughan et al. (2000), West et al. (1993), Vaughan et al. (1986b) and several additional case-control studies described in the text. With the exception of Hauptmann et al. (2004), most of the other cohort studies found little or no increased risk of NPC from exposure to formaldehyde. However, Hauptmann et al. (2004) employed different exposure metrics and based their analyses on conservative internal comparisons that limited the potential for the HWE to obscure true effects. The case-control studies that provide additional evidence of an association between NPC and formaldehyde have more power and generally rely on better diagnoses of NPC. Better ascertainment of histologic types of tumors can sometimes also be obtained if the cases are taken from cancer registries. The NPC risk is also supported by experimental evidence in animals in which formaldehyde induces nasal cancers (see Section 4.2.2). Since the physiology of the rat nasal passage is somewhat different from that of humans, it is not possible to obtain a direct site-specific correspondence between the species. However, in both species, the tumors are found within the same area of the URT where maximum exposure can be expected to occur.

Several researchers have challenged the conclusion of a relationship between formaldehyde and NPC. Those critical of the link argue that, given the wide variability in results across studies and competing explanations, conclusions about any link from the existing studies are premature. The difficulty in attaining consensus on whether formaldehyde influences the risk of NPC in humans arises from several limitations inherent in epidemiologic methods and exposure assessment, as well as from the characteristics of the disease. The most prominent of these limitations are the rarity of the cancer and imprecise estimates of exposure. Because NPC is a very rare cancer with an incidence of less than 1 per 100,000, it is difficult to obtain precise estimates of risk from cohort studies. Although case-control studies are better suited for studying rare conditions, they are limited in obtaining valid and precise exposure assessments. A further problem with exposure assessment is isolating formaldehyde exposure from other potential chemical or particulate exposures that may influence risk of NPC. Imprecise exposure assessment and the inability to isolate formaldehyde exposure from other exposures are largely the bases on which Marsh and coworkers have challenged the NCI cohort study (Marsh et al., 2007a, b, 2002, 1996; Marsh and Youk, 2005). Marsh and coworkers (Marsh et al., 2007a) show that subjectively assessed exposure to silversmithing is tentatively associated with NPC. Given that there were no prior citations of an association between silversmithing exposures and NPC in the medical literature and given the many post hoc reexaminations of alternative hypotheses to explain the original NCI findings, it is more likely that silversmithing is an artifactual potential confounder.

It may be expected that, without new approaches for obtaining more accurate and precise estimates of exposure, further follow-up of current cohorts and future epidemiologic studies of formaldehyde and NPC will face the same limitations and criticisms found with existing studies. These limitations notwithstanding, the epidemiologic studies reviewed here represent what may be currently discernable about a formaldehyde-NPC link in humans by using rigorous observational methods. As such, concluding any influence of formaldehyde must be made on the weight of all human and animal evidence in the face of known and expected limitations in study design and exposure assessment.

The results of two well-designed cohort studies found a positive association between formaldehyde-exposed professionals, such as pathologists, embalmers, and funeral directors, and LHP cancer, particularly ML. The largest cohort study of formaldehyde has the most extensive exposure assessment (Blair et al., 1986; Stewart et al., 1986), and the cohort was followed for a median duration of 35 years (Hauptmann et al., 2003). By using cumulative exposure measures not previously used and by using internal comparison groups, significant increases in the risk of cancer of the LHP system, particularly ML, were reported. This study demonstrated that formaldehyde was a risk factor for LHP cancers, independent of other risk factors, such as benzene and smoking. Hauptmann et al. (2003) found statistically significant dose trends for peak exposure and AIE. Pinkerton et al. (2004) also found a significant increase in the risk of ML in garment workers 20 years after their initial exposure and in workers with 10 or more years of exposure. Additionally, several studies of pathologists, embalmers, and other medical workers reported greater numbers of observed deaths from leukemia than expected although many studies of these groups suffer from a substantial HWE based on comparisons with external death rates. Two of these studies, Hayes et al. (1990) and Stroup et al. (1986), also report a significantly excess risk of ML in embalmers, funeral directors, and anatomists.

There is a range of biological plausibility for an agent whose primary action is at the POE. Acute leukemias (ALL and AML), believed to arise from transformation of stem cells in the bone marrow, are less plausible. In contrast chronic lymphatic leukemia, lymphomas, multiple myelomas (from plasma B cells), and unspecified cancers may involve an etiology in peripheral tissues to include cells, cell aggregates, germinal centers, and lymph nodes. An association of these cancers to an exogenous agent acting at the POE is biologically plausible.

It is the conclusion of this assessment that the weight of the epidemiologic evidence at this time supports a link between formaldehyde exposure and carcinogenicity in humans.

4.2. ANIMAL STUDIES

This section discusses the available laboratory animal data on the toxicity of inhalation, oral, and dermal exposures to formaldehyde. An extensive database of laboratory animal studies is available for formaldehyde, including numerous 2-year bioassays by both the inhalation and oral exposure routes. Although a large portion of the literature reports studies focused on toxic effects at the site of contact or portal of entry (POE), general systemic effects as well as neurobehavioral effects, reproductive and developmental effects, immunologic changes, and sensitization are represented in the literature as well. The organization and general content of the chapter is discussed below.

The first subject addressed for the animal studies is the occurrence of reflex bradypnea (RB) observed in rodents exposed to reactive gases, including formaldehyde (see Section 4.2.1). RB is a reduction in ventilation rate, minute volume, and other physiological parameters experienced by rodents exposed to an irritant/reactive gas. Although humans and nonhuman primates do not exhibit the same change in respiratory rate, these studies are included in order to better understand the effects on RB in interpreting rodent studies presented in the balance of the chapter. Additionally, although binding to the trigeminal nerve and subsequent downstream events do not result the acute signs of RB in humans, the mechanism itself may play a role in understanding other adverse health effects observed in humans including more subtle changes in pulmonary function, sensitization, and sensory irritation.

The available data for the inhalation and oral exposures confirm direct formaldehyde-induced toxicity in tissues present at the POE. These observations are consistent with the physicochemical characteristics, reactivity, and metabolic pathways of formaldehyde as discussed in Chapter 3. Indications of cell damage, cell proliferation, and inflammatory responses are similar for each route of exposure, therefore effects at the POE for inhalation and oral exposures are described first (see Sections 4.2.2 and 4.2.3, respectively). Given the well-established nature of these health effects and the wealth of literature for inhalation exposures, complete study summaries for respiratory tract effects are provided. Studies are organized by study duration—acute, subchronic and chronic—where some of the chronic bioassays were designed to address carcinogenic potential.

Although a majority of the oral and inhalation studies focus on health effects at the POE—respiratory tract and GI tract—the general systemic toxicity of formaldehyde is addressed where it was integral to the study. Therefore, body weight and organ weight changes, gross pathology, organ histopathology outside of the POE, blood and urine chemistry, and other biochemical measures may be included in these study summaries. An overview of general systemic findings is provided in Section 4.4 for all routes of exposure.

Studies addressing immune function, neurobehavioral effects, sensitization, and reproductive and developmental effects are addressed across routes of exposure. The specialized nature of these studies requires discrete treatment, and inclusion of data across routes of exposure allows for a synthesis of the available information, to better understand the toxic potential of formaldehyde exposure on these endpoints.

4.2.1. Reflex Bradypnea

Reflex bradypnea (RB), which is believed to be a protective response, is often observed in rodents exposed to reactive gases. It is primarily characterized by marked decreases in activity, respiratory rate, body temperature, and metabolic rate. RB is not seen in humans and nonhuman primates. An understanding of the RB is important to the interpretation of many of the animal bioassays examining formaldehyde-induced health effects. Of chief concern is that the physiological effects of RB, described below, may interfere with appropriate interpretation of adverse effects noted with formaldehyde exposure. It is important to distinguish between an effect directly related to RB versus formaldehyde exposure. Additionally the effects of RB may mask or alter formaldehyde-induced health effects. Secondly, differential respiratory effects of RB due to species and strain will result in differential inhaled doses at the same exposure level. This needs to be considered both when comparing the results of animal studies and in extrapolation to humans. Finally, although humans do not experience RB, the mechanism of RB as a reflex response to trigeminal nerve stimulation assists in understanding human health related to localized and reflex responses due to trigeminal nerve stimulation.

Irritant gases have been shown to decrease body temperature, heart rate, and blood pressure as well as alter blood chemistry in rodents (Pauluhn, 2003, 1996; Jaeger and Gearhart, 1982). Because of their small size, mice can rapidly lower their body temperatures and thus their metabolic rate and ventilation rate. The hypothermia that results from RB can directly affect nearly all biological processes (Gordon et al., 2008). Formaldehyde exposure can dramatically lower ventilation rate and reduce body temperature in mice by as much as 4°C, and it has been posited that decreased oxygen supply is likely to have profound effects on organisms with substantial oxygen demands (Jaeger and Gearhart, 1982). The effects of RB are reversible, though it may take several minutes to several hours to return to pre-exposure conditions (Pauluhn, 1996; Jaeger and Gearhart, 1982).

The literature on sensory irritation is broad; many studies have investigated species differences, dose response relationships, tolerance, and cross-tolerance to other sensory irritants (see Tables 4-8 and 4-9). This discussion focuses on the changes in respiratory rate and minute volume during formaldehyde exposure. Sensory irritation is often quantified as the statistically

derived exposure concentration that results in a 50% reduction in respiratory rate (RD_{50}) in rodents (ASTM, 2000; Kane et al., 1979). Kane and Alarie (1977) evaluated various aspects of sensory irritation, including establishing the RD_{50}, exploring the reproducibility of response, investigating the effect of tracheal cannulation, and determining the potential for tolerance with repeated exposure or pre-exposure in male Swiss-Webster mice, caused by formaldehyde and acrolein. The RD_{50} was established by exposing four mice for 10 minutes at each concentration across a range representing approximately 10 to 80% reduction in respiration and calculated by using least squares regression. The RD_{50} and its 95% CI for formaldehyde were calculated to be 3.1 (2.1−4.7) ppm (3.8 [2.58−5.77] mg/m^3). The tracheal cannulation experiments demonstrated that the effect on respiratory rate was caused by URT sensory irritation.

Table 4-8. Respiratory effects of formaldehyde-induced reflex bradypnea in various strains of mice

Species/strain	No./ group	Treatment[a]	Respiratory effects	Reference
Male Swiss-Webster mice	4	Duration: 10 minutes. Exposure: up to 100 ppm.	RD_{50}[a] = 3.1 ppm (95% CI: 2.1−4.7).	Kane and Alarie (1977)
Male Swiss-Webster mice	8	Duration: 3 hours/day for 3 days. Exposure: 0.52, 0.44, 1.16, 1.83, 3.10, 5.35, 5.60, and 11.2 ppm.	RD_{50} = 3.4 ppm (95% CI: 2.4−4.7).	Kane and Alarie (1977)
Male Swiss-Webster mice	4	Duration: 10 minutes (head only). Exposure: up to 10 ppm.	RD_{50} = 3.2 ppm (95% CI: 2.1−4.7).	Steinhagen and Barrow (1984)
Male Swiss OF_1 mice	6	Single 5-minute exposure to four unspecified concentrations.	RD_{50} = 5.3 ppm.	De Ceaurriz et al. (1981)
Male B6C3F1 mice	4	Duration: 10 minutes (head only). Exposure: Range up to 10 ppm.	RD_{50} = 4.9 ppm (95% CI: 3.9−6.4).	Steinhagen and Barrow (1984)
Male B6C3F1 mice	4	Duration: 10 minutes (head only). Exposure: up to 15 ppm Pretreatment: 2, 6, or 15 ppm 6 hours/day for 4 days.	Naïve mice: RD_{50} = 4.4 ppm (95% CI: 0.9−5.0) Pretreated mice: RD_{50} = 4.3 ppm (95% CI: 3.4−5.5).	Chang et al. (1981); Barrow et al. (1983)
Male C57BL6/F1 mice	3	Whole-body exposure for up to 2 hours.	After 1.25 hours: Tidal volume reduced by 33%; 68% reduction in respiratory frequency; CO_2 production reduced by 50%; percent; body temperature dropped from 37.8 to 34.7°C.	Jaeger and Gearhart (1982)

[a]Exposure concentration that results in a 50% reduction in respiratory rate.

Table 4-9. Respiratory effects of formaldehyde-induced reflex bradypnea in various strains of rats

Species/strain	No./ group	Treatment	Respiratory effects	Reference
Male Crl-CD rats	4		RD_{50} = 13.8 ppm.	
Male Wistar rats	4	30-minute nose-only exposure to a range of formaldehyde concentrations.	RD_{50} = 10.0 ppm.	Cassee et al. (1996)
Male F344 rats	4	Duration: 10 minutes (head only). Exposure: up to 56 ppm. Pretreatment:2, 6, or 15 ppm 6 hours/day for 4 days	Naïve rats: RD_{50} = 13.1 ppm (95% CI: 10.6−17.5) Pretreated rats: RD_{50} = 10.8 ppm (95% CI: 7.6−16.9)	Chang et al. (1981); Barrow et al. (1983)
Male F344 rats	4	Single 10-minute head-only exposure to a range of concentrations. Pretreatment: 15 or 28 ppm formaldehyde or 10 ppm chlorine.	Baseline RD_{50} = 31.7 ppm. Pre-exposure to formaldehyde-induced tolerance at 28 ppm (RD_{50} = 20.2 ppm) but not 15 ppm. Pre-exposure to chlorine-induced tolerance to formaldehyde (RD_{50} ranged from 64.5 to 115 ppm, depending on exposure duration).	Chang and Barrow (1984)
Male F344 rats	ND	10 minute exposure to acrolein or acetaldehyde (head only). Pre-exposed to formaldehyde at 15 ppm for 6 hours/day for 9 days.	Pre-exposure to formaldehyde-induced tolerance: Acetaldehyde (RD_{50} = 2,991 ppm in naive versus 10,601 ppm in preconditioned animals) Acrolein (RD_{50} = 6 ppm in naïve versus 29.6 ppm in preconditioned animals).	Babiuk et al. (1985)
Male Charles Rivers CD rats	3	Whole-body exposure for up to 2 hours.	After 0.7 hours: Tidal volume reduced by 22%; 20% reduction in respiratory frequency; CO_2 production unaffected.	Jaeger and Gearhart (1982)

Across the literature there is fairly good agreement on RD_{50} values for various strains of mice (see Table 4-8), ranging from 3.1 ppm in male Swiss-Webster mice to 4.9 ppm in male B6C3F1 mice. Rats are less sensitive, with RD_{50} values ranging from 10 ppm in male Wistar rats to 31.7 ppm in male F344 rats. No reported RD_{50} for female rodents exposed to formaldehyde exists.

Jaeger and Gearhart (1982) evaluated the effect of formaldehyde on respiratory rate, tidal volume, minute volume, carbon dioxide (CO_2) production (exhaled to air) as a reflection of total metabolism, and core body temperature in male Charles River CD rats and male C57BL6/F1

mice. Animals (three/concentration) were exposed to 15 ppm (18.4 mg/m^3) formaldehyde for up to 2 hours. Mice exhibited a greater decrease in respiratory frequency and minute volume compared with the rats. CO_2 production and body temperature were also affected to a greater extent in the mice (see Table 4-8). The authors postulated that the decreased body temperature in mice would likely lead to decreased biologic action of formaldehyde in the tissue.

4.2.1.1. *Tolerance*

Tolerance is defined as an increase in the concentration required to elicit the same degree of RB response and was evaluated by Kane and Alarie (1977). In the first set of experiments, mice (four/concentration) were exposed 3 hours/day for 4 days at the concentration associated with either a 30 or 50% decrease in respiratory frequency (specific concentrations not given) (Kane and Alarie, 1977). Naïve animals served as controls for each day. The maximum response increased with each additional day of exposure, and the diminution of response that was typically exhibited after 60 minutes of exposure in naïve animals was markedly delayed. In the second set of experiments, mice were exposed to a formaldehyde concentration at one-tenth the RD_{50} (i.e., 0.3 ppm) 3 hours/day for 3 days. On the fourth day the animals underwent a similar exposure protocol to identify the concentration that resulted in an RD_{50}, following the above protocol. No change in the RD_{50} was demonstrated. Both of these experiments indicate no change in tolerance with either type of pretreatment in Swiss-Webster mice.

Chang and Barrow (1984) tested whether tolerance would develop in male F344 (CDF[F344]Crl/Br) rats exposed to formaldehyde. Exposure to formaldehyde at 15 ppm (18.4 mg/m^3) for 6 hours/day, 5 days/week failed to induce tolerance. However, tolerance was observed following exposure to 28 ppm (34.4 mg/m^3) formaldehyde for 4 days. The concentration-response curve in these animals was significantly different than that of naïve animals, with an increase in the RD_{50} estimate for this exposure duration from 31.7 to 70.2 ppm.

4.2.1.2. *Cross-Species Differences in Inhaled Dose*

Formaldehyde-induced RB lowers both respiratory rate and tidal volume and thus reduces the inhaled dose of formaldehyde at a given exposure concentration. Chang et al. (1983) and Barrow et al. (1983) evaluated the species differences and the effective inhaled dose between rats and mice, since mice seem to be more sensitive to formaldehyde-induced RB and do not exhibit tolerance as shown in F344 rats. Groups (four/concentration) of male F344 rats and male B6C3F1 mice were exposed to formaldehyde concentration ranges of 6.2−48 ppm (7.6−59 mg/m^3) or 0.78−14.0 ppm (0.96−17.2 mg/m^3), respectively, for 10 minutes. Pretreated animals used in the tolerance experiments were exposed to formaldehyde at 2, 6, or 15 ppm

(2.45, 7.36, or 18.4 mg/m^3) 6 hours/day for 4 days prior to determination of the RD_{50} and concentration response across the same ranges.

A concentration-dependent decrease in respiratory rate was seen in both naïve and pretreated rats during formaldehyde exposure. Tolerance (defined as a decrease in respiratory rate followed by a subsequent return to control values) occurred after 4 minutes of exposure and was more pronounced at concentrations above 4 ppm. Concentration-response relationships were very similar for naïve and pretreated rats, and the RD_{50}s were similar for both groups (naïve = 13.1 ppm [95% CI: 10.6−17.5]; pretreated = 10.8 ppm [95% CI: 7.6−16.9]). In contrast, naïve or pretreated mice did not develop tolerance during exposures. An examination of concentration-response relationships for mice showed similar RD_{50} values (naïve = 4.4 ppm [95% CI: 0.9−5.0] and pretreated = 4.3 ppm [95% CI: 3.4−5.5]) compared with rats, although the slopes of the concentration-response regressions were statistically different (see Figure 4-5).

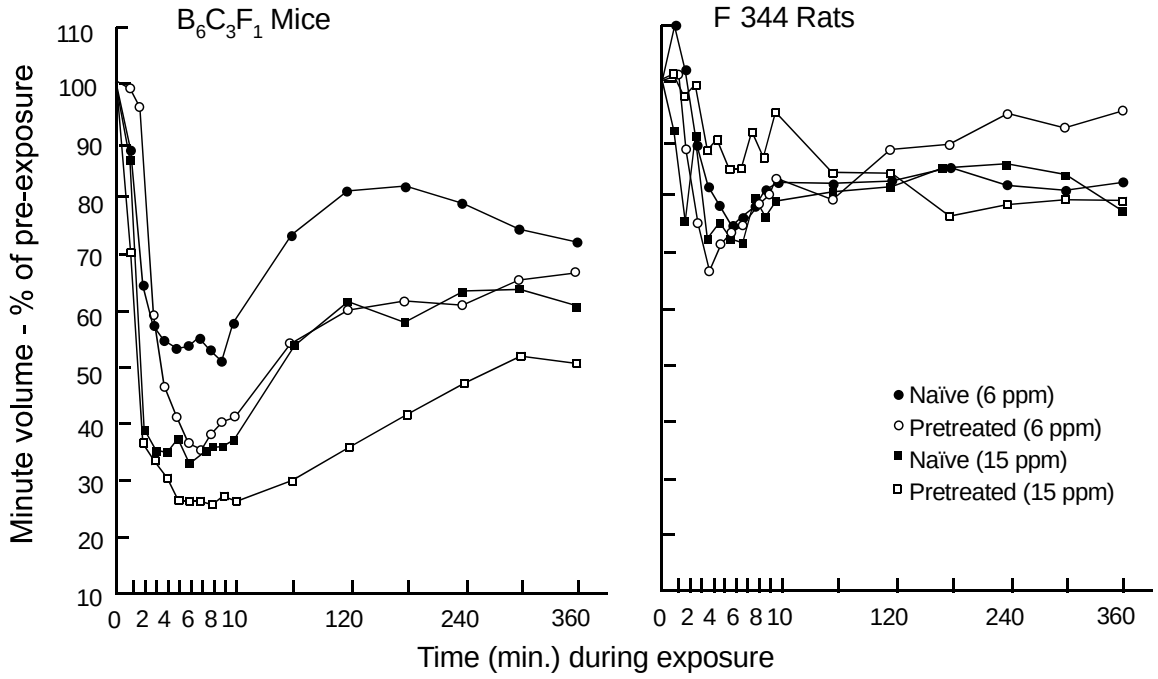

Figure 4-5. Formaldehyde effects on minute volume in naïve and formaldehyde-pretreated male B6C3F1 mice and F344 rats.

Source: Redrawn from Chang et al. (1983).

Exposure of naïve or pretreated rats resulted in an increased (compensatory) tidal volume. However, the increase in tidal volume did not compensate entirely for the decrease in ventilation rate and was only concentration dependent in pretreated rats. Comparison of tidal

volume from naïve and pretreated mice exposed to formaldehyde showed a slight increase in naïve animals but a decrease in pretreated ones. The effect of formaldehyde exposure on tidal volume was concentration dependent in both groups of mice. These results indicate that tidal volume does not compensate entirely for the decrease in respiratory rate and that the compensation is slightly greater in rats than in mice.

These studies (Barrow et al., 1983; Chang et al., 1983) showed that B6C3F1 mice sustain RB, whereas F344 rats develop tolerance more readily both during exposure and with pretreatment. Thus, these results suggest that the rat may be the more sensitive species for the effects of inhaled formaldehyde due in part to the difference in sensitivity between mice and rats as evidenced by an RD_{50} of 4.9 versus 31.7 ppm and the ability of rats to develop tolerance while mice appear to sustain RB. Barrow et al. (1983) used the results of these experiments to estimate an inhaled dose equivalent to the exposure concentration of 15 ppm for the strains of mice and rats used in the chronic formaldehyde bioassays by Kerns et al. (1983) and Monticello and Morgan (1994) described in Section 4.1.2 as follows:

Inhaled dose ($\mu g/min\text{-}cm^2$) =

$$\frac{\textit{HCHO concentration } (\mu g/L) \times \textit{minute volume } (L/min)}{\textit{Nasal cavity surface area } (cm^2)} \qquad (5\text{-}1)$$

As shown in Table 4-10, because mice were observed to be able to decrease their minute volume by approximately 75% as compared with 45% in rats, a twofold higher inhaled dose would be expected in rats versus mice. This difference may be relevant to the increased incidence of SCC in the nasal cavity seen in F344 rats when compared with B6C3F1 mice.

Table 4-10. Inhaled dose of formaldehyde to nasal mucosa of F344 rats and B6C3F1 mice exposed to 15 ppm

Parameter	F344 rats	B6C3F1 mice
HCHO concentration (µg/L)	18.4	18.4
Minute volume (L/min)	0.114	0.012
URT surface area (cm^2)	13.44	2.89
Inhaled dose ($\mu g/min/cm^2$)	0.156	0.076

Source: Barrow et al. (1983).

4.2.1.3. *Cross-Tolerance*

Cross-tolerance of chemically-induced reflex responses has been examined in several systems in order to better understand the specificity and nature of the interaction of reactive chemicals (such as formaldehyde with chlorine) with the trigeminal nerve involved in the RB. Development of cross-tolerance to formaldehyde following preexposure to chlorine or to chlorine following preexposure to formaldehyde was shown to be a function of the duration of the pretreatment in male F344 rats (Chang and Barrow, 1984) (see Table 4-11). A 7-day recovery period resulted in only a slight loss of cross-tolerance from a 4-day pre-exposure to either chlorine or formaldehyde (data not shown). The cross-tolerance between formaldehyde and chlorine demonstrated in the Chang and Barrow (1984) study suggests that these chemicals may act via a common mechanism and may involve the trigeminal nerve. In rats, cross-tolerance was induced after chlorine exposure but not after formaldehyde exposure, which suggests that the trigeminal nerve may have different reactive sites that are differentially activated, depending on the stimulus.

Table 4-11. Exposure regimen for cross-tolerance study

Pre-exposure			Chlorine RD_{50}	
			FA-pretreated	**Naïve**
Formaldehyde	15 ppm, 6 hours/day	1 day	22.6 ppm	10.9 ppm
		4 days	16.8 ppm	
		10 days	64.5 ppm	
Pre-exposure			**Formaldehyde RD_{50}**	
			Cl-pretreated	**Naive**
Chlorine	10 ppm, 6 hours/day	1 day	64.5 ppm	31.7
		4 days	66 ppm	
		10 days	115 ppm	

Source: Chang and Barrow (1984).

Babiuk et al. (1985) evaluated the potential for formaldehyde pretreatment to cause cross-tolerance with various other inhaled aldehydes, including acetaldehyde and acrolein. Male F344 rats were pretreated with 15 ppm (18.4 mg/m^3) formaldehyde 6 hours/day for 9 days and challenged on the 10^{th} day with the second aldehyde for 10 minutes at various concentrations (four rats/concentration) to establish an RD_{50}. Exposure to acetaldehyde and acrolein, the two smallest molecules in the series of aldehydes tested, resulted in cross-tolerance. The RD_{50} and

its 95% CI for acetaldehyde were estimated at 2,991 (95% CI: 2,411−3,825) ppm in the naïve rats, and this was increased by approximately 3.5-fold to 10,601 (95% CI: 7,902−15,442) ppm in the rats pretreated with formaldehyde. With acrolein, the RD_{50} increased approximately fivefold, from 6.0 (95% CI: 3.5−18.1) ppm to 29.6 (95% CI: 15.6−93.0) ppm. Cross-tolerance with formaldehyde has only been demonstrated with acetaldehyde, acrolein, and chlorine (Babiuk et al., 1985; Chang and Barrow, 1984), suggesting that it is not a generalized phenomenon.

Whether the phenomenon of tolerance involves modulation of specific trigeminal nerve receptors or whether it results from less specific chemical injury of the nasal mucosa has not been determined. For example, different mechanisms lead to stimulation of the trigeminal nerve and are likely to control the decrease in respiratory rate. In particular, acetaldehyde might interact with sensory nerves via an amino group (Steinhagen and Barrow, 1984; Schauenstein et al., 1977), whereas the receptor-binding site for formaldehyde and acrolein is believed to be a thiol group. Furthermore, different binding sites exist on the trigeminal nerve for different irritants (Nielsen, 1991). Thus, Bos et al. (1992) concluded that the data on tolerance or "desensitization" versus "sensitization" (as defined strictly on the basis of the respiratory apneic response) may be the result of adaptation or reversible/irreversible adverse changes. The mechanisms underlying sensitization or desensitization are not well characterized.

4.2.1.4. *Formaldehyde Binding and Activation of Trigeminal Nerve Afferent Activity*

Kane and Alarie (1978) evaluated the effect of 11 combinations of acrolein and formaldehyde on respiratory rate in outbred specific-pathogen-free male Swiss-Webster mice. Exposure concentrations ranged from 0.12−8.97 ppm (0.28−21 mg/m^3) for acrolein and 0.37−9.73 ppm (0.45−11.9 mg/m^3) for formaldehyde. The data were evaluated using a simple model of competitive antagonism. Comparing the observed and predicted responses indicated no apparent differences, and paired t-tests showed no statistical significance. The authors concluded that acrolein and formaldehyde acted at the same receptor site and acted as competitive antagonists when exposure occurred simultaneously.

Kulle and Cooper (1975) investigated the effects of formaldehyde on trigeminal nerve afferent activity in adult male Sprague-Dawley rats. The authors isolated both the ethmoid and nasopalatine branches of the trigeminal nerve and recorded afferent signaling as electrical activity while reactive gases (formaldehyde, ozone, and amyl alcohol) were passed through the nasal passages of the anesthetized animals. The authors reported that both branches of the trigeminal nerve responded similarly to all three chemicals, and they therefore conducted the balance of their experiments on the nasopalatine branch of the nerve. Nerve response was calculated as the difference between exposed and control activity, and the threshold for a positive

response was arbitrarily defined as an increase of 0.1 spikes per second. The sensory threshold was determined by extrapolation from the measured nerve response to a range of formaldehyde concentrations (0.5−2.5 ppm) or ozone (5.0−29 ppm) for an exposure duration of 2 minutes. Amyl alcohol exposure (0.3−10.0 ppm) lasted for 25 seconds. Threshold was arbitrarily defined as an increase of 0.1 spikes per second. The mean thresholds were 0.25 ppm for formaldehyde, 5.0 ppm for ozone, and 0.30 ppm for amyl alcohol, suggesting that the trigeminal nerve is highly sensitive to formaldehyde and amyl alcohol compared with ozone exposure.

In a second set of experiments, Kulle and Cooper (1975) investigated the effects of prolonged formaldehyde-exposure on the odor response to amyl alcohol. Rats were pre-exposed to a series of amyl alcohol concentrations (0.3, 0.7, 1.0, 3.3, 6.7, or 10.0 ppm [1.08, 2.52, 3.6, 11.9, 24, or 36 mg/m^3]) then a 1-hour continuous formaldehyde exposure (0, 0.5, 1.0, 1.5, or 2.0 ppm [0, 0.61, 1.23, 1.84, or 2.45 mg/m^3]). There was a progressive decrease in odor response to amyl alcohol with increasing stimulus of formaldehyde concentration ($p < 0.01$, analysis of variance [ANOVA]). The response to formaldehyde concentration was described by a power function $Y = 0.741 \times X^{1.47}$, where X is the formaldehyde concentration. The effects of exposure to 2.0 ppm were similar, regardless of whether it was presented immediately as a separate exposure or as the final concentration of a progressively increasing series. The response to amyl alcohol did not fully recover within the 1-hour extended recovery period. Thus, it appeared that the afferent function depression was not due to receptor adaptation or insufficient time for formaldehyde diffusion away from receptor sites.

In an attempt to elucidate the basis of the differential effects of various types of aldehydes on sensory irritation, Tsubone and Kawata (1991) recorded the afferent activity of the surgically isolated ethmoidal nerve (a branch of the trigeminal nerve) during delivery of 0.32−4.7 ppm (0.39−5.77 mg/m^3) formaldehyde, 0.18−7.2 ppm (0.41−16.5 mg/m^3) acrolein, and 134−2,232 ppm (241−4,021 mg/m^3) acetaldehyde into the cannulated URT of male Wistar rats (six/aldehyde) at a flow rate of 200 mL/minutes for 22 seconds. Only one aldehyde was used in each animal and each exposure was repeated two to four times at different concentrations. The activity of the nerve was recorded as the number of electrical discharges for a total period of 100 seconds, including preinhalation (30 second), inhalation (22 second), and postinhalation (48 second) periods. Nitrogen was used as the control gas and as the vehicle to dilute the aldehyde gases in order to not interfere with the gas chromatography used to analyze the exposures. The vapor concentrations associated with a 50% increase in nerve activity over the level of control gas were calculated as approximately 1.8, 1.2, and 908 ppm for formaldehyde, acrolein, and acetaldehyde, respectively. These results are consistent with the findings of

Steinhagen and Barrow (1984) and the hypothesis that the differences in RD_{50} are due to differences in chemical reactivity in the tissue.

In summary, RB is a phenomenon observed in rodents exposed to reactive gases, believed to be a protective response to the irritant properties of the gas. In comparative studies, mice have a more pronounced RB response to irritant gases than rats and generally respond at a lower concentration than rats. Interestingly, only rats appear to develop tolerance to irritant gases, while mice sustain an RB response. When formaldehyde exposure is studied in concert with other reactive gases like chlorine and other aldehydes like acetaldehyde and acrolein, cross-tolerance developed. However, the mechanism underlying this response is unknown. It is thought that RB may occur as a result of stimulation of the trigeminal nerve. Thus, although RB appears to be a phenomenon specific to rodents, the mechanism by which it occurs, trigeminal nerve stimulation, may be applicable to understanding MOAs in other species, such as primates and humans, particularly in regard to sensitization.

4.2.2. Respiratory Tract Pathology

The database for evaluating the POE toxicity in the respiratory tract of inhaled formaldehyde is robust, with well-designed studies that span a duration range of a few hours to chronic 2-year bioassays. Toxicity testing has been performed in various species, including mice, rats, hamsters, guinea pigs, dogs, and nonhuman primates. Although a few studies include examination of tissues outside of the URT, the majority of studies focus on changes in cell proliferation and cell pathology in the nasal mucosa. Both mice and rats are well-defined animal models with standard histologic sections established to evaluate various regions of the nasal passages, divided into Levels 1 to 5 and illustrated in Figure 4-6. Pathology of the nasal mucosa will be discussed with reference to these sections, and the region examined will be stipulated (e.g., nasoturbinates, maxilloturbinates, or ethmoid turbinates [ETs]). Additionally, pathology of the respiratory epithelium will be distinguished from effects on the olfactory epithelium, although the nature of the lesions is similar.

Direct effects of formaldehyde exposure on mucociliary clearance are presented first—as this may be the first interaction of the reactive chemical with elements of the upper respiratory tract. A discussion of changes in cell proliferation follows, as increased cell proliferation at low levels of exposure may be a more sensitive indicator of effects on the underlying epithelium. This balance of this section summarizes studies that have investigated cellular pathology in the URT and in the lung. Below, full study descriptions are provided for both short-term, subchronic and chronic duration studies (including, where appropriate, how cell proliferation relates to the observed formaldehyde-induced pathology).

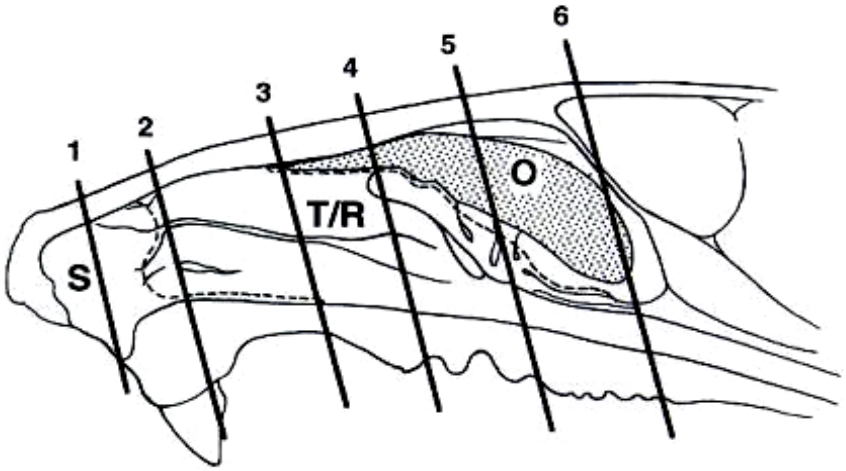

Figure 4-6. Sagittal view of the rat nose (nares oriented to the left).

Note: The figure shows the normal distribution of nasal mucosae and the section levels used in contemporary histopathology (Brenneman et al., 2000; Mery et al., 1994). Sections 1, 2, 4, and 5 correspond to Levels I, II, III, and IV as proposed by Young (1981). S = squamous, T/R = transitional/respiratory, O = olfactory mucosa.

Source: Brenneman et al. (2000).

4.2.2.1. *Mucociliary Clearance*

The mucociliary apparatus of the URT is the first line of defense against airborne toxicants. Comprising a thick mucus layer (epiphase), hydrophase, and ciliated epithelium, the mucociliary apparatus may entrain, neutralize, and remove particulates and airborne chemicals from inspired air (see Figure 4-7). The mucus serves to entrain or neutralize and remove exogenous agents from the nasal epithelium (e.g., particles, reactive chemicals). As reviewed by Kim et al. (2003), the nasal mucus contains proteins, glycoprotein, and lipids but is primarily water (95%) and is propelled along by movement of the underlying cilia. Degradation in the continuity or function of the mucociliary apparatus, which provides protection to the nasal epithelium, would result in higher levels of gases and particles reaching the nasal epithelium itself and greater penetration of chemicals into the respiratory tract. Therefore, breakdown and disruption of mucociliary function are adverse effects, since a key bodily defense to exogenous agents (including infectious agents) is damaged.

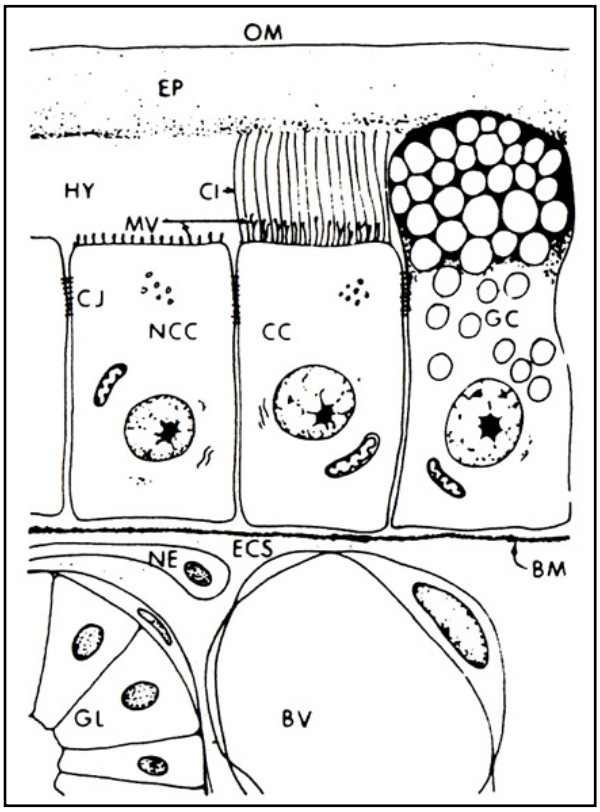

Figure 4-7. Main components of the nasal respiratory epithelium.

Note: OM = osmiophilic membrane; EP = epiphase; HY = hypophase; Cl = cilia; MV = microvilli; CJ = cell junction; CC = ciliated cell; NCC = nonciliated cell; GC = goblet cell; NE = nerve; GL = gland; BV = blood vessel; ECS = extracellular space; BM = basement membrane.

Source: Morgan et al. (1986d).

Mucus flow slows upon formaldehyde exposure, despite an increase in the ciliary beat of the underlying epithelial cells, which propel the mucus across the nasal epithelium (Morgan et al., 1986a, c, d; 1983). These findings are consistent with other studies since airborne pollutants and reactive gases have been shown to decrease mucus flow rates in several animal models (Mannix et al., 1983; Iravani, 1974; Carson et al., 1966; Dalhamn, 1956; Cralley, 1942). In addition to slowing flow, the mucus layer has been observed breaking up as it floats on the epiphase, creating gaps in the epiphase and revealing the hydrophase below (Morgan et al., 1986c, d). Formaldehyde reacts with glycoproteins in

the mucus of the epiphase, creating cross-links between these large molecules; this is believed to increase the viscosity of the mucus.

In their first experiments, Morgan et al. (1983) describe progressive mucostasis (slowing of mucous flow) and ciliastasis (disruption of ciliary beat) with increasing days of exposure to formaldehyde in male F344 rats (15 ppm; 6 hours/day for 1, 2, 4, or 9 days). Ciliastasis occurred with greater frequency and across more regions of the nasoturbinate with subsequent days of exposure. After 9 days, mucostasis was recorded in all but two regions evaluated. Although the severity and time course of these changes varied across regions of the nose, the process followed a similar pattern: decreased flow, increased ciliary action, mucostasis, and ciliastasis. Since the formaldehyde-induced deficits in mucociliary function increased with days of exposure, activity did not fully recover between exposures (18 hours) (Morgan et al., 1983). Therefore, the severity and extent of adverse effects are dependent on both the concentration of exposure and duration (in this case, days of repeated exposures).

In subsequent studies, Morgan et al. (1986c) examined the exposure-response relationship of formaldehyde effects on mucociliary function and functional recovery 18 hours after exposure ceased. Exposure regimens similar to the above experiment included additional exposure concentrations (0.5, 2, and 6 ppm) and an additional time point of 15 days duration. Exposure at 2 and 6 ppm resulted in the same progression of effects on mucus flow and ciliary beat. Considering both severity and extent of effects a clear exposure-response relationship was demonstrated. Additionally, within each exposure group, effects progressed both in severity and extent by duration of exposure to formaldehyde (from 1 to 4, 9, and 15 days of exposure) (Morgan et al., 1986c).

Flow and ciliary beat were not reduced, but rather increased, in epithelium from rats exposed to 0.5 ppm formaldehyde. Mucus flow in 2 of 10 areas assessed was clearly increased (275 and 200% of controls) after 4 days of exposure to 0.5 ppm formaldehyde. Two other epithelial regions showed a similar trend (150% of controls), but this change was not statistically significant. Interestingly, measurements made in corresponding areas after 9 days of exposure did not show an increase, and measurements in one region were reduced to 37% of control. Although it is not known whether the observed increase in mucus flow rate is a subtle indication of an adaptive response to a low level irritant, the increase appears to be transient. It is not known if flow rate would continue to decrease below control levels for repeated exposures at 0.5 ppm for longer than 9 days.

The regions affected at 15 ppm generally included the lateral aspects of the nasoturbinate and both the dorsal and medial aspects of the maxilloturbinate. In general there was an anterior to posterior effect with increasing concentration and time. Additionally, impaired mucociliary

function was more extensive with greater concentration and length of exposure. Nasal lesions were seen on the nasal epithelium and correlated with those areas where some inhibition of ciliary function was measured. Areas without mucus flow but that still retained ciliary function did not develop epithelial lesions. Morgan et al. (1986c) reported "coagulated mucus," viewed as a "continuous membrane" over the epithelium after 6 hours of exposure to 15 ppm formaldehyde. Minor cell damage and infiltrating neutrophils and monocytes were also seen in these areas. The coagulated mucus was not seen in similarly exposed rats that were allowed 18 hours of recovery before sacrifice. However, ciliated cells were damaged, and there was a greater presence of neutrophils and macrophages (MPs) after this recovery period. The authors noted that, as the exposure continued, these areas exhibited increased signs of inflammation and epithelial damage, eventually resulting in "severe degenerative changes."

Morgan et al. (1986a) refined their study design to implement a nose-only exposure to formaldehyde in order to better examine the progression of changes in mucociliary function during short-term exposure, allowing examination of mucus flow immediately following exposure. Three F344 rats/group were exposed to 15 ppm (18.4 mg/m^3) formaldehyde for 10, 20, 45, or 90 minutes or 6 hours. Two groups of rats were exposed to 2 ppm to determine a no effect level for 90 minutes or 6 hours. The extent and severity of mucostasis and ciliastasis seen after a 6-hour 15 ppm (18.4 mg/m^3) formaldehyde exposure and a 1-hour recovery period were similar to the earlier study (Morgan et al., 1986a), indicating that similar exposure conditions were reached with this nose-only apparatus. Ciliastasis and mucostasis were both less severe and less extensive in a time-dependent manner and at the earlier time points of 10, 20, 45, and 90 minutes. Significant recovery was seen in mucociliary function by allowing a 1-hour recovery between exposure and sacrifice. Regions of both the nasal septum and lateral wall, which exhibited no mucus flow when examined immediately after a 6-hour exposure, had measurable flow after the 1-hour recovery period. Similar recovery was seen at all durations of exposure. No decreases in mucociliary function were seen after exposure for either 90 minutes or 6 hours at 2 ppm formaldehyde. However, given evidence of recovery (Morgan et al., 1986a) and the time taken to dissect and view the tissues ex vivo may have obscured more subtle effects.

To assess more immediate effects on mucociliary apparatus, Morgan et al. (1984a) have examined formaldehyde effects on the mucociliary apparatus of isolated frog palates. This system allowed observation of mucociliary function during exposure. Unexposed frog palates were covered by a continuous sheet of mucus of variable thickness, which was observed to flow in streams across the palette, exhibiting a wave-like form in some areas of the epiphase. The authors reported particle movement in a lower, less viscous layer that was consistent with a less viscous underlying hydrophase, similar to that described in rat mucosa. The basal mucus flow

rate was 0−4 mm/minute, with localized ciliary activity. Short periods of increased mucus flow were associated with seemingly spontaneous increases in ciliary beat.

Formaldehyde exposure resulted in an initial increase in ciliary beat and mucus flow rate in all palates exposed at 1.37, 4.36, and 9.58 ppm formaldehyde (but not 0.23 ppm). With increasing formaldehyde concentration and time of exposure, mucostasis was evident as mucus became stiff and eventually rigid. Ciliary beat continued after mucostasis was reached until palates were exposed to 4.36 and 9.48 ppm formaldehyde, when ciliastasis was reached. The time course to peak mucus flow rate, mucostasis, and ciliastasis was concentration dependent, with mucostasis reached in less than 3 minutes at 9.48 ppm. In contrast, increased mucus flow peaked at 8 minutes in palettes exposed at 1.52 ppm formaldehyde, which, though declining, remained above basal levels after 25 minutes with no mucostasis or ciliastasis noted at this level.

Fló-Neyret et al. (2001) demonstrated reduced mucociliary clearance and decreased frequency of ciliary beats by using a similar isolated frog palette mucociliary apparatus. However the palates were exposed by formaldehyde in the Ringer's solution in which the palates were placed (0, 1.25, 2.5, or 5 ppm). Also, mucus was removed from the palettes and did not come into direct contact with the formaldehyde. Despite these differences, formaldehyde caused mucociliary clearance to decrease in a time- and concentration-dependent manner; mucostasis occurred after 60 minutes of exposure to 5 ppm formaldehyde (see Figure 4-8). Ciliary beat was decreased in a time-dependent manner at 2.5 and 5 ppm exposure but increased at 1.25 ppm formaldehyde (see Figure 4-8). Reduced mucociliary clearance at 2.5 and 5 ppm was consistent with the reduced ciliary beat. However, clearance decreased at 1.25 ppm formaldehyde, where there was an apparent increase in ciliary beat. The authors suggest this may be a result of disrupting the harmonic movement of the cilia, impairing effective mucociliary clearance. Based on study results, the authors hypothesize that changes in ciliary beat, including excitation at lower exposures, are likely to be a direct effect of formaldehyde on epithelial cells or other cellular components of the mucosa.

In summary, numerous studies have identified impaired mucociliary clearance activity associated with formaldehyde exposure (see Table 4-12). Although low-dose and short-term exposures first increase ciliary beat, impaired mucus flow, slowed ciliary beat, and eventual mucostasis and ciliastasis have been demonstrated in both in vivo and in vitro exposure systems. These effects are both concentration and duration dependent and can be seen in as few as 15 minutes from exposure. Repeated inhalation exposures in rats indicate the effect does not fully recovery in an 18-hour period between exposures, contributing to greater impairment over extended periods of exposure.

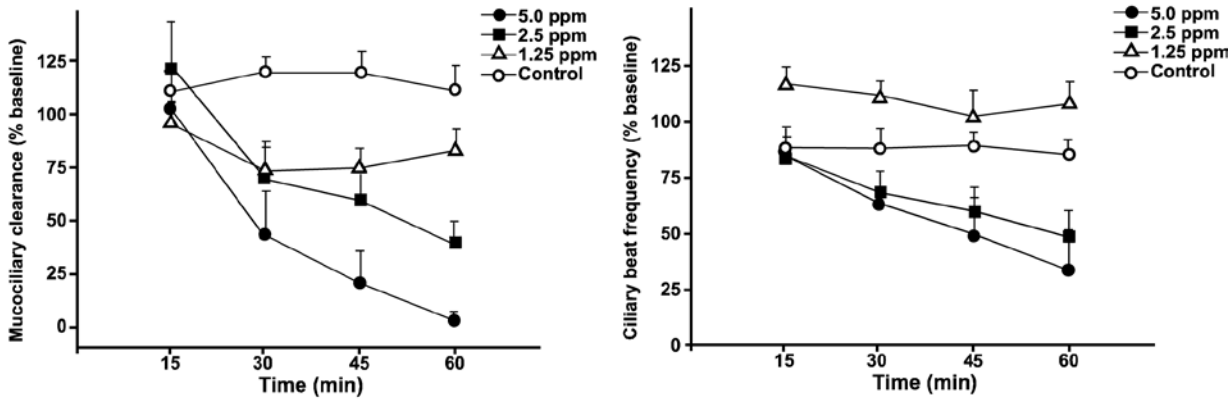

Figure 4-8. Decreased mucus clearance and ciliary beat in isolated frog palates exposed to formaldehyde after 3 days in culture.

Source: Fló-Neyret et al. (2001).

Table 4-12. Summary of formaldehyde effects on mucociliary function in the upper respiratory tract

Species	N[a]	Treatment	Measure of mucociliary function	Summary of results by location	Reference
Male F344 rats	10	15 ppm formaldehyde 6 hours/day for 1, 2, 4, or 9 days	Mucus flow and ciliary beat	Mucostasis in regions 2, 3, 4, 5, and 8 for all rats after a single dose. Mucostasis in all but two regions evaluated by day 9. Ciliastasis followed mucostasis.	Morgan et al. (1983)
Male F344 rats	6	0, 0.5, 2, 6, or 15 ppm formaldehyde 6 hours/day for 1, 4, 9, or 15 days	Mucus flow and ciliary beat and histopathologic analysis	Flow or ciliary beat were increased at 0.5 ppm. After1After 1 day, slowed or halted mucociliary flow at 15 ppm after 6 hours. After 9 days, slowed or halted mucociliary flow decreased or completely stopped in all nasal regions evaluated. Regions affected included lateral aspect of the nasoturbinate and dorsal and medial aspects of maxilloturbinate.	Morgan et al. (1986c)
Male F344 rats	3 per group	15 ppm formaldehyde for 10, 20, 45, or 90 minutes or 6 hours	Mucus flow and ciliary beat	Ciliastasis and mucostasis increased in a time- and concentration-dependent manner, with maximal response at 6 hours. Significant recovery was observed when a 1-hour recovery period occurred between exposure and sacrifice.	Morgan et al. (1986a)
Isolated frog palates	Not stated	0.23, 1.37, 4.36, or 9.58 ppm formaldehyde	Mucus flow rates and histopathology	Ciliary beat and mucus flow increased from baseline at 1.37, 4.36, and 9.58 ppm. Over time, mucus became rigid, and ciliastasis occurred	Morgan et al. (1984a)
Isolated frog palates	4	0, 1.25, 2.5, and 5 ppm formaldehyde every 15 minutes for 60 minutes	Mucociliary clearance and ciliary beat	Ciliary beat decreased in a time-dependent manner at 2.5 and 5,0 ppm but was increased at 1.25 ppm. Mucostasis occurred after 60 minutes at 5 ppm.	Fló-Neyret et al. (2001)

N = number of animals in study.

Morgan et al. (1983) suggested that the initial stimulation of ciliary activity may be a defensive response to the irritant gas, possibly indicating some penetration of formaldehyde to the underlying epithelial cells. Later effects of mucostasis may be a result of cross-linking of mucus glycoproteins by formaldehyde, creating a rigid mucus that is not able to flow even with a rigorous ciliary beat. It is unknown if the eventual cessation of ciliary beat is a result of compound-related effects on ciliated epithelium as formaldehyde diffuses through the mucus or an indirect effect associated with mucostasis. However, in vitro experiments by Fló-Neyret et al. (2001) indicate that formaldehyde in solution, supporting isolated frog palates without mucus, resulted in the same sequence of effects, including increased ciliary beat at the lowest exposure. These data suggest a role of formaldehyde beyond its ability to form protein cross-links in mucociliary proteins.

4.2.2.2. *Cell Proliferation*

Formaldehyde-induced cell proliferation has been demonstrated under range of exposure conditions in vivo and in vitro as well (see Chapter 3). Formaldehyde-induced mitogenesis may be a primary effect (as demonstrated in the in vitro work) or secondary to adaptive responses and tissue remodeling (Swenberg et al., 1983). This section provides a comprehensive discussion of formaldehyde effects on cell proliferation in the epithelial tissues in the respiratory tract. The majority of the work discussed investigates cell proliferation with in vivo labeling of proliferating cells, although additional methods, such as flow-cytometry, have been employed in some instances.

Swenberg et al. (1986) conducted a series of experiments in rodents to assess cell proliferation in the nasal mucosa after formaldehyde inhalation. Radiolabeled thymidine [^{3}H]-thymidine was injected intraperitoneally (I.P.) into male F344 rats and B6C3F1 mice after formaldehyde exposure to assess the extent of in vivo incorporation into proliferating cells. Two hours later, animals were sacrificed and the nasal passages were fixed, embedded, and sectioned to examine the nasal mucosa. Slides were exposed for 12 weeks and developed to identify cells that incorporated the radiolabeled thymidine. The percentage of labeled cells, as indicated by the presence of five or more grains over the nucleus, was determined by visual count. A total of 4,000 or 1,500 cells were counted per section for rats and mice, respectively.

The first set of studies reported by Swenberg et al. (1986) compared the dose response of rats and mice. Animals were exposed to 0, 0.5, 2, 6, or 15 ppm (0, 0.61, 2.45, 7.36, or 18.4 mg/m^3) formaldehyde 6 hours/day for 3 days. Tritiated thymidine for cell labeling was injected 2 hours after the end of exposure. No change in the percentage of labeled cells was seen after 0.5 or 2 ppm formaldehyde exposure. However, the nasal passages of rats exposed at 6 and

15 ppm showed 10- to 20-fold increases over controls in LI at level 2. A similar cell proliferation response was seen in mice treated with 15 ppm formaldehyde, although no increase over control was seen in mice exposed to 6 ppm formaldehyde. These findings are consistent with other data that indicate rats are more sensitive to formaldehyde exposure than mice. This may be due to differences in the reflex apneic response between the two species. As discussed in Section 4.2.1.1, mice maintain decreases in minute volume in response to formaldehyde, which results in a lower overall effective internal dose to the mice.

Comparing cell proliferation rates after 2 versus 18 hours of exposure, Swenberg et al. (1986) found that the longer exposure duration gave twice the cell proliferation rates after repeated exposures. Therefore, these researchers conducted a second dose-response study to examine cell proliferation 18 hours after exposure instead of the shorter exposure duration. The dose-response study varied dose as well as duration of treatment. Rats were exposed 6 hours/day to either 0.5, 2, or 6 ppm (0.61, 2.45, or 7.36 mg/m^3) formaldehyde over periods of 1, 3, or 9 days. Formaldehyde exposure at 0.5, 2, or 6 ppm for 1 day increased cell proliferation in the nasal epithelium. However, these increases were transient, and cell proliferation was not increased after 3 or 9 days of exposure to 0.5 ppm or 2 ppm formaldehyde. Although still elevated after a 3-day exposure to 6 ppm formaldehyde, cell proliferation returned to control values after 9 days of exposure to 6 ppm formaldehyde (Swenberg et al., 1986). Therefore, although concentration is a major determinant of cell proliferation, duration of exposure also influenced formaldehyde-induced cell proliferation in the nasal epithelium.

Swenberg et al. (1986) directly tested the effects of cumulative exposure versus concentration for both mice and rats. Animals were treated with one of three regimens, resulting in the same C × t product: 3 ppm × 12 hours, 6 ppm × 6 hours, or 12 ppm × 3 hours, each exposure resulting in 36 ppm-hours. The animals were exposed once a day for either 3 or 9 days. Tritiated thymidine was injected 18 hours after exposure to label of proliferating cells. Tissue sections from levels 1 and 2 of the nasal passages were examined in each case, and the percentage of cells labeled was reported as the percentage of proliferating cells (see Figure 4-9).

Cell proliferation at level 1 in the nasal cavity was much greater than at level 2 for all C × *t* combinations of formaldehyde exposure in both mice and rats (see Figure 4-9). The authors noted that level 1 is more anterior and lacks significant defense from the mucociliary apparatus, which may account for the observed greater sensitivity to formaldehyde. At all C × *t* exposure products, 3 days of exposure resulted in greater cell proliferation than 9 days of exposure. This was true for both species and for both examined levels of the nasal cavity. The decrease in cell proliferation by day 9 is consistent with data on rats labeled 18 hours postexposure (Swenberg et al., 1986).

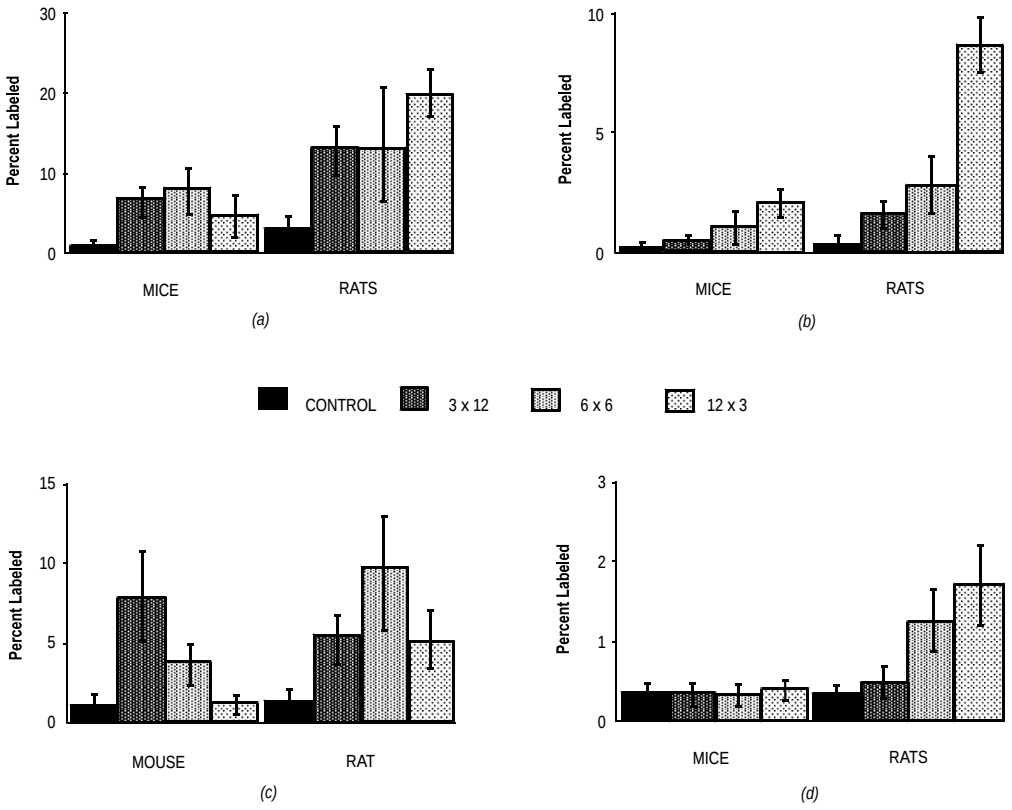

Figure 4-9. Effect of formaldehyde exposure on cell proliferation of the respiratory mucosa of rats and mice.

Note: *a* and *b* are data following 3 days of exposure; *c* and *d* are for 9 days of exposure. *a* and *c* are from level 1 (most anterior); *b* and *d* are from level 2. [^{3}H]-thymidine was administered 18 hours after the last exposure.

Source: Swenberg et al. (1986).

When comparing C × t exposures for a single species and location, the findings are more complex. Cell proliferation in level 2 of the nasal passages appeared to be more dependent on concentration than on duration or cumulative exposure, with the strongest response seen for 12 ppm formaldehyde in combination with the shortest exposure period, 3 hours (see Figure 4-9). This pattern was observed in both rats and mice after 3 days of exposure and in rats after 9 days of exposure. No increases in cell proliferation at level 2 were seen for any C × t combination in mice after 9 days. In contrast, increases in cell proliferation at level 1 of the nasal passages were not strictly concentration dependent. After a 3-day exposure, no clear differences were seen among different C × t treatments for either mice or rats, suggesting cumulative exposure may be

the important metric. Therefore, it may be concluded that cell proliferation for level 1 of the nasal passages, where there is less protection of the epithelium, is influenced by concentration, time, and duration of exposure. Cell proliferation at level 2 appeared to be more dependent on concentration than time of exposure (Swenberg et al., 1986).

Cassee and Feron (1994) reported a qualitative increase in histochemical staining for proliferating cell nuclear antigen (PCNA) in the respiratory epithelium of the nasoturbinates, maxilloturbinates, septum, and lateral wall at levels 2 and 3 of rat nasal passages after repeated exposures to 3.5 ppm (4.29 mg/m^3) formaldehyde 22 hours/day for 3 days. While no increases were seen in olfactory epithelium, frank necrosis, squamous metaplasia, and hyperplasia of both ciliated and nonciliated epithelium were noted at these section levels.

Quantitative cell proliferation studies have been conducted by several researchers in the same laboratory (Reuzel et al., 1990; Wilmer et al., 1989; Zwart et al., 1988; Wilmer et al., 1987; Woutersen et al., 1987) (see Summary Table 4-17). These studies build off of those of Swenberg et al. (1986), who labeled proliferating cells with [^{3}H]-thymidine in assessing cell proliferation within the nasal mucosa. The studies, all performed in male albino Wistar rats and using a similar experimental design, provide the basis for comparing different exposure levels and dose regimens across studies. Wilmer et al. (1987) demonstrate a concentration-dependent increase in cell proliferation after 3 days of repeated 8-hour exposures at 5, 10, or 20 ppm (6.13, 12.3, or 24.6 mg/m^3) formaldehyde, regardless of continuous versus interrupted exposure conditions (2.83, 8.87, and 19.8 versus 0.86% proliferation in controls). Similar trends were seen when the repeated continuous exposures were extended for 4 weeks, but cell proliferation was not maintained at the same levels. As observed by Swenberg et al. (1986), these results suggest that duration of repeated exposures may be an important determinant of cell proliferation rates.

Woutersen et al. (1987) reported that the majority of the dose-dependent increases in cell proliferation seen at section level 3 after 3 days of repeated 6-hour exposures to 10 and 20 ppm (12.3 and 24.6 mg/m^3) formaldehyde occurred in areas of the epithelium showing "clear squamous metaplasia and hyperplasia." Cell proliferation rates in metaplastic epithelium of 29.5 and 33.2% were much higher than the 1.4 to 2.8% proliferation in the visibly unaffected respiratory epithelium from rats exposed at 10 ppm formaldehyde. Although there was a slight trend towards increased cell proliferation in the visibly unaffected epithelium of exposed animals compared with unexposed controls, the majority of increased cell proliferation resulting from exposure to 10 and 20 ppm formaldehyde was attributed to the metaplastic epithelium.

Similarly, dose-dependent increases in cell proliferation seen at level 3 after 3 days of repeated 6-hour exposures at 0.3, 1, and 3 ppm (0.37, 1.23, and 3.68 mg/m^3) formaldehyde ($p < 0.001$) corresponded to focal basal cell hyperplasia and loss of cilia (Woutersen et al., 1987).

No necrosis or focal erosion was noted at these levels of formaldehyde exposure. Cell proliferation was not sustained at this location, and no lesions were noted after 13 weeks of repeated 6-hour exposures. The authors hypothesized that defensive mechanisms, such as the mucociliary apparatus, may have provided greater protection of the mucosa at level 3. Swenberg et al. (1986) drew a similar conclusion when evaluating extended exposures, suggesting that more posterior sections had a greater adaptive ability than those anterior sections with little mucociliary function. Both Woutersen et al. (1987) and Swenberg et al. (1986) reported sustained cell proliferation and development of lesions in the more anterior cross section. Repeated exposures to 3 ppm formaldehyde (6 hours/day) resulted in significant increases in cell proliferation in the epithelial cells at level 2, with accompanying disarrangement, focal hyperplasia, and squamous metaplasia (Woutersen et al., 1987). Although no cell death was observed at level 2 when viewed by light microscopy, "strongly indented and disarranged nuclei" were seen by electron microscopy, which may be consistent with apoptosis (Woutersen et al., 1987). However, later work in the same laboratory indicated no increased cell proliferation at levels 2 or 3 in male Wistar rats exposed to formaldehyde at 1 or 2 ppm (1.23 and 2.45 mg/m^3) (8-hour repeated exposures for 3 days or 13 weeks) and only minimal response in rats exposed at 4 ppm formaldehyde (interrupted 8-hour exposures for 3 days or 13 weeks) (Wilmer et al., 1989).

Reuzel et al. (1990) published the only report in which formaldehyde effects on cell proliferation were studied for longer daily exposure durations: 22 hours/day versus 6−8 hours/day. Male Wistar rats were exposed to formaldehyde, ozone, or the combination of the two 22 hours/day for 3 consecutive days. The concentrations of formaldehyde were 0.3, 1.0, or 3.0 ppm (0.37, 1.23, or 3.68 mg/m^3). Rats were injected with [^{3}H]-thymidine 2 hours rather than 18 hours after the last exposure. Cell proliferation was quantified by enumerating the percentage of labeled cells in fixed and stained tissue sections. Cell proliferation on the nasoturbinates, maxilloturbinates, lateral wall, and septum at levels 2 and 3 were quantified and reported separately. Cell proliferation was increased at all locations in level 2 at 3 ppm formaldehyde exposure ($p < 0.05$) but not at 0.3 or 1 ppm exposures (see Summary Table 4-17). Whereas proliferation of cells in the nasoturbinate, maxilloturbinate, and septum was nearly undetectable in control animals, 4, 5, and 3% proliferation was reported after repeated 22-hour exposures to 3 ppm formaldehyde. Basal proliferation in the lateral wall was greater than in other areas, approximately 1% increasing to 6% after exposure to 3 ppm formaldehyde. Although basal levels of cell proliferation were slightly higher in all areas of level 3, formaldehyde had no significant effects on cell proliferation in the level 3 areas evaluated. There was a slight trend for increases at 3 ppm, but all proliferation rates were below 1%. Exposure to

3 ppm formaldehyde also damaged the respiratory epithelium at levels 2 and 3, where cell disarrangement and hyperplastic and metaplastic lesions were reported.

Roemer et al. (1993) investigated the effects of formaldehyde exposure on cell proliferation in the trachea and lung in addition to nasal mucosa. Male Sprague-Dawley rats were exposed head only to 2, 6, or 20 ppm (2.45, 7.36, or 24.5 mg/m^3) formaldehyde 6 hours/day for either 1 or 3 days. Proliferating cells were labeled with 5-bromodeoxyuridine (BrdU), the label injected 16−22 hours after formaldehyde exposure ended. Free lung cells were harvested by tracheal lavage, and the majority of isolated cells were MPs (>97%). Epithelial cells were isolated from the nasal and tracheal mucosa by dissection, physical disaggregation, and enzyme treatment to release epithelial cells. All cells were fixed and stained with fluorescent dyes to detect BrdU and total DNA. Flow cytometry was used to determine the percentage of BrdU-labeled cells as a measure of cell proliferation. Cells undergoing unscheduled DNA synthesis (e.g., DNA repair) were excluded by cell cycle analysis.

The proportion of BrdU-labeled cells from the nose and trachea increased two- to threefold above control values after a single 6-hour exposure to formaldehyde (see Table 4-13). The lowest effective dose for increased cell proliferation was 2 ppm for nose and tracheal cell proliferation ($p < 0.05$). However, increased proliferation in the nasal mucosa at the lowest dose was transient, returning to control levels after a 3-day exposure. Cell proliferation remained increased in the nasal mucosa after exposure to 6 or 10 ppm (7.36 or 12.3 mg/m^3) formaldehyde for 3 days. In contrast, proliferation of tracheal cells appeared to be reduced as a result of a 3-day exposure to 2 or 6 ppm formaldehyde. A similar trend was seen in free lung cells, but the differences were not statistically significant.

Table 4-13. Cell proliferation in nasal mucosa, trachea, and free lung cells isolated from male Wistar rats after inhalation exposures to formaldehyde

1 Day[a]	Control	2 ppm	6 ppm	20 ppm
Nose	1.3[b]	2.4[c]	3.7[c]	2.7
Trachea	1.2	3.1[c]	2.1[c]	2.8
Lung[d]	1.8	2.6	3.3	3.1
3 Days	**Control**	**2 ppm**	**6 ppm**	**20 ppm**
Nose	1.3	1.4	2.5[c]	2.3[c]
Trachea	1.2	0.3[c]	0.6[c]	2.5[c]
Lung	1.8	2.2	2.4	5.1

[a]Exposures were 6 hours/day.
[b]Proliferation is measured as the percent of BrdU-labeled cells.
[c]Statistically different from controls ($p < 0.05$).
[d]The majority of free lung cells were MPs (97%).
Source: Roemer et al. (1993).

The flow cytometry employed by Roemer et al. (1993) allowed for subtle changes in proliferation rates to be measured with good discrimination. However, the method of cell isolation did not allow examination of proliferation rates in discrete regions of the mucosa, which may have attenuated the magnitude of the response. Additionally, proliferation rates represent a mix of cell types that were not separated in this analysis, making the findings difficult to interpret. This may be especially noteworthy in the free lung cells that were reportedly primarily MPs.

Monticello et al. (1990) investigated whether changes in cell proliferation rate correlated with areas of cell injury or with areas that developed tumors due to formaldehyde exposures by using a unique metric of cell proliferation. They hypothesized that treatment-related effects on cell populations could influence the apparent cell proliferation measured as LI, even though no proliferative effect had occurred. For example, cell death could give an apparent increased proliferation as a LI (% cells proliferating) by reducing the total number of cells present. This would be especially true for a stratified epithelium, where the number of basal cells in active proliferation may not change but cells above the basal layer might die or slough off, thereby reducing the overall number of population of cells counted. The unit length labeling index (ULLI) metric was developed to normalize proliferation rates against length of basal membrane rather than cell population. However, application of a ULLI to the pseudostratified epithelium of the nasal mucosa introduced additional complexities. First, undamaged mucosa has a single layer of epithelial cells that have the capability for cell proliferation. Second, cells only become layered in response to cell damage as a protective measure. Therefore, the total cells present and the linear cell density should be considered, as well as the number and density of proliferating cells, in developing an understanding of the proliferative response of these tissues to toxic insult.

Monticello et al. (1990) directly compared the apparent effects of formaldehyde exposure on cell proliferation when quantified as an LI or as a ULLI. Male F344 rats were divided into groups (n = 6) and exposed to 0, 2, 6, or 15 ppm (0, 2.45, 7.36, or 18.4 mg/m^3) formaldehyde 6 hours/day, 5 days/week for 12 weeks. Rats were administered [^{3}H]-thymidine continuously for the last 5 days of exposure by surgically implanted osmotic pumps. After sacrifice, nasal passages were fixed, and sections from standard level 3 were prepared for examination. Cell proliferation was quantified at the midseptum and the lateral meatus at this level. Basement membrane length, total number of cells present, and number of labeled proliferating cells were recorded for each location. Each of these areas also was scored for the presence of nasal lesions.

The formaldehyde-related lesions included epithelial hyperplasia, squamous metaplasia, and acute inflammation. These lesions were most severe in animals exposed to 15 ppm, mild at 6 ppm, but absent at 2 ppm. Cell proliferation, measured either as LI or ULLI, was increased in

the level 3 septum and lateral meatus after 13 weeks of exposure to 15 ppm formaldehyde but not to 6 or 2 ppm (see Table 4-14). There was a slight increase in both cell number and labeled cells in the lateral meatus of rats exposed to 6 ppm formaldehyde, but both measures of proliferation were unchanged from controls. The increased proliferation in the lateral meatus at 15 ppm was entirely due to an increased number of labeled cells. Total cells were unchanged at 15 ppm; therefore, both LIs demonstrated a similar increase over control. In addition to increased labeled cells in the septum at 15 ppm, total cells were increased from 470 to 640 ($p < 0.05$). Where the total cells and linear cell density were increased, the ULLI was proportionally increased over the LI. These observations are consistent with the development of squamous metaplasia and hyperplasia seen at 15 ppm. However, while both LI and ULLI showed an eightfold increase in cell proliferation in the lateral meatus, they gave different results in the septum where cell number was increased by formaldehyde treatment. LI increased 19-fold and ULLI 25-fold with repeated exposures to 15 ppm formaldehyde. Although these data are based on only 5−6 animals/group, and only in an extended study, the results suggest that the ULLI and LI may not be proportional under all conditions studied. In similar experiments the LI and ULLI provided different indices of proliferation in the olfactory epithelium after methyl bromide exposure (Monticello et al., 1990). Methyl bromide exposure decreased cell number/mm of basement membrane in a time-dependent manner, and the LI and ULLI were not proportional across these changes. At day 3 there was an increase in labeled cells but a decrease in total cells; therefore, the LI was increased greater than 20-fold, where the ULLI was only increased eightfold. The authors endeavored to explain why the ULLI and LI yielded different findings. Where ULLI is a more time-efficient method of assessing cell proliferation, the authors suggested that representative areas should be quantified by LI to better understand the nature of increased ULLI.

Monticello et al. (1990) reported similar results in a contemporary abstract; although treatment groups were slightly different than in the above experiments, the findings were similar. Rats were exposed to 0, 0.7, 2, 6, 10, or 15 ppm (0, 0.86, 2.45, 7.36, 12.3, or 18.4 mg/m^3) formaldehyde 6 hours/day for 4 days, 6 weeks, or 3 months. ULLIs were determined in the septum and lateral meatus (methods not detailed). It is not stated whether [^{3}H]-thymidine labeling was carried out by injection or continuous infusion. Significant increases in cell proliferation were reported after repeated exposures to 6, 10, and 15 ppm for 4 days and 6 weeks. After 3 months of exposure, cell proliferation was still increased in rats exposed to 10 and 15 ppm formaldehyde. The authors noted that, although increased cell proliferation was seen at earlier time points, sustained increased cell proliferation was only seen at 10 and 15 ppm, which they considered the clearly carcinogenic doses.

Table 4-14. The effect of repeated formaldehyde inhalation exposures for 3 months on cell count, basal membrane length, proliferation cells, and two measures of cell proliferation, LI and ULLI, in male F344 rats

	Formaldehyde exposure level (6 hours/day, 5 days/week for 3 months)			
	0 ppm	**2 ppm**	**6 ppm**	**15 ppm**
Lateral meatus				
Total cells	1,800 ± 100	1,800 ± 150	2,300[a] ± 1700	1,900 ± 160
BM length (mm)[b]	12.7 ± 0.6	11.9 ± 0.5	13.4 ± 0.3	11.6 ± 0.7
Cells/mm BM	150 ± 5	150 ± 10	170 ± 10	150 ± 5
Labeled cells	130 ± 10	130 ± 20	210 ± 30	1,400 ± 130
LI	7.2%[c]	7.2%	9.1%	73.7%
ULLI	10.2 cells/mm[d]	10.9 cells/mm	15.7 cells/mm	120.7 cells/mm
Septum				
Total cells	470 ± 20	460 ± 30	470 ± 20	640[a] ± 20
BM length (mm)	2.9 ± 0.1	2.7 ± 0.1	2.9 ± 0.1	2.9 ± 0.1
Cells/mm BM	160 ± 10	170 ± 10	160 ± 3	220[a] ± 10
Labeled cells	20 ± 1	40 ± 10	10 ± 2	250 ± 50
LI	4.3%	8.7%	2.1%	39%
ULLI	6.9 cells/mm	14.8 cells/mm	3.45 cells/mm	86.2 cells/mm

[a]Different from control, $p < 0.05$.
[b]BM is basal membrane length in mm.
[c]Calculated from group averages: LI = (labeled cells)/total cells.
[d]Calculated from group averages: ULLI = (labeled cells)/BM length.

Source: Monticello et al. (1990).

Monticello et al. (1991) applied the ULLI measurements in evaluating formaldehyde effects on cell proliferation after short-term and subchronic repeated exposures. Six male F344 rats/group were exposed to 0, 0.7, 2, 6, 10, or 15 ppm (0, 0.86, 2.45, 7.36, 12.3, or 18.4 mg/m^3) formaldehyde 6 hours/day for 1, 4, or 9 days and for 6 weeks, using a 5 days/week regimen. Rats were injected with [^{3}H]-thymidine 18 hours postexposure to label proliferating cells. All animals were sacrificed 2 hours later. Nasal passages were fixed, and sections from levels 2 and 3 were prepared for examination. Cell proliferation was quantified for three locations in level 2 (specifically, the lateral meatus, midseptum, and medial aspect of the maxilloturbinate) and for two regions of level 3 (the lateral wall and midventral septum). Each of these areas also was scored for the presence of nasal lesions.

As discussed above, proliferating cells were visually identified by the number of grains over the nucleus, 10 grains indicating a proliferating cell. Cell proliferation was quantified as the number of proliferating cells per length of basement membrane (cells/mm) and reported as a ULLI. The report does not indicate the length of membrane viewed for each section as an indication of how representative the counts are for each region. Lesions associated with formaldehyde exposure may change the density of cells/mm of basement membrane (Monticello et al., 1990). Areas of disarranged cells, erosion, metaplasia, or layering of epithelial cells may exhibit different cell profiles. These processes would alter cell density, and therefore the ULLI, independent of differential proliferation rates. As such, it is not expected to be proportional to cell proliferation rates across conditions that have the potential to change cell density (Monticello et al., 1990).

No formaldehyde-induced epithelial lesions or increases in the ULLI were seen in rats exposed to 0.7 or 2.0 ppm formaldehyde, regardless of duration (see Table 4-15). Formaldehyde-induced lesions were present in all regions of the nasal epithelium after exposures to 10 and 15 ppm formaldehyde, regardless of duration (Monticello et al., 1991). Incidence and severity of the lesions increased with concentration and duration of treatment and were correlated to areas with increased cell proliferation. Rats exposed to 6 ppm formaldehyde developed lesions in the level 2 nasal passages, where the ULLI was clearly elevated, but not in the deeper level 3 passages. For example, no formaldehyde-related lesions were seen at the lateral meatus and septum of level 3 at 1, 4, and 9 days of repeated exposure at 6 ppm, although cell proliferation was increased. This transient increase in ULLI returned to near-control levels after 6 weeks of repeated exposure (see Table 4-15). Monticello et al. (1991) suggested that cell proliferation is a more sensitive indicator of cellular response and not necessarily dependent on cellular necrosis.

The sustained cell proliferation at the lateral meatus and midseptum in rats exposed to 10 and 15 ppm formaldehyde, locations where SCCs are known to arise, supports a role for compensatory cell proliferation in tumor development. However, Monticello et al. (1991) noted that regional differences in sustained cell proliferation do not always correspond to the occurrence of nasal tumors, primarily SCCs, in formaldehyde-exposed rats. Where sustained cell proliferation has been demonstrated in the medial maxilloturbinate (MMT) at level 2 (Monticello et al., 1991), SCCs have not been found to originate in this area at similar exposures (Monticello et al., 1996; Woutersen et al., 1989). Monticello et al. (1991) suggested that the findings of Bermudez and Allen (1984), indicating that the epithelial cells of the maxilloturbinate are more resistant to the genotoxic effects of DEN, support the possibility that differences in regional tissue susceptibility may contribute to site specificity of formaldehyde-related SCCs.

Table 4-15. Formaldehyde-induced changes in cell proliferation (ULLI) in the nasal passages of male F344 rats exposed 6 hours/day

Location[a]	Exposure concentration					
	0 ppm	0.7 ppm	2 ppm	6 ppm[b]	10 ppm[b]	15 ppm[b]
Level 2: lateral meatus						
1 day	2.16	1.31	2.36	16.9[b]	11.2[b]	12.7[b]
4 days	1.46	1.37	1.72	30.5[b]	20.9[b]	25.8[b]
9 days	1.44	1.20	1.73	23.5[b]	28.6[b]	24.6[b]
6 weeks	0.91	0.88	1.36	14.4[b]	23.9[b]	28.7[b]
Level 2: midseptum						
1 day	1.08	1.01	1.69	3.85	17.9[b]	16.7[b]
4 days	1.03	0.97	0.67	10.0[b]	26.1[b]	29.1[b]
9 days	1.09	0.80	0.97	10.9[b]	19.6[b]	29.1[b]
6 weeks	0.41	0.24	0.68	2.10	21.4[b]	25.9[b]
Level 2: medial maxilloturbinate						
1 day	2.49	1.75	2.81	18.15[b]	5.9	5.3
4 days	1.36	1.54	1.09	25.03[b]	20.3[b]	19.4[b]
9 days	1.38	0.80	1.48	22.54[b]	21.0[b]	28.7[b]
6 weeks	1.02	1.21	1.11	16.32[b]	26.1[b]	25.1[b]
Level 3: lateral meatus						
1 day	1.83	1.72	2.46	7.53[b,,c]	14.5[b]	16.4[b]
4 days	1.10	1.27	1.09	8.77[b,,c]	20.0[b]	30.8[b]
9 days	1.36	1.40	1.74	7.35[b,,c]	30.6[b]	40.4[b]
6 weeks	0.98	0.91	0.86	2.08	24.2[b]	34.8[b]
Level 3: midseptum						
1 day	3.02	1.74	2.39	4.20	24.4[b]	19.3[b]
4 days	2.81	3.09	1.43	9.22[b,,c]	18.7[b]	34.4[b]
9 days	1.68	1.06	1.43	9.50[b,,c]	28.6[b]	32.5[b]
6 weeks	2.18	1.54	2.57	2.58	14.0[b]	27.5[b]

[a]ULLI is expressed as the number of labeled cells/mm of basement membrane.
[b]Indicates significantly different from control, $p < 0.05$.
[c]Indicates a location where epithelial lesions were not seen by light microscopy.

Source: Monticello et al. (1991).

Monticello et al. (1996) further explored the correlation between measures of cell proliferation and tumor site by modifying the ULLI to take into consideration the total number of cells in a region that may be subject to increased cell proliferation. The population weighted ULLI (PWULLI) is the product of the expected number of cells on a three-dimensional surface in the nasal mucosa and the ULLI of a cross section of that surface. For this series of experiments, six male F344 rats/group were exposed to 0, 0.7, 2, 6, 10, or 15 ppm (0, 0.86, 2.45, 7.36, 12.3, or 18.4 mg/m^3) formaldehyde for up to 24 months with interim sacrifices at 3, 6, 12,

and 18 months. Before each interim sacrifice [^{3}H]-thymidine was continuously injected for the last 5 days of exposure through a surgically implanted pump. Nasal passages were prepared, and six standard sections were taken and developed as above for [^{3}H]-thymidine-labeled cells. Stained tissue sections were viewed in order to map all nasal tumors. A ULLI was determined for each region (details not provided). The total cell population of each nasal region was estimated from control animals sacrificed at 3 months (see Table 4-16). Cell profiles were counted across 0.5 mm of basement membrane length at two locations for each region (site not specified). Total cells per region were estimated from these counts and the modeled surface area expected in each region (Fluid Dynamics Analysis Package version 7.0). It is unclear if one or more rats were used to quantify cell population. Cell counts and variability were not reported.

Table 4-16. Cell population and surface area estimates in untreated male F344 rats and regional site location of squamous cell carcinomas in formaldehyde-exposed rats for correlation to cell proliferation rates

Nasal region	Total cells (number)[a]	Area (mm^2)[b]	Cell density (cell/mm^2)	SCC incidence[c]	
				10 ppm	15 ppm
Anterior lateral meatus	976,000	59.5	16,400	12	17
Anterior midseptum	184,000	10.5	17,500	0	1
Anterior dorsal septum	128,000	3.84	33,300	0	3
Anterior medial maxilloturbinate	104,000	7.63	13,600	0	4
Posterior lateral meatus	508,000	38.1	13,300	2	9
Posterior midseptum	190,000	10.8	17,600	0	1
Maxillary sinus	884,000	38	23,300	0	0
Region not specified[c]	--	--	--	6	25

[a]Total cell number determined in unexposed rats as a product of representative cell counts and expected surface area of the region.
[b]Modeled surface area of the defined region by FDIP version 7.0.
[c]The number of animals bearing a tumor located in the region. Animals were exposed 6 hours/day for 24 months prior to sacrifice.

Source: Monticello et al. (1996).

ULLIs were quantified by region of the nasal passages in order to correlate with regional localization of tumors. For example, the anterior midseptum included cells from the midseptum from approximately standard section levels 2 to 3. An anterior to posterior pattern of formaldehyde effects, especially differences in cell proliferation rates, has been well established. As such, cell proliferation rates would be expected to vary across the nasal regions used in this

analysis. Areas considered to possibly be preneoplastic were not quantified for this work. Monticello et al. (1996) reported increased ULLIs in the ALM and the MMT of rats exposed to 10 or 15 ppm at all time points (3, 6, 12, and 18 months) but provided no indication of variability or a statistical analysis, making it difficult to determine where true differences may exist. Some caution should be used in interpreting the ULLI counts assigned for each region.

The PWULLI was calculated by multiplying the reported ULLIs by the calculated cell populations by region. SCC incidence by region had a greater correlation to the calculated PWULLI than the ULLI, $R^2 = 0.88$ versus $R^2 = 0.46$, respectively. The authors noted that the relative lack of correlation with the ULLI was influenced by findings at the maxilloturbinate where cell proliferation was high but SCC incidence was low. Other tumor types were not included in the analysis (polypoid adenomas, adenocarcinomas, and rhabdomyosarcomas). Additionally, 54 of the SCC tumors could not be accurately localized and were excluded from the analysis, resulting in exclusion of 30 and 39% of animals with SCCs in the 10 and 15 ppm treatment groups, respectively. The authors cautioned that the absence of these data might have skewed the regional analysis of tumor location. Although the purpose of weighting the ULLIs by total population of cells available in each region is to better represent the chance of a tumor arising in each region, the cancer incidence was represented by the number of animals, not the number of tumors, per region. Based on the exclusion of location data (up to 40% of the animals), lack of variability and significance reported for the ULLI for cell counts, and SCC incidence considered by animal rather than by tumor, the significance of a greater correlation by PWULLI versus ULLI is of questionable value.

Monticello et al. (1989) also assessed formaldehyde-induced cell proliferation and regional site location of lesions in the respiratory tract of rhesus monkeys (see Section 4.2.2.2, Figures 4-11 and 4-12 for a full study description). LIs from the histoautoradiograms indicated increased cell proliferation in transitory, respiratory, and olfactory epithelial cells after the 6-week formaldehyde exposure. Similar trends were seen after only 1 week but were statistically significant only in the respiratory epithelium. Although increased proliferation in the trachea and carina was statistically significant after 1 week of exposure, the greater increases seen after 6 weeks of exposure were not statistically significant. A small sample size ($n = 3$) and high variability may have contributed to the lack of statistical significance. The authors noted that increased cell proliferation was seen in locations with minimal histologic changes, indicating proliferation may be a more sensitive predictor of adverse health effects of formaldehyde exposure (see Figures 4-11 and 4-12).

Table 4-17. Summary of formaldehyde effects on cell proliferation in the upper respiratory tract

Species	N[a]	Treatment[b]	Measure of cell proliferation	Summary of results by location[c]	Reference
Male F344 rats; male B6C3F1 mice	NR[d]	0.5, 2, 6, or 15 ppm 6 hours/day for 3 days	LI: percent labeled cells on tissue sections (^{3}H-thymidine I.P.[d] 2 hours postexposure)	Level 2: Rats exhibited greater increased cell proliferation than mice. No increase seen in rats or mice at 0.5 or 2.0 ppm. No increase seen in mice at 6 ppm, but rats had 20-fold increase in proliferation. 10- to 20-fold increase seen in both rats and mice at 15 ppm.	Swenberg et al. (1986)
Male F344 rats	NR	0.5, 2, or 6 ppm 6 hours/day 1, 3, or 9 days	LI: percent labeled cells on tissue sections (^{3}H-thymidine I.P. 18 hours postexposure)	Level 2: Transient increase in cell proliferation on day 1 at 0.5 and 2.0 ppm. Increase in cell proliferation on days 1, 3, and 9 by 6 ppm.	Swenberg et al. (1986)
Male F344 rats; male B6C3F1 mice	NR	3 ppm for 12 hours, 6 ppm for 6 hours, or 12 ppm for 3 hours 3 or 9 days	LI: percent labeled cells on tissue sections (^{3}H-thymidine I.P. 18 hours postexposure for 2 hours)	Level 1: 3 days: Greater increased proliferation in rats than mice. Increases similar for various concentrations yielding the same C × t product. 9 days: Mice exhibited duration-dependent increases in proliferation, inverse to concentration for constant C × t. Level 2 3 days: Concentration-dependent increase in cell proliferation. 9 days: Concentration-dependent increase in cell proliferation in rats; no increase in mice.	Swenberg et al. (1986)
Male F344 rats	4−5	15 ppm 6 hours/day 1 or 5 days	LI: percent labeled cells on tissue sections (^{3}H-thymidine I.P. 18 hours postexposure for 2 hours)	Level 2: Increase in cell proliferation in respiratory epithelium, nasoturbinates, maxilloturbinates, and lateral wall. 1 day: 5.51[f] versus 0.43% in controls 5 days: 10.1%[f]	Chang et al. (1983)[e]
Male BC3F1 mice	4−5	15 ppm 6 hours/day 1 or 5 days	LI: percent labeled cells on tissue sections (^{3}H-thymidine I.P. 18 hours postexposure for 2 hours)	Level 2: Increase in cell proliferation in respiratory epithelium, nasoturbinates, maxilloturbinates, and lateral wall. 1 day: 2.14[f] versus 0.27% in controls 5 day: 3.42%[f]	Chang et al. (1983)[e]
Male albino Wistar rats	5[d]	3.5 ppm 8 hours, twice a day for 3 days	Qualitative staining for PCNA on tissue sections	Levels 2 and 3: Increase in cell proliferation in respiratory epithelium, nasoturbinates, maxilloturbinates, septum, and lateral wall.	Cassee and Feron (1994)[e]

Table 4-17. Summary of formaldehyde effects on cell proliferation in the upper respiratory tract (continued)

Species	N[a]	Treatment[b]	Measure of cell proliferation	Summary of results by location[c]	Reference
Male albino Wistar rats	3	0, 5, or 10 ppm 8 hours/day continuously for 3 days or 4 weeks, or 0, 10, or 20 ppm 8 hours/day intermittent[g] for 3 days or 4 weeks	LI: percent labeled cells on tissue sections (^{3}H-thymidine I.P. 18 hours postexposure for 2 hours)	Section level not stipulated in report. 3 days: 0.86% in controls 2.83%[f] at 5 ppm continuous 8.87%[f] at 10 ppm continuous 9.80%[f] at 10 ppm interrupted 19.8%[f] at 20 ppm interrupted 4 weeks: 0.68% in controls 1.33% at 5 ppm continuous 8.85%[h] at 10 ppm continuous 3.41%[f] at 10 ppm interrupted 13.9%[f] at 20 ppm interrupted	Wilmer et al. (1987)[e]
Male albino Wistar rats	2	0, 1, 10, or 20 ppm 6 hours/day for 3 days	LI: percent labeled cells (18 hour postexposure ex vivo ^{3}H-thymidine labeled excised mucosa)	Level 3 Metaplastic epithelium: increased proliferation 31.4% at 10 ppm, 37.6% at 20 ppm Visibly unaffected respiratory epithelium 1.6% in controls 2.6% at 10 ppm, 2.8% at 20 ppm	Woutersen et al. (1987)[e]
Male albino Wistar rats	5	0, 1, or 2 ppm 8 hours/day continuously for 3 days or 4 weeks, or 0, 2, or 4 ppm 8 hours/day intermittent[g] for 3 days or 4 weeks	LI: percent labeled cells on tissue sections (^{3}H-thymidine I.P. 18 hours postexposure for 2 hours)	Level 2 3 days: No change from controls 0.60% in controls 0.34% at 1 ppm continuous 0.61% at 2 ppm continuous 0.29% at 2 ppm interrupted 0.58% at 4 ppm interrupted 4 weeks: no change from controls 1.03% in controls 0.81% at 1 ppm continuous 0.91% at 2 ppm continuous 1.16% at 2 ppm interrupted 2.86% at 4 ppm interrupted	Wilmer et al. (1989)[e]
Male and female albino Wistar rats	5	0, 0.3, 1, 3 ppm 6 hours/day, 5 days/week for 3 days or 13 weeks.	LI: percent labeled cells on tissue sections (^{3}H-thymidine I.P. 18 hours postexposure for 2 hours)	Level 2: Increased cell proliferation at days 3 and 13 weeks ($p < 0.001$). Level 3: Transient dose-dependent increase at 1 and 3 ppm; only seen at day 3 ($p < 0.001$). Note: Results pooled by sex. Data shown graphically on log-normal scale.	Zwart et al. (1988)[e]

Table 4-17. Summary of formaldehyde effects on cell proliferation in the upper respiratory tract (continued)

Species	N[a]	Treatment[b]	Measure of cell proliferation	Summary of results by location[c]	Reference
Male Wistar rats	5	0, 0.3, 1, or 3 ppm 22 hours/day for 3 days	LI: percent labeled cells on tissue sections (2 hours postexposure ex vivo ^{3}H-thymidine-labeled excised mucosa)	Level 2: 3 ppm increased cell proliferation in nasoturbinates, maxilloturbinates, septum, and lateral wall ($p < 0.05$). Level 3: No significant increases in cell proliferation.	Reuzel et al. (1990)
Male Sprague-Dawley rats	3−5	0, 2, 6, or 20 ppm 6 hours/day for 1 or 3 days	LI: percent labeled cells by flow cytometry (5-bromodeoxyuridine I.P. 18 hours postexposure for 2 hours)	Respiratory and olfactory epithelial cells. 1 day: 1.3% in controls 2.4% at 2 ppm[f] 3.7% at 6 ppm[f] 2.7% at 20 ppm[f] 3 days: 1.4% at 2 ppm 2.5% at 6 ppm[f] 2.3% at 20 ppm[f] Tracheal epithelial cells 1 day: 1.2% in controls 3.1% at 2 ppm[f] 2.1% at 6 ppm 2.8% at 20 ppm[f] 3 days: 0.3% at 2 ppm[f] 0.6% at 6 ppm[f] 2.5% at 20 ppm[f] Free lung cells (>97% MPs): no significant change.	Roemer et al. (1993)
Male F344 rats	6	0.7, 2, 6, 10, or 15 ppm 6 hours/day, 5 days/week for 1, 4, or 9 days or 6 weeks	ULLI (unit length LI) (^{3}H-thymidine I.P. 18 hours postexposure for 2 hours)	Level 3 No increases in cell proliferation at 0.7 or 2 ppm. Level 4 ULLI increases in locations without lesions at 6 ppm. Increases in ULLI at all locations at 10 and 15 ppm.	Monticello et al. (1991)

Table 4-17. Summary of formaldehyde effects on cell proliferation in the upper respiratory tract (continued)

Species	N[a]	Treatment[b]	Measure of cell proliferation	Summary of results by location[c]	Reference
Male rhesus monkeys	3	6 ppm 6 hours/day for 1 or 6 weeks	LI: percent labeled cells on tissue sections (^{3}H-thymidine I.P. 18 hours postexposure for 2 hours)	Nasal passages: Duration-dependent increase in cell proliferation at all levels (B−E) in transitional, respiratory, and olfactory epithelium. Increased cell proliferation in areas with minimal lesions. Larynx: trend for increased proliferation Trachea: increased cell proliferation 1 week : 1.14 versus 0.55% in controls 6 weeks: 3.73% Carina of trachea: increased cell proliferation. 1 week: 1.34 versus 0.43% in controls 6 weeks: 3.60% Respiratory bronchioles: no increase in proliferation.	Monticello et al. (1989)

[a]N = number of animals per treatment group.
[b]Treatment is given as the concentration of formaldehyde, duration of exposure each day, and length of the experiment in days and weeks.
[c]Standard section levels of the nasal passages as shown in Figure 4-6 are given for experiments in rats or mice.
[d]NR = not reported; I.P. = intraperitoneally.
[e]Study is described in full in Section 4.2.1.2.2.4.
[f]Different from control, $p < 0.05$.
[g]Intermittent exposures were 30 minutes per hour for 8 hours.
[h]Data from one animal only.

4.2.2.3. *Short-Term Studies*

Inhalation of formaldehyde for a few hours has been shown to result in damage of the nasal mucosa, depending on the exposure concentration. Bhalla et al. (1991) observed changes in cell morphology in male Sprague-Dawley rat nasal epithelia after a single 4-hour exposure to 10 ppm (12.3 mg/m^3) formaldehyde. Three exposed rats were sacrificed 1 hour and 24 hours after exposure, with two control rats at each time point. Noses were fixed, decalcified, and sliced along the midsagittal plane through the nasal septum. The exposed turbinates were examined by scanning electron microscopy. Transverse sections through the hard palate, at the level of the incisive papillae, were prepared for light microscopy from similarly exposed rats (n = 10). The authors provided detailed descriptions of cell epithelial organization in untreated rat turbinates and changes observed in formaldehyde-treated rats, as set forth below. No statistical analysis was provided.

Scanning electron microscope examination of nasoturbinates showed increased mucus, erythrocyte infiltration, swelling of microvillus cells, and some cell separation in formaldehyde-treated rats. Nasoturbinates examined 1 day after exposure showed greater effects, including cell damage, matted cilia, and blebbing of cell membranes. Damage to microvillus cells of the maxilloturbinate included deformed cilia, cell swelling and rupture, and lack of typical microvilli on the cell margins. As in the nasoturbinates, damage was more marked 24 hours after exposure. The epithelium of the ETs exhibited less cell damage than in the nasal and maxillary regions, with the slight lesions noted in the upper (ET1) portion and little to no damage noted on the mid and lower (ET2 and ET3) regions. Examination of transverse tissue sections revealed swollen goblet cells and stretched epithelial cells that formed an epithelial lining approximately 40% taller than the lining seen in control rats. There was also a patchy loss of ciliated cells in the respiratory epithelium, where columnar cells were present.

Buckley et al. (1984) investigated the respiratory tract lesions associated with several sensory irritants. As part of this investigation, male Swiss-Webster mice were exposed to 3.13 ppm (3.85 mg/m^3) formaldehyde 6 hours/day for 5 days. A total of nine chemicals were tested in parallel. The report indicates there were 24−34 mice in each group, although not detailed for each chemical. One-half of the treatment group and unexposed controls were sacrificed immediately after the last exposure. The remaining exposed mice were sacrificed 72 hours later. The head, trachea, and lungs were fixed and heads decalcified. Five sections were taken of each nose at levels equivalent to standard levels 2−6 (see Figure 4-6) and were examined by light microscopy. Details on lung and trachea sections were not given. Formaldehyde induced lesions in the respiratory epithelium of exposed mice, including inflammation, exfoliation, erosion, ulceration, necrosis, and squamous metaplasia. The section

level for these effects was not given. No effects were reported in the squamous epithelium, olfactory epithelium, trachea, or lungs of formaldehyde-exposed mice.

Monteiro-Riviere and Popp (1986) evaluated damage to the respiratory epithelium due to acute formaldehyde exposures. Male F344 (CDF [F344]/CrlBr) rats (three to five per group) were exposed at 0.5, 2.0, 6.0, or 15 ppm (0.62, 2.5, 7.4, or 18.5 mg/m^3) formaldehyde 6 hours/day for either 1, 2, or 4 days. Rats were sacrificed either immediately after exposure or 18 hours later (see Table 4-18). After fixation and decalcification, blocks of tissue were collected from transverse sections of the skull. The first block of tissue, 1 µm thick, was taken just posterior of the incisor teeth. The second block was taken halfway between the first block and the incisive papillae. The dorsal nasal conchae, lateral wall, and ventral nasal conchae were microdissected, postfixed, and viewed by transmission electron microscopy (Monteiro-Riviere and Popp, 1986).

Table 4-18. Concentration regimens for ultrastructural evaluation of male CDF rat nasoturbinates

Formaldehyde[a,b]	Duration	Time of sacrifice	Observations
0.5 ppm (3)	6 hours for 1 day 6 hours for 4 days	18 hours later	No lesions. Altered ciliary configuration.
2.0 ppm (3)	6 hours for 1 day 6 hours for 4 days	18 hours later	No lesions. Altered ciliary configuration.
6 ppm (5 each group)	6 hours for 1 day 6 hours for 1 day 6 hours for 2 days 6 hours for 4 days	Immediately 18 hours later	Focal lesions on dorsal and nasal conchae and lateral wall. Severity of lesions increased with exposure duration.
15 ppm (5 each group)	6 hours for 1 day 6 hours for 2 days	18 hours later	Focal lesions on dorsal and nasal conchae and lateral wall. Severity of lesions increased with exposure duration. Severity of lesions increased with concentration.

[a]Number of exposed rats is shown in parentheses.
[b]Five control rats were examined for each experiment.

Source: Monteiro-Riviere and Popp (1986).

No lesions were observed at either 0.5 or 2.0 ppm formaldehyde for either 1 day or 4 days, evaluated 18 hours after exposure. However, an unusual altered ciliary configuration, including blebbing of the cell membrane, was observed in almost all formaldehyde-treated rats, whereas it was only "occasionally noted" in control rats. Focal lesions in the dorsal and ventral

conchae and lateral wall were seen in rats exposed at 6 and 15 ppm for 1 day and sacrificed immediately after exposure. These lesions included cytoplasmic and autophagic vacuoles, loss of microvilli, and hypertrophy. Lesions increased in severity with both exposure concentration and duration. Neutrophil infiltration and intercellular edema were seen after 1 day at 6 and 15 ppm. Nonkeratinized squamous metaplasia was noted after 4 days at 6 and 15 ppm in treated rats. Cell death and sloughing were noted after only 2 days of exposure at 6 ppm formaldehyde.

As described above, Cassee and Feron (1994) examined the effects of intermittent exposure to formaldehyde (3.5 ppm [4.3 mg/m^3]), ozone (0.44 ppm [0.86 mg/m^3]), or a combination of the two on changes to the rat nasal epithelium. Exposure occurred through six consecutive 12-hour cycles in which rats were exposed for 8 hours and then not exposed for a further 4 hours. Rats were weighed before the first and after the last exposure periods and sacrificed immediately after the last exposure. To collect tissue for biochemical analysis, skulls were split sagittally and the respiratory epithelium collected. Tissues from six rats were pooled and homogenized to enable the measurement of glutathione (GSH) and the activities of the following enzymes: glutathione S-transferase (GST), glutathione peroxidase (GPX), glucose-6-phosphate dehydrogenase (G6PDH), glutathione reductase (GR), alcohol dehydrogenase (ADH), and formaldehyde dehydrogenase (FALDH). The remaining heads were fixed, decalcified, and sectioned (standard cross sections [see Figure 4-6]).

All groups, including controls, lost weight during the course of treatment. Rats exposed to formaldehyde, ozone, or both lost more weight than controls ($p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively). Formaldehyde treatment alone increased GPX from 48.6 to 64.0 µmole/minute-mg protein ($p < 0.05$) (see Table 4-19). Formaldehyde exposure, in conjunction with ozone, decreased GST from 490 to 389 µmole/minute-mg protein ($p < 0.05$). No other enzyme activities or tissue GSH levels were affected by formaldehyde exposure.

Formaldehyde-exposed rats exhibited lesions in the nasal epithelium at levels 2 and 3 of the nose, with effects slightly more severe in level 2. Lesions observed include necrosis, hyperplasia accompanied by squamous metaplasia, and rhinitis. Exposure to formaldehyde in the presence of ozone resulted in more severe squamous metaplasia (statistics not given). These findings are similar to those of Monteiro-Riviere and Popp (1986), indicating that single or repeated exposures can result in cell damage and death. Cell death and increased cell proliferation were seen here after 3 days of repeated exposures to 3.5 ppm formaldehyde. While no increases were seen in olfactory epithelium, frank necrosis, squamous metaplasia, and hyperplasia of both ciliated and nonciliated epithelium were noted at level 2 and 3.

Table 4-19. Enzymatic activities in nasal respiratory epithelium of male Wistar rats exposed to formaldehyde, ozone, or both

Enzyme	Controls[a]	Formaldehyde (3.5 ppm)	Ozone (0.4 ppm)	Both[b]
ADH	2.66 (0.99)	3.53 (0.13)	3.40 (0.33)	2.42 (0.61)
GST	490 (32)	494 (24)	514 (4)	389 (28)[c]
GPX	48.6 (4.3)	64.0 (7.9)[c]	55.6 (2.0)	54.5 (0.3)
G6PDH	58.9 (7)	60.8 (4.7)	65.8 (1.0)	45.5 (6.8)
GR	275 (16)	288.2 (16)	279 (17)	236 (14)
FALDH	0.77 (0.03)	0.68 (0.04)	0.68 (0.07)	0.80 (0.08)

[a]Values shown are the means and SDs of three measurements of a pooled sample. Units are μmole/minute/mg of cytosolic protein.
[b]Rats were exposed intermittently, 12-hour cycles of 8 hours exposed and 4 hours unexposed, for 3 days.
[c]Different from control, $p < 0.05$.

Source: Cassee and Feron (1994).

Javdan and Taher (2000) exposed male and female albino Wistar rats (five/group) at 0, 2, or 5 ppm (0, 2.5, or 6.2 mg/m^3) formaldehyde 8 hours/day for either 3 or 30 days. Transverse tissue sections at the base of the incisive teeth and the first palatine folds were examined by light microscopy. Lesions reported after 3 days of exposure to 2 ppm formaldehyde included chorion congestion, cell disarrangement, squamous hyperplasia, atypical mitosis, and epithelial hyperplasia. Similar lesions were seen after 30 days but were more severe. Effects at 5 ppm formaldehyde included goblet cell proliferation, olfactory epithelial hyperplasia, calcified regions, and an abscess on the chorion. These lesions were more severe after 30 days of exposure.

Kamata et al. (1996a, b) conducted several high-dose studies by inhalation in rats. Specifically they exposed male F344 rats to 0, 128.4, or 294.5 ppm (0, 158, or 362 mg/m^3) formaldehyde for 6 hours (Kamata et al., 1996a). In a subsequent study in the same laboratory, male F344 rats were exposed to either 0, 15, or 145 ppm (0, 18.5, or 178 mg/m^3) formaldehyde nose only for 6 hours (Kamata et al., 1996b). Congestion was noted in the nasal cavities of formaldehyde-exposed rats and was more severe at 145.6 ppm (Kamata et al., 1996b). Rats exposed to 15 ppm formaldehyde had lesions in the nasal turbinate and trachea (not detailed) (Kamata et al., 1996b). A slight hypersecretion of mucus was noted in the tracheal epithelium in the absence of histopathologic changes. Rats exposed to 145.6 ppm had more dramatic lesions that penetrated more deeply into the respiratory tract. Hyperkeratosis of the squamous epithelium was found at level 1 of the nasal cavity. Hypersecretion, desquamation, and irregular

mucosal epithelium were seen in levels 2, 3, 4, and 5 of the nasal cavity, with more severe changes noted in the nasal septum. Increased secretion and desquamation of mucosal cells occurred in the trachea, and a slight hyperplasia of the alveolar wall was noted in rats exposed to 145.6 ppm formaldehyde (Kamata et al., 1996b)

Hester et al. (2003) carried out a transcriptional analysis of the nasal epithelium of male F344 rats 24 hours after nasal instillation of 40 µL of 400 mM formaldehyde. Immediately after sacrifice, cells were harvested from the nasal cavity for RNA extraction. The authors found several phase I and II enzymes, indicative of oxidative stress, to be elevated. They also reported the greatest increase in inflammatory genes, such as iNOS and neuropeptides. In an effort to phenotypically link any gene changes to pathology, Hester et al. (2003) noted that this exposure scenario has been demonstrated to induce regenerative hyperplasia with minimal cytotoxicity. In this regard, they observed no significant change in nine genes involved in three apoptotic pathways.

In an expansion of their earlier study, Hester et al. (2005) carried out a transcriptional analysis of the nasal epithelium of male F344 rats that had been exposed to formaldehyde by nasal instillation for a single exposure, 5 days of exposures, or 28 days of exposure. In addition, this study also attempted to characterize the comparative toxicity of glutaraldehyde with structurally similar formaldehyde (van Birgelen et al., 2000). Thus, four animals per group were instilled with 40 µL of deionized water (control group), 40 µL of 400 mM formaldehyde, or 40 µL of 20 mM glutaraldehyde. Phenotypically, both aldehydes induced similar histopathologic changes.

Both aldehydes induced similar changes in DNA repair and apoptotic pathways initially, but the patterns of gene changes were different after about 5 days of exposure. Eight genes were differentially expressed between formaldehyde and glutaraldehyde that indicated different pathways for DNA repair, including recombination, base excision repair, and nucleotide excision repair. Within this group, replication protein 70 and DNA excision repair ERCC1 showed a twofold induction by formaldehyde compared with glutaraldehyde. Since both of these genes and their products function by recognizing and removing damaged DNA bases, Hester et al. (2005) hypothesized that formaldehyde-exposed cells may remove damaged bases more efficiently than glutaraldehyde-exposed cells

In addition to nasal pathology, several researchers specifically investigated formaldehyde-induced effects in the trachea, bronchi, and pulmonary tissues of the deep respiratory tract in a variety of species (Lino dos Santos Franco et al., 2006; Kamata et al., 1996a, b; Schreibner et al., 1979; Ionescu et al., 1978).

Ionescu et al. (1978) described progressive damage in pulmonary tissue of adult male rabbits exposed to an aerosol of 3% formaldehyde solution 3 hours/day for up to 50 days (method of aerosol generation or particle size were not provided). An equivalent air concentration was not reported and cannot be derived from the information given. Animals were sacrificed at several time points (3, 7, 15, 20, 30, and 50 days), and fragments of the caudal lobes of both lungs were taken to examine bronchi (intrapulmonary and distal) and lung parenchyma. Enzymatic activity was characterized in frozen sections for β-galactosidase, adenosine triphosphatase (ATPase), adenosine monophosphatase (AMPase), lactate dehydrogenase (LDH), malate dehydrogenase, succinate dehydrogenase (SDH), acid phosphatase, Tween-60 esterase, naphthol-AS-D-acetate esterase, proline oxidase, hydroxyproline epimerase, leucyl aminopeptidase, and β-glucuronidase. A portion of the lung was fixed and sectioned and viewed by light microscopy to determine changes in cell populations and tissue pathology.

In addition, biochemical analysis revealed that enzymatic activity of β-galactosidase, ATPase, AMPase, LDH, malate dehydrogenase, and SDH were all unchanged by formaldehyde exposure across the course of treatment (Ionescu et al., 1978). The activities of several enzymes were increased through the course of exposure, including acid phosphatase, Tween-60 esterase, naphthol-AS-D-acetate esterase, proline oxidase, and hydroxyproline epimerase. Although no details were reported, the authors described the changes as progressive, with the increase in proline oxidase and hydroxyproline epimerase seen only in the second half of the treatment course. The activities of two enzymes, leucyl aminopeptidase and β-glucuronidase, were observed to decrease rapidly (time frame not provided) (Ionescu et al., 1978)

Histologic changes in the lung tissue were noted after only 3 days of exposure and were generally progressive throughout the course of treatment. Early changes in the bronchial epithelium included increased mucus secretion, hyperplasia, and hypertrophy of epithelial cells. Lymphocyte infiltration was noted in many areas, and a limited thickening of the alveolar walls was reported after 3 days of exposure. Epithelial cell lesions, thickening of the alveolar, and infiltration of lymphocytes increased as exposure continued. Mucus cells increased as much as 40% after 40 days of treatment. After 40 days of treatment, Ionescu et al. (1978) observed "destructive and fibrotic lesions" and provided a detailed description of progressive lesions.

Schreiber et al. (1979) also examined histologic changes in lung tissue after high formaldehyde exposures. Syrian golden hamsters (34, sex not stated) were exposed to 250 ppm (308 mg/m^3) formaldehyde 1 hour/day for 1, 2, 5, or 15 days. Five hamsters in each treatment group were sacrificed 2 days after exposure was ended. Three hamsters in each group were sacrificed 1, 2, or 6 weeks after exposure ended to determine if formaldehyde-induced changes regressed over time. Tracheal washing was carried out to collect cytologic samples in each

animal prior to sacrifice. Samples were fixed, stained, and examined by light microscopy. Lungs and tracheae were removed en bloc and fixed, and 20, 1 µm thick cross sections were taken (location not detailed). The remaining respiratory tissue was sectioned at 200 µm intervals. Sections were stained and viewed by light microscopy.

Abnormal epithelial cells were found in tracheal washings from formaldehyde-exposed hamsters. Schreibner et al. (1979) described cells with lobulated nuclei and a coarse chromatin pattern, especially in cells showing signs of degeneration (e.g., vacuolization of nuclei and cytoplasm) (Schreiber et al., 1979). Cell number and damage were not quantified, and there was no discussion of the effects of exposure duration on treatment, if any, on these observations. Tracheal washing was normal 2 and 6 weeks after the end of exposure, indicating that the cytological changes were reversible (Schreiber et al., 1979).

Formaldehyde exposure caused multifocal lesions in the mucociliary epithelium in the trachea and larger bronchi. Dysplastic and poorly differentiated squamous metaplastic foci replaced ciliated epithelium (Schreiber et al., 1979). Abnormal nuclear membranes, tonofibrils around the nuclei, the appearance of nucleoli, and heterochromatin condensation were distinct in the formaldehyde-treated hamsters. These changes, observed 2 days after formaldehyde exposure, were reversible over time and not seen 2 and 6 weeks later.

Because of the similarity of form and physiology of rhesus monkey URTs to the human respiratory tract, the effects of short-term formaldehyde exposure were evaluated in both nasal and lung tissue in these monkeys by Monticello et al. (1989). Male rhesus monkeys (nine/group) were exposed to 6 ppm formaldehyde (7.4 mg/m^3) 6 hours/day for 5 days/week for either 1 or 6 weeks. Control animals were exposed to the same regimen of filtered air for 1 week. Monkeys were weighed during the course of exposure and observed for clinical signs of irritation or sickness. Monkeys were intravenously injected with [^{3}H]-thymidine 18 hours after the last formaldehyde treatment to evaluate induced cell proliferation. Sections of the nasal passages, trachea, larynx, lung carina, and duodenum were processed for histoautoradiography. Tissues fixed and sectioned for examination by light microscopy included nose, adrenal, sternum (bone marrow), duodenum, esophagus, eyes, gallbladder, heart, kidney, liver, lymph nodes, pancreas, stomach, spleen, and tongue. The nose was cut into a series of transverse sections, 3 µm thick, and sections from five levels were examined (see Figure 4-10). Lung lobes were trimmed midsagittally and sectioned with care to include airway bifurcations. Sections of the nasal passages, trachea (cross section), larynx (cross section), lung carina (frontal section), and duodenum were also processed for histoautoradiography.

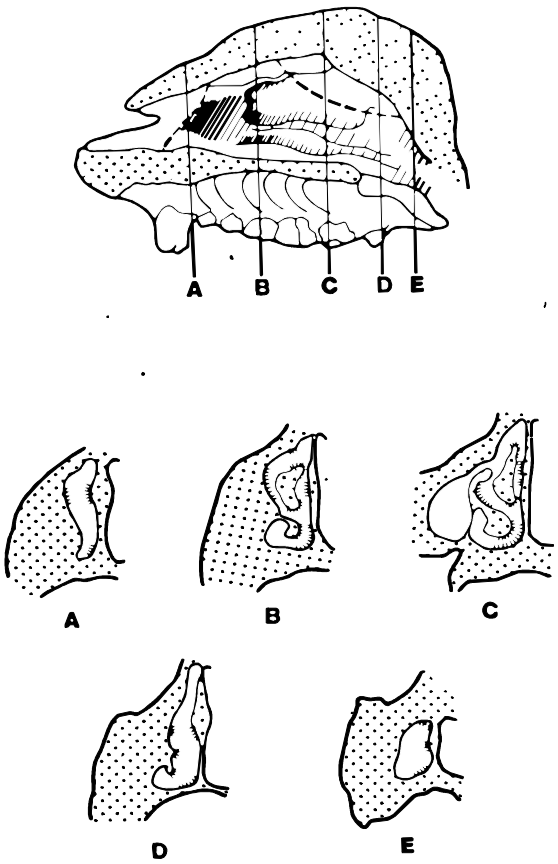

Figure 4-10. Diagram of nasal passages, showing section levels chosen for morphometry and autoradiography in male rhesus monkeys exposed to formaldehyde.

Source: Redrawn from Monticello et al. (1989).

There were no significant changes in body weight over the course of the experiment. Oronasal breathing was noted in the first 15 minutes of formaldehyde exposure (Monticello et al., 1989). Monkeys did experience eye irritation (mild lacrimation and conjunctival hyperemia) during exposure.

Formaldehyde-related lesions were reported in the nasal passages, tracheas, and in the larynx of treated animals (see Figure 4-11) (Monticello et al., 1989). Nasal epithelium from treated animals exhibited many of the histologic lesions described in rodent studies, including loss of goblet cells, loss of cilia, epithelial hyperplasia, squamous metaplasia, and neutrophilic inflammatory response in the respiratory epithelium. The lesions were more severe after 6 weeks of exposure and were present over a greater percentage of the epithelium compared with the 1-week exposure group ($p < 0.05$) (see Figure 4-11).

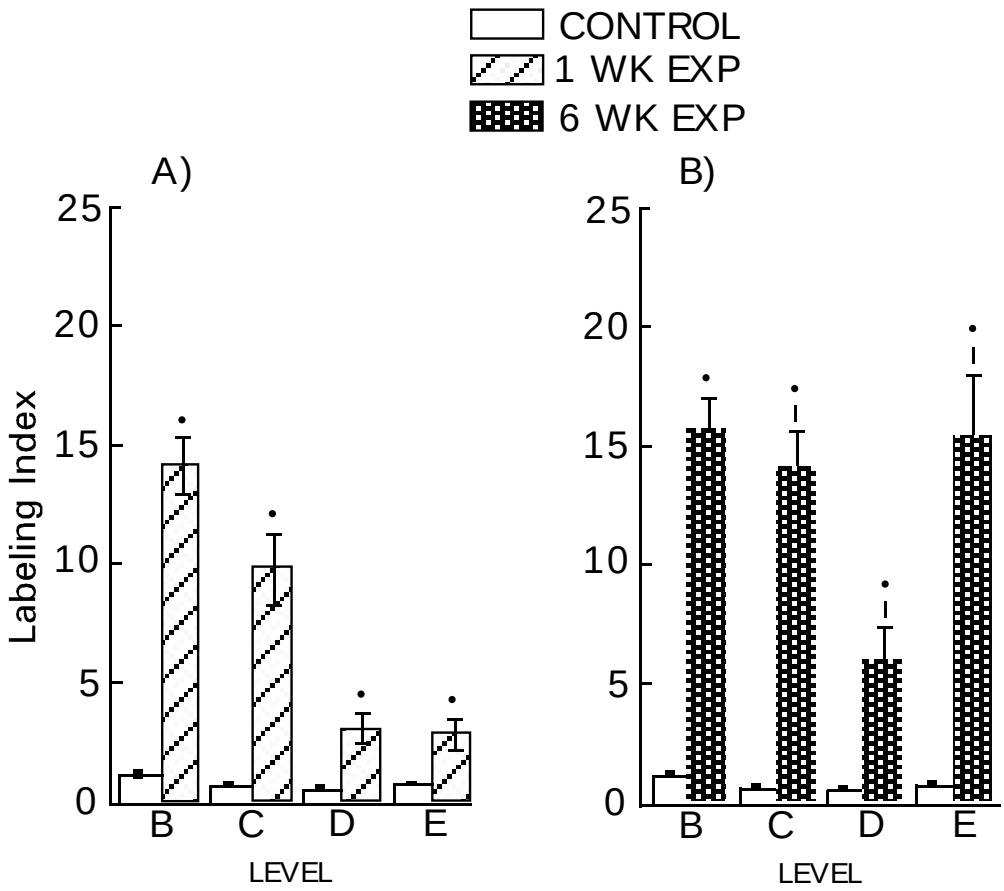

Figure 4-11. Formaldehyde-induced cell proliferation in male rhesus monkeys exposed to formaldehyde

Note: Animals were exposed to 6 ppm formaldehyde 6 hours/day, 5 days/week for 1 or 6 weeks. Bar graph depicting mean labeling indices for the respiratory epithelium at levels B−E. A: One-week exposure group. B: Six-week exposure group. *Statistically different from controls ($p \leq 0.05$). Statistically different from 1-week exposure group ($p \leq 0.05$).

Source: Redrawn from Monticello et al. (1989).

There was a distinct anterior to posterior gradient in both 1-week and 6-week treatment groups in which the anterior regions had a higher percentage of impacted epithelium (Monticello et al., 1989). However, the longer duration exposure produced significantly more lesions in the larynx and trachea compared with those observed after only 1 week of exposure ($p < 0.05$). No formaldehyde-related lesions were reported for the epithelium of the maxillary sinus, a structure not present in rodents. Labeling indices (LIs) from the histoautoradiograms indicated increased cell proliferation in transitory, respiratory, and olfactory epithelial cells after the 6-week

formaldehyde exposure (see Figure 4-12) (Monticello et al., 1989). Similar trends were seen after only 1 week but were statistically significant only in the respiratory epithelium. Although increased proliferation in the trachea and carina was statistically significant after 1 week of exposure, the greater increases seen after 6 weeks of exposure, compared with controls, were not statistically significant. A small sample size (n = 3) and high variability may have contributed to the lack of statistical significance. Monticello et al. (1989) noted that increased cell proliferation was seen in locations with minimal histologic changes, indicating proliferation may be a more sensitive predictor of adverse health effects of formaldehyde exposure.

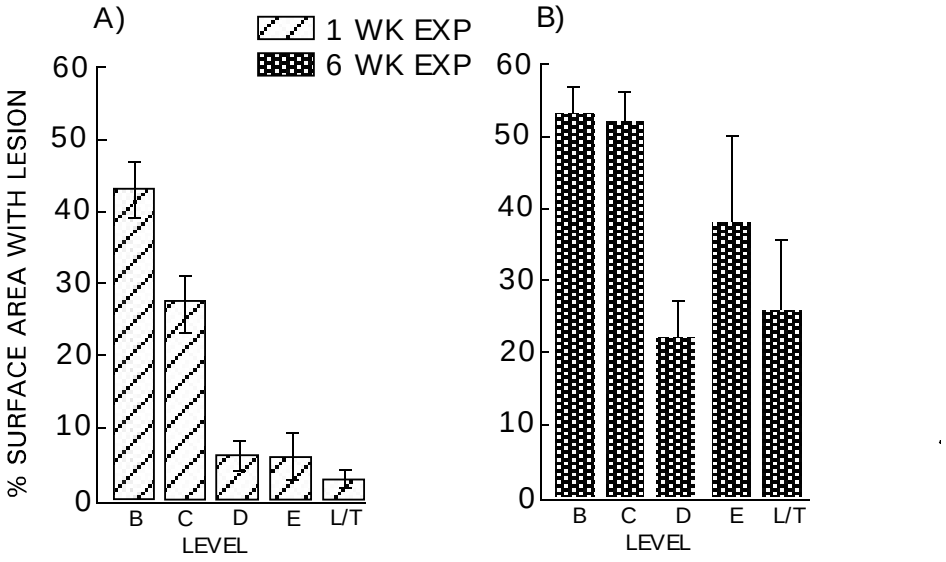

Figure 4-12. Formaldehyde-induced lesions in male rhesus monkeys exposed to formaldehyde.

Note: Animals were exposed to 6 ppm formaldehyde 6 hours/day, 5 days/week for 1 or 6 weeks. Bar graph showing levels B−E of the nasal passages and the larynx/trachea (L/T), depicting percent surface area with formaldehyde-induced lesions. Morphometry of level A was excluded due to the similarity of normal features of transitional epithelium to formaldehyde-induced lesions in the respiratory epithelium. A: One-week exposure group. B: Six-week exposure group.

*Statistically different from controls ($p \leq 0.05$).

| Statistically different from 1-week exposure group ($p \leq 0.05$).

Source: Redrawn from Monticello et al. (1989).

There are two reports in the literature assessing changes in pulmonary tissues after acute formaldehyde exposures (Kamata et al., 1996a, b). Kamata et al. (1996a) exposed male F344

rats to 0, 128.4, or 294.5 ppm (0, 158, or 362 mg/m^3) formaldehyde for 6 hours. Lung lavage samples were collected and the fluid analyzed for the lipids, free cholesterol, phosphatidyl ethanolamine, phosphatidyl choline, sphingomyelin, and triglyceride.

The bronchoalveolar lavage (BAL) was analyzed for triglycerides, cholesterol, and phosphatidyl choline. As in the first experiment (Kamata et al., 1996a), triglyceride concentration was reduced in the lavage of treated animals, in this case, to 16% of controls in lavage in those rats exposed to 145.6 ppm formaldehyde (see Table 4-20). Cholesterol concentration was unchanged and phosphatidyl choline was increased to 220% of that of control rats as a result of exposure to 145.6 ppm formaldehyde. However, BAL lipids were unchanged in 15 ppm exposed rats. Triglycerides were reduced in unwashed lung tissue from formaldehyde-treated rats in a concentration-dependent manner and free fatty acids were reduced in rats exposed to 145.6 ppm formaldehyde. Neither triglyceride nor sphingomyelin was detected in lung lavage fluid from the high treatment group.

Table 4-20. Lipid analysis of lung tissue and lung lavage from male F344 rats exposed to 0, 15, or 145.6 ppm formaldehyde for 6 hours

	Control[a]	15 ppm[a]	145 ppm[a]
Lung tissue			
Free fatty acids (mg/g lung)	3.30 (0.7)	3.11 (1.23)	1.41 (0.63)[b]
Triglyceride (mg/g lung)	1.55 (0.23)	0.74 (0.14)[c]	0.62 (0.17)[c]
Cholesterol (mg/g lung)	1.72 (0.10)	1.41 (0.25)	1.16 (0.55)
Phosphatidyl ethanolamine (mg/g lung)	7.41 (1.81)	7.46 (2.28)	5.49 (1.78)
Phosphatidyl choline (mg/g lung)	11.0 (1.49)	9.65 (3.21)	7.53 (3.52)
Sphingomyelin (mg/g lung)	3.44 (0.75)	3.13 (1.28)	2.51 (0.95)
Lung lavage			
Triglyceride (mg/lung)	0.31 (0.10)	0.24 (0.09)	0.05 (0.02)[c]
Cholesterol (mg/lung)	0.04 (0.01)	0.04 (0.01)	0.04 (0.01)
Phosphatidyl choline (mg/lung)	0.66 (0.23)	0.84 (0.35)	1.45 (0.31)[c]

[a]SD given in parentheses.
[b]Significant difference from controls ($p < 0.05$).
[c]Significant difference from controls ($p < 0.01$).
Source: Kamata et al. (1996b).

Concentration-dependent decreases were seen in nonprotein sulfhydryl (SH) groups and lipooxygenase in nasal mucosa homogenate and nonprotein SH groups in lung tissue

homogenate (see Table 4-21). Increases in both lipooxygenase and LDH activities were found in lung tissue homogenate from formaldehyde-exposed rats.

Table 4-21. Formaldehyde effects on biochemical parameters in nasal mucosa and lung tissue homogenates from male F344 rats exposed to 0, 15, or 145.6 ppm formaldehyde for 6 hours

	Control[a]	15 ppm[a]	145 ppm[a]
Nasal mucosa[b]			
Nonprotein SH groups (μM/g tissue)[c]	1.64 (0.50)	1.29 (0.28)	0.73 (0.21)[f]
Lipid peroxides (μM/g tissue)	118 (23)	71 (16)[f]	59 (18)[f]
Glucose-6-dehydrogenase (U/g tissue)[d]	1.96 (0.10)	1.87 (0.07)	2.07 (0.13)
Lung[e]			
Nonprotein SH groups (μM/g tissue)[c]	1.83 (0.18)	1.70 (0.11)	1.29 (0.28)[f]
Lipid peroxides (μM/g tissue)	72 (8)	95 (15)[f]	93 (8)[g]
Glutathione reductase (U/g tissue)[d]	0.42 (0.25)	0.25 (0.05)	0.22 (0.05)
Lactate dehydrogenase (U/g tissue)[d]	77.37 (9.28)	88.69 (7.66)	93.62 (4.99)[f]

[a]SD given in parentheses.
[b]5 or 10% nasal mucosa homogenates.
[c]nmol malonaldehyde/g tissue.
[d]Units per gram tissue.
[e]20% lung homogenates.
[f]Significant difference from controls ($p < 0.01$).
[g]Significant difference from controls ($p < 0.05$).

Source: Kamata et al. (1996b).

Lino dos Santos Franco et al. (2006) studied the effects of inhaled formaldehyde on lung injury and changes in airway reactivity in rats. The extent of local and systemic inflammation was assessed by changes in leukocyte counts in BAL fluid, blood, bone marrow, and spleen. Changes of reactivity of isolated tracheae and intrapulmonary bronchi in response to methacholine were monitored in response to formaldehyde exposure. The authors exposed male Wistar rats to formaldehyde generated from a 1% solution of formalin. However, they provided insufficient information for the exposure concentration to be determined. Groups of six animals were exposed to formaldehyde for either 0, 30, 60, or 90 minutes on 4 consecutive days. All experiments were carried out 24 hours after the final exposure.

The authors reported a significantly increased number of leukocytes in the BAL fluid of animals exposed to formaldehyde via inhalation. The effect reached a maximum for the longer exposure duration (90 minutes). Compared with controls, rats exposed to formaldehyde 90 minutes/day for 4 days also displayed an increase in the number of total blood leucocytes ($1.4 \pm 0.06 \times 10^4$ versus $0.8 \pm 0.01 \times 10^4$ cells/mm^3). These values are means ± standard error of

the mean (SEM) for six animals/group. The effect appeared to reflect changes in the mononuclear cell population ($1.1 \pm 0.02 \times 10^4$ versus $0.6 \pm 0.003 \times 10^4$ cells/mm^3) rather than peripheral blood neutrophils ($0.2 \pm 0.003 \times 10^4$ cells/mm^3 in test animals and controls). There was also an apparently compound-related increase in the total cell count in the spleen ($112.7 \pm 4.4 \times 10^6$ versus $94.2 \pm 5.5 \times 10^6$ cells). However, a change in the number of cells eluted from bone marrow did not reach statistical significance ($54.6 \pm 1.3 \times 10^6$ versus $45.0 \pm 4.3 \times 10^6$ cells). Lino dos Santos Franco et al. (2006) provided data on dose-dependent changes in methacholine-induced contractions in isolated tracheae and bronchi obtained from formaldehyde-exposed and control rats. Although the maximal contractile response induced by methacholine in tracheae of formaldehyde-treated rats was unchanged compared with controls, contractions in isolated bronchi were significantly weaker than those observed in controls.

The authors examined the effect of formaldehyde inhalation on rat lung mast cells. Degranulation and significant neutrophil infiltration were features of the response to formaldehyde (see Table 4-22).

Table 4-22. Mast cell degranulation and neutrophil infiltration in the lung of rats exposed to formaldehyde via inhalation

Treatment group	Mast cell degranulation (cells/mm^2)[a]	Neutrophil infiltration (cells/mm^2)[a]
Controls	0[b]	0.3 ± 0.2
Formaldehyde-exposed	2.0 ± 0.4[c]	5.2 ± 1.7[c]

[a]Values are means ± SEM; $n = 6$.
[b]4.2 ± 0.6 cells/mm^2 intact mast cells were found in the lungs of controls.
[c]No statistical analysis was provided by the authors for these changes.

Source: Lino dos Santos Franco et al. (2006).

Selected pharmacological agents were used to explore the mechanism by which exposure to formaldehyde might have brought about the observed lung infiltration and bronchial hyporesponsiveness. Lino dos Santos Franco et al. (2006) provided data showing that separate pretreatment of the animals with compound 48/80, sodium cromoglycate (SCG), and indomethacin reduced the formaldehyde effect on neutrophil release into BAL but had no effect on mononuclear cell counts. Compound 48/80 and SCG also reversed the formaldehyde-induced reduction in bronchial response to methacholine, but indomethacin had the opposite effect (causing an additional decrease in bronchial responsiveness). In broad terms, these findings were thought to implicate mast cells as a possible mediator of the toxicological effects of

formaldehyde. Histologically, a significantly increased number of degranulated mast cells were evident in the pulmonary tissue of rats that were exposed to formaldehyde.

Lino dos Santos Franco et al. (2006) also examined the regulatory role of NO on formaldehyde-induced bronchial activity. Nitrites generated by cultured cells of BAL from formaldehyde-treated rats increased about threefold compared with those from controls. However, pretreatment with the NO synthase inhibitor, *N*-nitro-L-arginine methyl ester, prevented the formaldehyde-induced bronchial hyporesponsiveness to methacholine but had no effect on pulmonary leukocyte recruitment. These data implicate the existence of distinct mechanisms for the induction of lung inflammation versus bronchial hyporeactivity. Further support for this concept came from an experiment in which rats were pretreated with capsaicin to examine the involvement of sensory fibers in lung inflammation and the bronchial hyporesponsiveness induced by formaldehyde inhalation. Although the treatment did not influence formaldehyde-induced bronchial hyporesponsiveness to methacholine, the number of leukocytes recovered in the BAL fluid were reduced compared with those of rats exposed to formaldehyde alone.

Extrapulmonary effects

Kamata et al. (1996a) exposed male F344 rats to 0, 128.4, or 294.5 ppm (0, 158, or 362 mg/m^3) formaldehyde for 6 hours. In addition, blood samples were monitored for hematology and clinical chemistry parameters, including red blood cell (RBC) count, hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC) count, and plasma levels of total protein (TP), albumin (ALB), blood urea nitrogen (BUN), glucose, phospholipids, triglycerides, total cholesterol, cholinesterase, and LDH. Male rats exposed to 294.5 ppm formaldehyde had increased RBC count, Hb, hematocrit (HCT), MCV, and serum glucose ($p < 0.05$) compared with controls (Kamata et al., 1996a). There were concentration-related decreases in serum measures of TP, ALB, and phospholipids ($p < 0.05$). BUN was decreased in rats exposed to 128.4 ppm but increased in the higher treatment group ($p < 0.05$). Phospholipid analysis of the lung surfactant indicated a decrease in the production in formaldehyde-treated animals ($p < 0.05$). Total free cholesterol, phosphatidyl ethanolamine, and phosphatidyl choline were reduced to 60, 55, and 38% of controls for rats treated with 294.5 ppm formaldehyde ($p < 0.05$). Sphingomyelin was reduced to 32% of controls in the low treatment group ($p < 0.05$).

In a subsequent study in the same laboratory (Kamata et al., 1996b), male F344 rats were exposed to either 0, 15, or 145 ppm (0, 18.5, or 178 mg/m^3) formaldehyde nose only for 6 hours (Kamata et al., 1996b). Fifteen animals were treated at each level and separated into subgroups

of five animals each for tissue collection and the determination of other endpoints. Blood samples were collected from one subgroup to determine such hematological and clinical chemistry parameters as RBC count, Hb, PCV, MCV, MCHC, WBC count, and plasma levels of TP, ALB, BUN, glucose, phospholipids, triglycerides, total cholesterol, LDH, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and G6PDH. BAL was collected from five animals of each group and analyzed for phospholipids. Lung homogenate from five animals in each treatment group was analyzed for nonprotein SH groups, lipid peroxides, and total lipids. The 20,000 × g supernatant of the lung homogenate was assayed for the activities of GR, G6PDH, and LDH. Similarly, nonprotein SH groups and lipid peroxidase were measured in homogenates of excised nasal mucosa. At autopsy, organs (brain, heart, lung, liver, kidney, spleen, and testis) were weighed and tracheae and nasal turbinates examined. After fixation and decalcification, five sections across the nose were taken, corresponding to standard sections 1−5 (see Figure 4-6).

Several blood parameters were affected after these acute exposures. The WBC count was slightly increased, from 4.7×10^3 cells/mm^3 in control rats to 5.1×10^3 cells/mm^3 and 6.1×10^3 cells/mm^3 at 15 and 145.6 ppm formaldehyde, respectively (Kamata et al., 1996b). Serum levels of AST and LDH decreased in an apparent concentration-dependent manner (AST 68 and 54% of controls and LDH 48 and 28% of controls, respectively). Serum levels of G6PDH and ALT were decreased similarly across exposure groups at 45 and 78% of controls, respectively.

A synopsis of respiratory pathology findings following short-term exposure to formaldehyde is presented in Table 4-23.

4.2.2.4. *Subchronic Studies*

In a study by Maronpot et al. (1986), female and male B6C3F1 mice (10/group) were exposed at 0, 2, 4, 10, 20, or 40 ppm (0, 2.46, 4.92, 12.3, 24.6, or 49.2 mg/m^3) formaldehyde 6 hours/day, 5 days/week for 13 weeks. Clinical observations were made daily, and mice were weighed weekly. At autopsy, tissue sections from each organ system (approximately 50 tissues per mouse) were fixed, stained, and examined by light microscopy. Noses were fixed, decalcified, and transversely trimmed at three levels: the incisor teeth, midway between the incisor teeth and first molar teeth, and the second molar teeth (corresponding to sections 2, 3, and 4 in Figure 4-6).

Although control mice gained weight, mice exposed to 40 ppm formaldehyde lost weight during the 13-week exposures. Expressed by the authors as a percent of weight gain in controls, the weight losses were −235% in males and −168.6% in females. Early mortality for both male and female mice exposed to 40 ppm was 80%. Although gross and histochemical effects in excised pieces from each organ system were evaluated, endometrial hypoplasia in mice treated

Table 4-23. Summary of respiratory tract pathology from inhalation exposures to formaldehyde—short-term studies

Species/strain	No./ group	Treatment	Respiratory effects	LOAEL/NOAEL	Reference
			Nasal pathology		
Male Sprague-Dawley rats	3	Single 4-hour exposure to 10 ppm formaldehyde.	Marked histopathologic changes to the nasoturbinates, maxilloturbinates, ethmoidal turbinates, and goblet and microvillus cells.	LOAEL = 10 ppm.	Bhalla et al. (1991)
Male Swiss-Webster mice	24−34	0 or 3.13 ppm formaldehyde 6 hours/day for 5 days.	Histopathologic lesions to the respiratory epithelium, including inflammation, exfoliation, erosion, ulceration, necrosis, and squamous metaplasia.	LOAEL = 3.13 ppm.	Buckley et al. (1984)
Male F344 rats	3−5	0, 0.5, 2, 6, or 15 ppm 6 hours/day for 1, 2, or 4 days.	Histopathologic lesions to the nasal conchae, lateral wall, and ventral nasal conchae.	NOAEL = 2 ppm for focal lesions. Some changes in ciliary configuration were evident at all exposures.	Monteiro-Riviere and Popp (1986)
Male Wistar rats	20	0 or 3.5 ppm formaldehyde through six consecutive 12-hour cycles in which rats were exposed for 8 hours; 10 were unexposed for 4 hours.	The activity of GPX was increased in respiratory epithelium homogenates. The nasal respiratory epithelium showed frank necrosis.	LOAEL = 3.5 ppm.	Cassee and Feron (1994)
Male F344 rats	5	0, 6, or 15 ppm [^{14}C]-formaldehyde 6 hours/day for a single day (naïve group). A pretreated group was exposed to 6 or 15 ppm formaldehyde 6 hours/day for 4 days prior to [^{14}C]-formaldehyde exposure.	Cellular necrosis to the nasal epithelium. 10.05% cellular proliferation.	LOAEL = 6 ppm.	Chang et al. (1983)
Male and female Wistar rats	5/sex	0, 2, or 5 ppm formaldehyde 8 hours/day for 3 or 30 days.	Cell disarrangement, squamous hyperplasia, atypical mitosis, and epithelial hyperplasia.	NOAEL = 2 ppm.	Javdan and Taher (2000)
Male F344 rats	15	0, 15, or 145.6 ppm formaldehyde for a single 6-hour exposure.	Histopathologic lesions in the nasal turbinates and trachea	LOAEL = 15 ppm.	Kamata et al. (1996b)
Male rhesus monkeys	9	0 or 6 ppm formaldehyde 6 hours/day for 1 or 6 weeks. [^{3}H]-thymidine was injected prior to sacrifice.	Histopathologic lesions, including loss of goblet cells, loss of cilia, epithelial hyperplasia, squamous metaplasia, and neutrophilic inflammation.	LOAEL = 6 ppm.	Monticello et al. (1989)

Table 4-23. Summary of respiratory tract pathology from inhalation exposures to formaldehyde—short-term studies (continued)

Species/strain	No./ group	Treatment	Respiratory effects	LOAEL/NOAEL	Reference
Tracheal and lung pathology					
Syrian golden hamsters (sex unstated)	5	0 or 250 ppm 1 hour/day for 1, 2, 5, or 15 days.	Abnormal cells in tracheal lavage, an effect that was reversed on cessation of treatment.	LOAEL = 250 ppm.	Schreiber (1979)
Male rabbits (strain unstated)	ND	Aerosol generated from a 3% formaldehyde solution 3 hours/day for up to 50 days (air concentration unknown).	Necrosis of the bronchi and lung parenchyma. Increased activities of acid phosphatase, Tween-60 esterase, naphthol-AS-D-acetate esterase, proline oxidase, and hydroxyproline epimerase. Reduced activities of leucyl aminopeptidase and β-glucuronidase. Adverse histopathologic changes.	ND.	Ionescu et al. (1978)
Male F344 rats	6	0, 128.4, or 294.5 ppm for a single 6-hour exposure.	Phospholipid content was reduced in lung surfactant, for example, sphingomyelin to 43% of controls in the low-concentration group.	LOAEL = 128.4 ppm.	Kamata et al. (1996a)
Male F344 rats	15	0, 15, or 145.6 ppm formaldehyde for a single 6-hour exposure.	Biochemical changes in lung homogenates. Altered lipid content of BAL in high concentration rats.	LOAEL = 15 ppm.	Kamata et al. (1996b)
Male Wistar rats	6	Aerosol generated from a 1% formalin solution 0, 30, 60, or 90 minutes/day on 4 consecutive days (air concentration unknown).	Increased leukocyte count in bronchoalveolar fluid. Degranulation of mast cells and increased neutrophil infiltration.	ND.	Lino dos Santos Franco et al. (2006)
Extrapulmonary effects					
Male F344 rats	6	0, 128.4, or 294.5 ppm for a single 6-hour exposure.	.	LOAEL = 128.4 ppm.	Kamata et al. (1996a)
Male F344 rats	15	0, 15, or 145.6 ppm formaldehyde for a single 6-hour exposure.	.	LOAEL = 15 ppm.	Kamata et al. (1996b)

ND = not determined; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level.

with 40 ppm was the only effect noted outside the respiratory system. The authors considered this effect secondary to the observed respiratory tract lesions and frank toxicity at 40 ppm formaldehyde.

While no statistical comparison was provided, respiratory tract lesions showed an increased incidence with concentration, as well as an increased distribution throughout the respiratory tract (see Table 4-24). No lesions were seen in the nasal cavity, larynx, trachea, or lung of control mice or mice treated with 2 ppm formaldehyde. Minimal squamous metaplasia in the nasal cavity was noted in 1 of 10 male mice treated with 4 ppm formaldehyde, but none were observed in the female mice. However, squamous metaplasia was observed in all mice in the higher treatment groups (10, 20, and 40 ppm). Lesions became more severe and penetrated more deeply into the respiratory tract as exposure concentration increased. Where lesions were present in the nasal cavities of all mice exposed to 10 ppm, similar lesions were reported in the larynx and trachea of some animals exposed to 20 ppm and all animals exposed to 40 ppm formaldehyde. Mice exposed to 40 ppm formaldehyde exhibited lesions as deep as the lung, including squamous metaplasia, submucosal fibrosis inflammation, and epithelial hyperplasia.

The findings of Maronpot et al. (1986) indicated a no-observed-adverse-effect level (NOAEL) of 4 ppm and a LOAEL of 10 ppm in mice, based on squamous metaplasia in the nasal epithelium. Although a LOAEL of 10 ppm was observed, there was 80% mortality for both sexes at 40 ppm, indicating a very narrow range between the first observed adverse health effects and frank effect concentrations in mice for this 13-week treatment.

In a study by Woutersen et al. (1987), male and female albino SPF Wistar rats (10/group) were exposed to 0, 1, 10, or 20 ppm (0, 1.23, 12.3, or 24.6 mg/m^3) formaldehyde 6 hours/day, 5 days/week for 13 weeks. Rats were checked daily and weighed weekly. Three longitudinal sections of lungs, trachea, and larynx and six standard cross sections of the nose were taken for microscopic examination. Two rats per exposure group were similarly treated for 3 days and sacrificed 18 hours later, and nasoturbinates were dissected to measure cell proliferation. Woutersen et al. (1987) noted that the majority of the dose-dependent increases in cell proliferation seen at section level 3 after 3 days of repeated 6-hour exposures to 10 and 20 ppm (12.3 and 24.6 mg/m^3) formaldehyde occurred in areas of the epithelium showing "clear squamous metaplasia and hyperplasia." Cell proliferation rates in metaplastic epithelium of 29.5 and 33.2% were much higher than the 1.4 to 2.8% proliferation in the visibly unaffected respiratory epithelium from rats exposed at 10 ppm formaldehyde. Although there was a slight trend towards increased cell proliferation in the visibly unaffected epithelium of exposed animals compared with unexposed controls, the majority of increased cell proliferation resulting from exposure to 10 and 20 ppm formaldehyde was attributed to the metaplastic epithelium (Woutersen et al., 1987).

Table 4-24. Location and incidence of respiratory tract lesions in B6C3F1 mice exposed to formaldehyde

Location of respiratory tract lesions	Control		2 ppm		4 ppm		10 ppm		20 ppm		40 ppm	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Nasal cavity												
Squamous metaplasia	–[a]	–	–	–	1/10	–	10/10	10/10	10/10	10/10	10/10	10/10
Seropurulent inflammation	–	–	–	–	–	–	4/10	–	10/10	8/10	10/10	10/10
Larynx												
Squamous metaplasia	–	–	–	–	–	–	–	–	6/9	3/9	10/10	7/8
Trachea												
Squamous metaplasia	–	–	–	–	–	–	–	1/10	3/10	5/10	10/10	10/10
Epithelial hyperplasia	–	–	–	–	–	–	–	–	4/10	2/10	2/10	---
Seropurulent inflammation	–	–	–	–	–	–	–	–	–	–	8/10	5/10
Submucosal fibrosis	–	–	–	–	–	–	–	–	–	–	9/10	5/10
Lung (Bronchus)												
Squamous metaplasia	–	–	ND[b]	ND	ND	ND	–	–	–	–	4/10	3/10
Inflammation	–	–	ND	ND	ND	ND	–	–	–	–	3/10	2/10
Submucosal fibrosis	–	–	ND	ND	ND	ND	–	–	–	–	2/10	–

[a]Dash indicates no lesions recorded in that treatment group.
[b]ND = no data.

Source: Maronpot et al. (1986).

Statistically significant increases were seen in focal respiratory epithelial hyperplasia and keratinization in both male and female rats at the highest treatment level (20 ppm) (see Table 4-25). Male rats also had statistically significant increases in observed respiratory epithelial squamous metaplasia, focal olfactory epithelial thinning, and rhinitis. Both male and female rats treated with 10 ppm formaldehyde showed statistically significant increases in squamous metaplasia, hyperplasia, and keratinization of the respiratory epithelium (Woutersen et al., 1987).

Disarrangement of the respiratory epithelium was only significantly increased in female rats, but this change was observed at both the 10 and 20 ppm treatment levels. Although some lesions were observed in animals treated with 1 ppm formaldehyde, their incidences were not statistically significant and the findings were equivocal.

Feron et al. (1988) examined recovery of formaldehyde-induced nasal lesions after subchronic exposures. Male albino SPF Wistar rats (50−55/group) were exposed to 0, 10, or 20 ppm (0, 12.3, or 24.6 mg/m^3) formaldehyde 6 hours/day, 5 days/week for either 4, 8, or 13 weeks. All groups were observed for a total of 130 weeks, including treatment and recovery. Rats were weighed weekly for the first 13 weeks and monthly thereafter. Rats (five/group) were sacrificed immediately after the end of exposure (4, 8, or 13 weeks). The balance of the rats were sacrificed after 130 weeks, inclusive of exposure time. At sacrifice, noses were fixed and sectioned by using standard section levels.

Formaldehyde exposure (20 ppm) was associated with reduced body weight throughout the exposure period (4, 8, or 13 weeks). However, body weight in these groups matched that of controls after 8, 40, and 100 weeks, respectively. Rats exposed to 10 ppm for 8 or 12 weeks had slightly decreased body weight (further details not given).

Nonneoplastic lesions were reported in the nasal mucosa of rats exposed to either 10 or 20 ppm formaldehyde and examined immediately after exposure was discontinued (4, 8, or 13 weeks). Lesions increased in severity with both exposure duration and concentration (details of severity and incidence were not provided). Rhinitis, hyperplasia, and squamous metaplasia of the respiratory epithelium were seen in rats from both dose groups, but changes in olfactory epithelia were only seen in rats exposed to 20 ppm, where cell disruption, thinning of the epithelium, and simple cuboidal or squamous metaplasia were also reported. Changes in the dorsomedial region, at the junction of the respiratory and olfactory epithelium, were similar to those seen in the olfactory epithelium of rats exposed to 20 ppm formaldehyde. A similar concentration- and duration-dependent increase in histopathologic changes in nasal epithelium was observed after the full 130 weeks, which included 126, 122, or 117 weeks of recovery for the three duration groups, 4, 8, and 13 weeks, respectively (see Table 4-25).

Table 4-25. Formaldehyde effects (incidence and severity) on histopathologic changes in the noses and larynxes of male and female albino SPF Wistar rats exposed to formaldehyde 6 hours/day for 13 weeks

		Concentration of formaldehyde (ppm)							
		0	1	10	20	0	1	10	20
Respiratory epithelium	***Severity***	***Males***				***Females***			
Diffuse squamous metaplasia	Slight	–[a]	–	–	–	–	–	–	3
	Moderate	–	–	–	5[b]	–	–	–	4
	Severe	–	–	–	5[b]	–	–	–	3
Focal squamous metaplasia	Very slight	–	1	–	–	–	–	1	–
	Slight	–	1	6[b]	–	–	–	7[c]	–
	Moderate	–	–	4	–	–	–	2	–
Focal hyperplasia	Very slight	–	–	1	1	–	–	2	1
	Slight	–	–	6[b]	7[c]	–	1	6[b]	6[b]
	Moderate	–	–	1	–	–	–	–	–
Focal disarrangement	Very slight	–	–	1	–	–	–	2	1
	Slight	–	–	3	–	–	1	6[b]	6[b]
	Moderate	–	–	1	–	–	–	–	–
Focal keratinization	Very slight	–	2	6[b]	1	–	–	6[b]	6[b]
	Slight	–	–	3	6[b]	–	–	2	4
	Moderate	–	–	–	1	–	–	–	–
Olfactory epithelium									
Focal thinning	Slight	–	–	–	2	–	–	–	2
	Moderate	–	–	–	1	–	–	–	2
	Severe	–	–	–	5[b]	–	–	–	2
Focal squamous metaplasia	Slight	–	–	–	4	–	–	–	3
	Moderate	–	–	–	4	–	–	–	1
Focal keratinization	Very slight	–	–	–	1	–	–	–	–
	Slight	–	–	–	2	–	–	–	–
Rhinitis		–	2	5[b]	10[c]	–	–	3	2
Larynx									
Squamous metaplasia	Very slight	–	–	–	3	–	NE[d]	NE	–
	Slight	–	–	–	1	–	NE	NE	–
	Moderate	–	–	–	1	–	NE	NE	–
Keratinization	Slight	–	–	–	2	–	NE	NE	–

[a] Dash indicates no lesions reported.
[b] Different from control, $p < 0.05$.
[c] Different from control, $p < 0.01$.
[d] NE = not evaluated.
Source: Woutersen et al. (1987).

Feron et al. (1988) did not provide a direct comparison among lesions reported at the interim sacrifice and terminal sacrifice after the extended recovery period. However, similar lesions were reported after the recovery period, including focal hyperplasia and stratified squamous metaplasia of the respiratory epithelium, stratified cuboidal or squamous metaplasia in the dorsomedial area, and replacement of olfactory epithelium. The incidence and severity of these lesions in rats exposed to 20 ppm formaldehyde were statistically different from control animals, regardless of exposure duration (see Table 4-26).

Table 4-26. Formaldehyde-induced nonneoplastic histopathologic changes in male albino SPF Wistar rats exposed to 0, 10, or 20 ppm formaldehyde (6 hours/day, 5 days/week) and examined at the end of 130 weeks inclusive of exposure

	4 Weeks			8 Weeks			13 Weeks		
Formaldehyde, ppm	**0**	**10**	**20**	**0**	**10**	**20**	**0**	**10**	**20**
Total noses examined	**44**	**44**	**45**	**45**	**44**	**43**	**45**	**44**	**44**
Respiratory epithelium focal hyperplasia									
Very slight	0	0	0	0	1	3	0	5[a]	2
Slight	0	3	8[b]	2	2	12[b]	1	6	14[b]
Moderate	0	0	1	0	1	0	0	0	4
Respiratory epithelium focal stratified squamous metaplasia									
Very slight	3	6	14[b]	8	16	17[a]	2	10[a]	2
Slight	4	2	19[b]	2	1	20[b]	3	18[b]	26[b]
Moderate	0	2	3	0	0	2	1	5	14[b]
Severe	0	0	0	0	0	0	0	0	1
Respiratory/olfactory epithelium stratified cuboidal or squamous metaplasia	0	0	4	0	0	17[b]	0	2	23[b]
Rhinitis	7	7	18[a]	4	6	22[a]	8	11	23[b]
Olfactory epithelium replacement by respiratory epithelium and regeneration									
Very slight	0	0	0	0	0	2	0	0	1
Slight	1	0	6	0	0	14[b]	0	0	12[b]
Moderate	0	0	1	0	0	3	0	0	12[b]
Severe	0	0	0	0	0	1	0	0	1

[a]Significantly different from control, $p < 0.05$.
[b]Significantly different from control, $p < 0.01$.

Source: Feron et al. (1988).

Although a slight increase in changes to the olfactory epithelium and dorsomedial area was seen in rats treated with 20 ppm formaldehyde for only 4 weeks, these differences were significant and more severe in the 8- and 13-week treatment groups. Replacement of olfactory epithelium by respiratory epithelium was described as slight after 8 weeks of exposure and slight to moderate after 13 weeks of exposure in the 20 ppm treatment groups. Therefore, formaldehyde-induced lesions were not resolved after a considerable nonexposure recovery period of up to 126 weeks (Feron et al., 1988).

Feron et al., (1988) derived a correlation between the development of nonneoplastic changes in nasal epithelium and the development of nasal tumors as a result of these subchronic formaldehyde exposures. Two SCCs were reported in rats exposed to 10 ppm formaldehyde but were not considered to be formaldehyde related because of their locations (nasolacrimal duct, incisor tooth). Six tumors were observed in the 20 ppm, 13-week exposure group (see Table 4-27) of which three of the tumors were SCCs similar to those observed as a result of chronic formaldehyde exposure. Two polypoid adenomas also were reported in rats exposed to 20 ppm formaldehyde. Feron et al. (1988) concluded that subchronic exposures to 20 ppm formaldehyde could result in an increase in nasal tumors, an effect that followed observation of cellular proliferation.

Table 4-27. Formaldehyde-induced nasal tumors in male albino SPF Wistar rats exposed to formaldehyde (6 hours/day, 5 days/week for 13 weeks) and examined at the end of 130 weeks inclusive of exposure

Tumor type	0 ppm	10 ppm	20 ppm
No. of rats exposed for 4 weeks	**44**	**44**	**45**
Polypoid adenoma	0	0	1[a]
SCC	0	0	1
No. of rats exposed for 8 weeks	**45**	**44**	**43**
Polypoid adenoma	0	0	1[a]
SCC	2	1	1
No. of rats exposed for 13 weeks	**45**	**44**	**44**
SCC	0	1	3[a]
Cystic squamous cell carcinoma	0	0	1
Carcinoma in situ	0	0	1[a]
Ameloblastoma	0	0	1

[a]Tumor considered to be associated with formaldehyde exposure.

Source: Feron et al. (1988).

A companion study from the same laboratory examined the effects of lower concentration formaldehyde exposures (Zwart et al., 1988). Male and female albino Wistar rats (50/group) were exposed to 0, 0.3, 1, or 3.0 ppm (0, 0.37, 1.2, or 3.7 mg/m^3) formaldehyde 6 hours/day, 5 days/week for 13 weeks. Body weight, general condition, and behavior were recorded weekly. No effects of formaldehyde exposure on body weight changes were noted, and growth was considered comparable among different exposure groups and controls. Rats were sacrificed during week 14, and noses were fixed and sectioned (exact time after exposure ended not given). Six standard cross sections were examined for each animal by light microscopy, anterior to posterior. Noses were fixed and decalcified, and six standard cross sections were taken and developed.

No formaldehyde-related lesions were reported in the respiratory epithelium at section level 3 after 13 weeks of formaldehyde exposure (0.1 ppm, 1 ppm, or 3 ppm). Signs of inflammation (rhinitis, sinusitis, mononuclear cell infiltrates) were observed in formaldehyde-treated rats, but there was no concentration-response relationship (data not provided). Formaldehyde-related pathology in the anterior part of level 2 epithelium was reported in 37/50 males and 21/50 female rats exposed to 3.0 ppm for 13 weeks. Both keratinized and unkeratinized squamous metaplasia were present, and disarranged cells and hyperplastic respiratory epithelium were found in the transitional zone between squamous and pseudostratified epithelium at level 2. Foci of keratinized squamous epithelium, glandularization of goblet cells, and deciliated epithelium were observed by electron microscopy in anterior sections of level 2 of rats exposed to 3 ppm formaldehyde. Epithelial cells with irregularly shaped and strongly indented nuclei were described at level 2 in animals exposed to 0.3 and 1 ppm formaldehyde and were considered to be disarranged as well at 3 ppm formaldehyde exposures.

Although early cell proliferation at level 3 corresponded to basal cell hyperplasia at 3 days, neither effect persisted for the course of the exposure. The authors speculate that this is an indication of an adaptive response, perhaps through increased function of the mucociliary apparatus present at level 3. In contrast, the early changes at section level 2 were less dramatic but persisted through 13 weeks, including clear formaldehyde-related pathology.

Concentration times time (C × t) issues have been investigated for histopathology as well as for cellular proliferation, outlined above. Specifically, Wilmer et al. (1989, 1987) compared the effects of 8-hour continuous and 8-hour intermittent formaldehyde exposure in two studies. Fifty male albino Wistar rats (10/group) were exposed to different exposure regimens to achieve similar compound-related C × t products. A C × t product of 40 ppm-hours (49.2 mg/m^3-hours) was attained by an 8-hour exposure to 5 ppm (6.2 mg/m^3) or a 4-hour exposure to 10 ppm (12.3

mg/m^3) (Wilmer et al., 1987). Similarly, an 80 ppm-hours (98.4 mg/m^3-hours) C × t product was attained from continuous 10 ppm exposure or intermittent 20 ppm (24.6 mg/m^3) exposure. Rats were exposed to one of these regimens 8 hours/day for either 3 days (two/group) or 4 weeks (eight/group). Eighteen hours after exposure ended, rats were injected with [^{3}H]-thymidine and sacrificed 2 hours later. Noses were fixed and decalcified, and six standard cross sections were taken and developed.

Thinning and disarrangement of the respiratory epithelium, squamous metaplasia, basal cell hyperplasia, and rhinitis were seen in formaldehyde-treated rats. Lesions were most severe in group 4 (20 ppm intermittent). Groups 2 and 3 had similar lesions (10 ppm intermittent and continuous). Rats in group 1 had mild lesions. Formaldehyde concentration was the major determinate in severity of nasal lesions. Formaldehyde effects were less severe in group 1 than in group 3, even though the C × t product was the same, indicating concentration rather than duration or cumulative exposure correlates to severity. Epithelial lesions in group 3 rats were similar among rats exposed to 10 ppm, regardless of duration (groups 2 and 3).

In a follow-up study, Wilmer et al. (1989) assessed both cellular proliferation and histologic lesions in Wistar rats exposed to formaldehyde in groups that differed by concentration and time. Group A served as a control group (0 ppm). Group B was exposed to 1 ppm for 8 hours, group C to 2 ppm for 8 hours, group D to 2 ppm for 4 hours (30 minutes for 8 hours), and group E to 4 ppm for 4 hours (30 minutes for 8 hours). The experimental design and cellular proliferation results are illustrated in Table 4-28. Intermittent exposures at 2 and 4 ppm resulted in formaldehyde-related histopathologic lesions similar to those reported by Zwart et al. (1988). Disarrangement and squamous metaplasia in respiratory epithelium were observed at 4 ppm (see Table 4-28). Disarrangement, nest-like infolds, goblet cell hyperplasia, and rhinitis were observed at 2 ppm. Rats exposed continuously for 8 hours at 2 ppm formaldehyde had fewer lesions than rats intermittently exposed to 2 ppm and were not statistically different from controls. Although lesions were noted in rats given the continuous 1 ppm, 8-hour treatment, their incidence was not significantly different from the controls (see Table 4-28). It should be noted that the control rats in this study were reported to have a higher frequency of lesions than controls in two previous studies from this laboratory employing the same techniques (Zwart et al., 1988; Woutersen et al., 1987). For example, lesions noted in the respiratory epithelium of 25 control rats included 13 disarrangements, 13 basal cell hyperplasia, and 5 each of goblet cell hyperplasia, nest-like infolds, and squamous metaplasia. This is in contrast to the data of Woutersen et al. (1987), who reported no lesions in the respiratory epithelium of 20 control rats (male and female). Although Zwart et al. (1988) discussed inflammatory lesions in control rats, no mention was made of the other scored lesions in control animals. Overall, Wilmer et al.

Table 4-28. Formaldehyde effects on nasal epithelium for various concentration-by-time products in male albino Wistar rats

Respiratory epithelium at cross section level 2	Exposure regimen (number of animals)				
	A (25)	B (22)	C (24)	D (23)	E (25)
	0 ppm	1 ppm	2 ppm	2 ppm	4 ppm
		8-Hour continuous	8-Hour continuous	8-Hour intermittent	8-Hour intermittent
Disarrangement					
Focal	12	4	8	3[a]	8
Diffuse	1	1	0	15[b]	11[c]
Necrosis					
Focal	4	3	0	2	3
Diffuse	0	0	0	2	2
Basal cell hyperplasia					
Focal	9	4	6	11	10
Diffuse	4	0	0	4	11
Squamous metaplasia					
Focal	5	0	1	7	16[c]
Keratinization	0	0	1	0	3
Nest-like infolds					
Focal	5	4	11	14[c]	7
Diffuse	0	3	1	0	1
Goblet cell hyperplasia					
Focal	0	1	1	2	1
Diffuse	5	2	8	13[b]	10
Rhinitis	3	2	3	16[c]	8

[a] $p < 0.05$, compared with group A.
[b] $p < 0.001$, compared with group A.
[c] $p < 0.01$, compared with group A.

Source: Wilmer et al. (1989).

(1989) reported clear adverse effects at 2 ppm formaldehyde, resulting from intermittent exposure for 8 hours/day, 5 days/week for 13 weeks. The indication of no effects at 1 ppm and 2 ppm continuous exposure should be considered with some caution, given the unusual incidence of lesions in the control animals.

The results reported by Wilmer et al. (1989, 1987) indicate a greater influence of concentration, rather than exposure regimen (continuous versus intermittent) on formaldehyde toxicity. However, these studies were conducted as repeated 8-hour exposure regimens over a course of days or weeks. Therefore both regimens allowed for a 16-hour recovery time before

the next reexposure and do not represent a true continuous exposure. This research group has speculated that defensive adaptation of the nasal mucosa may include the function of the mucociliary apparatus (Feron et al., 1989). Morgan et al. (1986a) have shown formaldehyde effects on mucus flow and ciliary beat in F344 rats to result from hourly exposures to 15 ppm formaldehyde. However, effects seen in repeated 8-hour exposures may not correspond to those effects resulting from shorter duration exposures to higher formaldehyde concentrations.

Rusch et al. (1983a, b) performed a comparative study of formaldehyde effects on the nasal epithelium in F344 rats, Syrian golden hamsters, and cynomolgus monkeys. Groups of animals were exposed at 0, 0.2, 1, or 3 ppm (0, 0.25, 1.2, or 3.7 mg/m^3) formaldehyde 22 hours/day, 7 days/week for 26 weeks. Six male monkeys, 10 male and 10 female hamsters, and 20 male and 20 female rats were exposed at each exposure level. The experiment was run in two trials, each with its own control group: trial 1 at 0.2 or 1 ppm and trial 2 at 3 ppm. Animals were weighed weekly and physically assessed (details not given). At sacrifice, organ weights were recorded for the kidney, adrenals, heart, and liver. Tissue sections of the lung (4), trachea, and nasal turbinates (4) of each animal were examined by light microscopy (section locations not given). Additionally, sections were examined by electron microscopy for rats in the control and 1 ppm treatment groups (five rats per group).

Body weights of both male and female rats in the 3 ppm treatment group were depressed by 20% between week 2 and the end of the 26-week exposure. Absolute liver weights were decreased in these animals as well (26% lower in males and 12% lower in females, $p < 0.05$). This decrease in liver weight remained significant for male rats when normalized for body weight (a ratio of 2.9 in treated versus 3.16 in controls) but not for female rats. No significant body weight or organ weight changes were seen in hamsters or monkeys. Increased incidences of congestion (36/156), hoarseness (32/156), and nasal discharge (62/156) were observed in monkeys in the 3.0 ppm treatment group versus no hoarseness or congestion and only five observations of nasal discharge in 156 observations for control monkeys. Increased nasal congestion was noted in the two lower treatment groups of monkeys: 30/156 and 45/156 observations, respectively, versus 9/156 observations in nasal discharge in the controls. The authors reported an increase in nasal discharge and lacrimation in treated hamsters but no increases in symptoms in rats. However, observations of adverse symptoms in the control rats were greater than 10% on some measures.

Rhinitis increased in rats in the 3 ppm treatment group, and the incidence in controls was notable (see Table 4-29). All groups of monkeys showed some rhinitis, and no treatment effects were observed in either monkeys or hamsters. Monkeys and rats in the high treatment group (3 ppm) had a greater incidence of lesions in the nasoturbinate epithelium (see Table 4-30).

Rusch et al. (1983a, b) noted that most lesions were mild to moderate but were "somewhat more severe" in the high treatment group. Hamsters did not exhibit a similar increase, with few lesions noted in the nasal epithelium. Overall, these studies show a clear increase in adverse health effects at 3 ppm for rats and monkeys, with no adverse effects seen in hamsters at this treatment level or rats and monkeys at the lower concentrations (0.2 ppm and 1 ppm).

Table 4-29. Rhinitis observed in formaldehyde-treated animals; data pooled for male and female animals

	F344 rats	Cynomolgus monkeys	Syrian golden hamsters
Trial 1:			
I, Control	17/38	4/6	0/14
II, 0.2 ppm	14/39	4/6	0/4
III, 1 ppm	14/38	5/6	0/11
Trial 2:			
IV, Control	12/40	2/6	0/9
V, 3 ppm	25/39	4/6	2/16

Source: Rusch et al. (1983a, b).

Table 4-30. Epithelial lesions found in the middle region of nasoturbinates of formaldehyde-treated and control animals; data pooled for males and females

	F344 rats		Cynomolgus monkeys	Syrian golden hamsters
	Basal cell hyperplasia	Squamous metaplasia/ hyperplasia	Squamous metaplasia/ hyperplasia	Nasal epithelium
Trial 1:				
I, Control	0/38	2/38	0/6	No lesions noted
II, 0.2 ppm	0/38	1/38	0/6	
III, 1 ppm	0/36	3/36	1/6	
Trial 2:				
IV, Control	4/39	3/39	0/6	No lesions noted
V, 3 ppm	25/37	23/37	6/6	

Source: Rusch et al. (1983a, b).

Andersen et al. (2008) examined the effect of formaldehyde exposure at several concentrations and durations. This study comprised histopathology and cell proliferation data, as well as genomic analyses at Level II of the nasal cavity. Toxicogenomics analysis was performed only at Level II because this was the region where the most severe lesions have been

reported in chronic bioassays (Andersen et al., 2008; Monticello et al., 1991; Kerns et al., 1983). More specifically, Andersen et al. (2008) stated that the histopathologic and cell proliferation effects at Levels II and III (with similar tissue structure) (Monticello et al., 1991) provided phenotypic anchoring for the genetic analysis. Table 4-31 summarizes many of the broad phenotypic findings.

The primary conclusions of this study with regard to the histopathology and cell proliferation are as follows:

- The presence of inflammatory cell infiltrates in the nasal epithelial tissue of F344 rats is highly variable and provides no coherent pattern with dose or duration at levels below 6 ppm.
- Hyperplasia was observed following exposure to ≥2 ppm.
- Metaplasia was observed at 6 ppm on day 5, but not before or after.
- Cell proliferation (as measured by labeling indices) was significantly elevated in Levels I−III at 6 ppm on day 5 and Level I on day 15, leading to the conclusion that significant changes in cell proliferation may not occur at exposures to ≤2 ppm.
- A significant decrease in cell density was observed at Level I in animals exposed to 6 ppm formaldehyde for 15 days, which was posited to be related to tissue remodeling in response to this concentration.

Based on their analysis of the microarray data, Andersen et al. (2008) concluded that no genes were significantly altered by exposure to 0.7 ppm from 1 to 15 days. Exposure to 2 ppm primarily resulted in gene changes at 5 days of exposure, but not thereafter. One gene was significantly increased on day 1, but the authors did not identify that gene. At 6 and 15 ppm, 42 and 745 genes were altered at day 1, respectively. After 5 days, gene changes were only observed at 6 ppm (15 ppm was not examined after day 1). These findings support conclusions reached by their laboratory in an earlier analysis. Thus, the primary conclusion in the Andersen et al. (2008) study is that genomic changes, including those suggestive of mutagenic effects, did not temporally precede or occur at lower doses than phenotypic changes in the tissue. The implications of this finding will be examined later in Section 4.5.

Studies have also investigated the ability for formaldehyde to induce pathology in the trachea, bronchi, and lung tissue. These studies have reported tracheal tissue changes, lung inflammation, necrosis, changes to the biochemistry of BAL fluid and lung surfactant in a variety

Table 4-31. Cellular and molecular changes in nasal tissues of F344 rats exposed to formaldehyde

ppm / Response	D1					D1R					D5					D6					D6R					D15				
	0	**0.7**	**2**	**6**	**15**	**0**	**0.7**	**2**	**6**	**15**	**0**	**0.7**	**2**	**6**	**15**	**0**	**0.7**	**2**	**6**	**15**	**0**	**0.7**	**2**	**6**	**15**	**0**	**0.7**	**2**	**6**	**15**
I	0	1	6	8	–	4	2	1	7	–	1	1	5	8	–	5	2	4	7	–	6	1	3	7	–	3	1	0	5	–
H	0	0	0	0	–	0	1	3	8	–	0	0	3	8	–	0	0	1	8	–	0	0	2	8	–	0	0	2	7	–
M	0	0	0	0	–	0	0	0	0	–	0	0	0	7	–	0	0	0	0	–	0	0	0	0	–	0	0	0	0	–
P1											39±9	37±15	65±40	155±89[a]												79±55	56±37	51±44	119±38[a]	
P2											–	–	–	[a]												–	–	–	–	
P3											–	–	–	[a]												–	–	–	–	
CD											321±30	336±64	377±141	400±61												362±61	340±57	321±37	293±53[b]	
G	–	0	1	42	745	–	0	0	0	–	–	0	15	28	–	–	0	0	9	–	–	–	–	–	–	–	0	0	54	

D = day; R = recovery.
I = infiltrations (number out of 8 total animals); H = hyperplasia (number/8); M = metaplasia (number/8).
P1−P3 = proliferation at levels I−III (ULLI).
CD = cell density (cells/mm) at Level I.
G = genes significantly altered at Level II of nasal epithelial tissue.
[a]Significantly elevated ULLI and LI at Level I on day 5 or significantly elevated lLIl at Level I on day 15; index [a] without numerical value indicates significant increases in ULLI in all subregions of Levels II and III at day 5.
[b]Statistically significant difference from control ($p < 0.05$).

Source: Andersen et al. (2008).

of species. Özen et al. (2003a) noted changes in zinc concentration in the lung tissue following exposure for formaldehyde. Dallas et al. (1989) and Dinsdale et al. (1993) observed changes in P450 enzyme activity in the lung associated with formaldehyde exposure.

Özen et al. (2003a) measured zinc, copper, and iron content in lung tissue from formaldehyde-exposed Wistar rats. Adult male rats were exposed to 0, 5, or 15 ppm (0, 6.2, or 18.5 mg/m^3) formaldehyde 8 hours/day, 5 days/week for either 4 or 13 weeks. Rats were checked daily and weighed weekly. At sacrifice, rats were autopsied and examined for gross pathological changes. Lung tissue was homogenized and analyzed for zinc, copper, and iron.

Body weight gain was depressed in all treatment groups in a concentration-dependent manner ($p < 0.001$) (see Table 4-32). Formaldehyde-exposed rats consumed less food and water than controls and showed unsteady breathing, increased nose cleaning, excessive licking, frequent sneezing, and nasal mucosa hemorrhages. Significant decreases were seen in the zinc content of lungs after either 5 or 10 ppm formaldehyde exposure (see Table 4-33). Copper content was unchanged from controls in all treatment regimens, whereas iron content was increased after 4 weeks of 5 ppm exposure and after 13 weeks of either 5 or 10 ppm formaldehyde exposure (Özen et al., 2003a).

Table 4-32. Percent body weight gain and concentrations of iron, zinc, and copper in cerebral cortex of male Wistar rats exposed to formaldehyde via inhalation for 4 and 13 weeks

Exposure (mg/m^3)	Weight gain (%)[a]	Zinc (mg/kg)[a]	Copper (mg/kg)[a]	Iron (mg/kg)[a]
4-week data				
0	20.11 ± 2.87	120 ± 6.03	4.60 ± 0.42	25.07 ± 2.83
6.1	7.27 ± 1.49[e]	130 ± 7.26[c]	5.60 ± 0.50[b]	23.00 ± 2.32
12.2	5.24 ± 1.52[e]	185 ± 10.36[e]	5.80 ± 0.60[d]	22.14 ± 1.95[b]
13-week data				
0	60.53 ± 7.84	123 ± 6.22	4.67 ± 0.38	24.92 ± 2.84
6.1	38.41 ± 2.53[e]	155 ± 7.94[e]	5.41 ± 0.56[c]	22.00 ± 2.41
12.2	25.87 ± 1.32[e]	163 ± 6.03[e]	6.10 ± 0.73[e]	21.00 ± 1.96[b]

[a]Values are means ± SDs ($n = 7$).

Statistical significance of differences versus controls, as calculated by the authors:
[b]$p < 0.05$.
[c]$p < 0.02$.
[d]$p < 0.002$.
[e]$p < 0.001$.

Source: Özen et al. (2003b).

Table 4-33. Zinc, copper, and iron content of lung tissue from formaldehyde-treated male Wistar rats

Concentration	Duration[a]	Zinc[b,c]	Copper[b,c]	Iron[b,c]
0 ppm	Control	20.7 (1.6)	0.39 (0.05)	12.5 (0.8)
5 ppm	4 weeks	16.1 (1.3)[d]	0.32 (0.07)	12.9 (1.0)
10 ppm	4 weeks	13.8 (1.2)[e]	0.36 (0.04)	17.5 (1.3)[e]
0 ppm	Control	20.0 (1.6)	0.39 (0.05)	12.7 (0.4)
5 ppm	13 weeks	15.3 (1.4)[e]	0.37 (0.04)	17.9 (1.1)[e]
10 ppm	13 weeks	13.0 (1.1)[e]	0.39 (0.05)	22.4 (1.4)[e]

[a]Rats were exposed 8 hours/day, 5 days/week for the number of weeks indicated.
[b]Concentrations are expressed as moles/mg of tissue, wet basis.
[c]Values are means (n = 7); SDs shown in parentheses.
[d]$p < 0.005$, compared with controls, as calculated by authors.
[e]$p < 0.001$, compared with controls, as calculated by the authors.

Source: Özen et al. (2003a).

There are two reports of lung cytochrome P450 levels after formaldehyde exposure. The first report by Dallas et al. (1989) describes concentration- and duration-dependent changes in P450 levels. Male Sprague-Dawley rats were exposed at 0, 0.5, 3.0, or 15 ppm (0, 0.62, 3.7, or 18.5 mg/m^3) formaldehyde 6 hours/day, 5 days/week for 1 day, 4 days, 12 weeks, or 24 weeks. There were six rats in each exposure group, but the experiment was run in two parts, with three rats in each subgroup. Rats were sacrificed after 1 day, 4 days, 12 weeks, or 24 weeks of exposure, and liver microsomes were prepared. TP and P450 content were determined on each sample.

Average P450 levels in control groups ranged from 17−76 pmol P450/mg protein. However, no P450 was detected in lung from formaldehyde-treated animals after 1 day of exposure, with a method detection limit of approximately 10 pmol P450/mg protein. In contrast, P450 levels were elevated significantly above controls in a concentration-dependent manner after 4 days of formaldehyde exposure (see Table 4-34). Although P450 levels remained elevated in some experimental groups after 12 and 24 weeks of exposure, results were variable and less dramatic.

A later study by Dinsdale et al. (1993) attempted to confirm the increase in P450 levels reported by Dallas et al. (1989). In their first experiment, Dinsdale et al. (1993) treated male Sprague-Dawley rats at approximately 10 ppm (12.3 mg/m^3) formaldehyde 6 hours/day for 4 days. The formaldehyde vapor was generated from formalin by a concentric jet atomizer. For the second experiment, Dinsdale et al. (1993) similarly exposed rats to formaldehyde, but the gas

Table 4-34. Total lung cytochrome P450 measurements of control and formaldehyde-treated male Sprague-Dawley rats

Formaldehyde	1 Day[a,b]		4 Days		12 Weeks		24 Weeks	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2
0 ppm	17 (6)	44 (13)	39 (11)	23 (3)	29 (10)	19 (23)	76 (49)	18 (11)
0.5 ppm	ND	ND	103 (52)	137 (14)[e]	87 (11)[d]	35 (7)	172 (12)[c]	38 (9)
3.0 ppm	ND	ND	357 (10)[e]	278 (100)[e]	91 (10)[d]	67 (34)	92 (103)	30 (15)
15 ppm	ND	ND	362 (38)[e]	334 (4)[e]	130 (2)[e]	56 (6)	151 (9)	48 (7)[c]

[a]Rats were exposed 6 hours/day, 5 days/week for the duration shown.
[b]Cytochrome P450 expressed as pmol P450/mg of protein. Values are means (SDs) (n = 3).
[c]Different from control, $p < 0.05$.
[d]Different from control, $p < 0.01$.
[e]Different from control, $p < 0.001$, as calculated by the authors.
ND = not detected above the limit of detection, approximately 10 pmol/mg protein.

Source: Dallas et al. (1989).

was generated by the thermal depolymerization of paraformaldehyde as was done by Dallas et al. (1989). The concentration of P450 and activity of several P450 isozymes were measured in lung microsomes (pentoxyresorufin O-dealkylase, benzyloxyresorufin O-dealkylase, ethoxyresorufin O-dealkylase, and 2-aminofluorene *N*-hydroxylation). ALP and γ-glutamyl transpeptidase activity were measured in BAL fluid collected from each animal. No changes were seen in BAL enzyme activity or the activity of lung microsomes for the P450 substrates tested. Cytochrome P450 levels were unchanged in experiment 1, where formaldehyde was generated from formalin. Cytochrome P450 levels were increased in experiment 2 with formaldehyde generated from paraformaldehyde (see Table 4-35).

Table 4-35. Cytochrome P450 levels in formaldehyde-treated rats

Group	Experiment 1 (formalin)[a]	Experiment 2 (paraformaldehyde)[a]
	(nmol/mg protein)	
Control	82 ± 30	85 ± 5
10 ppm formaldehyde	73 ± 27	125 ± 23[b]

[a]Values are means ± SDs (n = 3−5).
[b]Different from controls, $p < 0.05$.

Source: Dinsdale et al. (1993).

Extrapulmonary toxicity

Several studies have investigated toxicity in organs other than those associated with the respiratory tract. An earlier cross-species study examined changes in lung tissue resulting from continuous exposure (Coon et al., 1970). Animals were exposed to 3.7 ppm (4.6 mg/m^3) formaldehyde for 90 days. Five species of animals were studied: male and female Sprague-Dawley and Long-Evans derived rats (15), male and female Princeton-derived guinea pigs (15), male New Zealand albino rabbits (3), male squirrel monkeys (*Saimiri sciureus*) (3), and purebred male beagle dogs (2). Blood samples were taken for Hb concentration, HCT, leukocyte counts, and serum levels of BUN, AST, ALT, ALP, and LDH. Sections of heart, lung, liver, kidney, and spleen were fixed and examined from each species (details of method not provided). Brain, spinal cord, and adrenal tissue also were examined in monkeys and dogs as well as thyroid from dogs. Liver and kidney sections were stained for reduced nicotinamide adenine dinucleotide, lactate, isocitrate, and β-hydroxybutyrate. Tissue sections of the nasal mucosa were not examined in this study.

Hematological parameters were unaffected by formaldehyde treatment. The lung tissue of all species exhibited interstitial inflammation after 90 days of formaldehyde exposure (detailed description not provided). Formaldehyde-treated rats and guinea pigs also had focal chronic inflammation in heart and kidney tissue sections. However, the authors were uncertain whether the observed changes to heart and kidney were due to formaldehyde exposure.

As mentioned above, Woutersen et al. (1987) exposed male and female albino SPF Wistar rats (10/group) to 0, 1, 10, or 20 ppm (0, 1.23, 12.3, or 24.6 mg/m^3) formaldehyde 6 hours/day, 5 days/week for 13 weeks. Rats were checked daily and weighed weekly. During week 13, blood samples were taken for Hb, PCV, RBC count, and a differential count of leukocytes. Urine samples were also analyzed. At sacrifice, blood samples were analyzed for ALB, creatinine, glucose, TP, BUN, and the enzyme activities (AST, ALT, and ALP). GSH and protein content were determined in liver homogenates. Organs were examined and weighed: adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, spleen, testes, thymus, and thyroid.

No gross pathological changes were seen upon autopsy, but body weights decreased in both male and female rats at the 20 ppm treatment level. Of the organs weighed, 6 of 11 had significantly increased relative rates in male rats exposed to 20 ppm formaldehyde. Relative brain weight was increased in female rats at the same treatment level (Woutersen et al., 1987).

Clinical chemistry parameters of liver and kidney function and hematological parameters were also measured after the 13-week treatment by Woutersen et al. (1987). Compared with those of controls, activities of AST, ALT, and ALP were significantly elevated in plasma from the 20 ppm treated male rats (by 124, 132, and 126%, respectively; $p < 0.05$). Total plasma

protein was reduced to 95% of controls in the same animals. Although there was an observed increase in BUN in male rats treated with 1 ppm, this was not considered a treatment effect. Furthermore, no statistically significant differences were seen for these parameters in female rats at any concentration level (Woutersen et al., 1987).

Sul et al. (2007) exposed Sprague-Dawley rats to 0, 5, and 10 ppm formaldehyde for 6 hours/day (5 days/week) for 2 weeks and collected lung samples for tissue damage and genomic analysis. According to their results, 21 genes were altered in a dose-dependent manner by microarray analysis; 2 were up regulated and 19 were down regulated in the lung tissue of animals exposed to formaldehyde. However, six of the nine genes further analyzed by PCR did not show dose dependency (authors did not comment). Although the authors briefly describe the functions and potential implications for changes in the expression of some of the altered genes, there is no discussion of the relationship between these altered genes (i.e., there is no pathway analysis).

In 2006, Im et al. (2006) published a proteomic analysis using the same exposure protocols (possibly using the same animals as in the Sul et al. [2007] study, although neither study makes reference to the other). Im et al. (2006) examined DNA damage in lymphocytes and liver tissues, as well as protein and lipid oxidation in plasma and liver samples. Similar to changes reported in the lung (discussed elsewhere), using two-dimensional electrophoresis and matrix-assisted laser desorption ionization time-of-flight mass spectrometry, the authors also reported dose-dependent changes in the levels of 32 proteins in plasma (19 up, 13 down). None of the changes in plasma proteins correspond to the changes in lung reported by Sul et al. (2007). Again, no pathway analysis was provided. Interestingly, Im and colleagues (2006) also demonstrated a dose-dependent increase in plasma IL-4 and dose-dependent decrease in IFNγ, perhaps indicative of Th-2-mediated inflammatory response. An overview of formaldehyde exposure-related pathology in the respiratory system of laboratory animals is presented in Table 4-36.

4.2.2.5. *Chronic Inhalation Bioassays*

The respiratory pathology observed in chronic bioassays is consistent with the subchronic studies. As exposure concentration and duration of exposure are increased, the pathology becomes more severe and penetrates more deeply into the respiratory tract. These effects are progressive over time. Tumors are reported in several bioassays, primarily SCCs. Experimental results regarding both the severity of respiratory tract pathology as well as the tumor incidence vary by species strain and experimental design. As discussed above rodents experience RB, and species differences in respiratory and physiological depression would result in differences in

Table 4-36. Summary of respiratory tract pathology from inhalation exposures to formaldehyde, subchronic studies

Species/strain	No./ group	Treatment[a]	Respiratory effects	LOAEL/NOAEL	Reference
Nasal pathology					
B6C3F1 mice (male and female)	10	0, 2, 4, 10, 20, or 40 ppm formaldehyde 6 hours/day, 5 days/week for 13 weeks	Minimal squamous metaplasia in 1 of 10 mice (4 ppm). Squamous metaplasia observed in all mice at 10 and 20 ppm.	NOAEL = 4 ppm	Maronpot et al. (1986)
SPF Wistar Rats (male and female)	10	0, 1, 10, or 20 ppm formaldehyde 6 hours/day, 5 days/week for 13 weeks	Increased respiratory epithelial hyperplasia and keratinization at 20 ppm; squamous metaplasia at 10 ppm in males and females.	NOAEL = 1 ppm	Woutersen et al. (1987)
SPR Wister rats (male)	50−55	0, 10, or 20 ppm formaldehyde for 6 hours/day, 5 days/week for 4, 8, or 13 weeks	Rhinitis, hyperplasia, and squamous metaplasia in respiratory epithelium at all doses (number of weeks not specified). Squamous metaplasia of olfactory epithelium at 20 ppm (number of weeks not specified)	NOAEL = 1 ppm	Feron et al. (1988)
Wistar rats (male and female)	50	0, 0.3, 1, or 3.0 ppm formaldehyde 6 hours/day, 5 days/week for 13 weeks	Keratinized and nonkeratinized squamous metaplasia in level 2 epithelium in 37/50 male and 21/50 female rats at 3 ppm for 13 weeks.	NOAEL = 1 ppm	Zwart et al. (1988)
Wistar rats (male)	10	40 ppm-hours (8 hours at 5 ppm, 4 hours at 10 ppm) or 80 ppm hours (10 ppm continuous or 20 ppm intermittently)	Thinning and disarrangement of respiratory epithelium, squamous metaplasia, most severe in 20 hours intermittent exposure	NOAEL = 10 ppm	Wilmer et al. (1987)
Wistar rats (male)	10	0, 8, or 16 ppm, given either continuously or intermittently	Disarrangement and squamous metaplasia at 4 ppm. Continuous exposure yielded less severe lesions than intermittent exposure	LOAEL = 8 ppm	Wilmer et al. (1989)
F344 rats (male and female), Syrian golden hamsters (male and female), cynomolgus monkeys	20 rats, 10 hamsters, 6 monkeys	0.0.2, 1, or 3 ppm 22 hours/day, 7 days/week, 26 weeks	Rats: rhinitis at 3 ppm, increased incidence of nasal lesions at 3 ppm. Monkeys: rhinitis at all doses, increased incidence of nasal lesions at 3 ppm. Hamsters: no significant nasal lesions.	NOAEL = 1 ppm	Rusch et al. (1983a, b)
Tracheal and lung pathology					
Wistar rats (male)	6	0, 5, 15 ppm for 8 hours/day, 5 days/week, 4 or 13 weeks	Significant decreases in zinc content in lung, copper unchanged, iron increased in lung.	LOAEL = 5 ppm	Özen et al. (2003)

Table 4-36. Summary of respiratory tract pathology from inhalation exposures to formaldehyde, subchronic studies (continued)

Species/strain	No./ group	Treatment[a]	Respiratory effects	LOAEL/NOAEL	Reference
Sprague-Dawley rats (male)	6 but *n* = 5 in some trials	0, 0.5, 3.0, 15 ppm 6 hours/day, 5 days/week, for 1 day, 4 days, 12 weeks, 24 weeks	Increased P450 levels after 4 days at 3 ppm.	NOAEL = 0.5 ppm	Dallas et al. (1989)
Sprague-Dawley rats (male)	5	0 or 10 ppm 6 hours/day, 4 days using both formalin and paraformaldehyde	P450 levels increased at 10 ppm only in groups treated with paraformaldehyde.	LOAEL = 10 ppm	Dinsdale et al. (1993)
Extrapulmonary effects					
Rats and guinea pigs	15	3.7 ppm for 90 days	Focal chronic inflammation in heart and kidney tissue.	LOAEL = 3.7 ppm	Coon et al. (1970)
SPF Wistar rats (male and female)	10	0, 1, 10, or 20 ppm 6 hours/day, 5 days/week for 13 weeks	Relative brain weight increased in female rats at 20 ppm; increased AST, ALT, ALP in plasma at 20 ppm.	NOAEL = 10 ppm	Woutersen et al. (1987)

absorbed dose in the respiratory tract, given the same exposure concentration (Chang and Barrow, 1983). Additionally, differences in the nasal architecture result in species-dependent variation of formaldehyde absorption (flux) within the respiratory tract (see Section 3.4). Therefore, chronic studies are discussed by species for greater clarity.

4.2.2.5.1. ***Mice.***

Early experiments by Horton et al. (1963) subjected mice (C3H, sex unspecified) to extreme formaldehyde concentrations (0, 0.05, 0.1, and 0.2 mg/L or 41−163 ppm) in an attempt to simulate lung pathology reported in humans exposed to cigarette smoke. The mice were exposed 1 hour/day, 3 days a week for up to 35 weeks. The authors did not note the effects of RB or provide any information on pathology of the URT. There was a clear increase in histologic changes in the tracheobronchial epithelium by exposure, including basal-cell hyperplasia, stratification squamous cell metaplasia and atypical metaplasia. Subsequent exposures to various combinations of formaldehyde and coal tar did result in squamous cell tumors. The findings of Horton et al. (1963) suggest a role for formaldehyde in lung cancer under some conditions. However, the exposure design and early deaths in the treatment groups severely limit the usefulness of these data in human health risk assessment.

In a comprehensive study conducted by Swenberg et al. (1980) (also reported in Kerns et al. [1983]) in conjunction with Chemical Industry Institute of Toxicology (CIIT) and Battelle Columbus Laboratories, male and female C57BL/6 × C3H F_1 (B6C3F_1) mice (approximately 120/sex/concentration) were exposed to 0, 2.0, 5.6, or 14.3 ppm (0, 2.45, 6.87, or 17.5 mg/m^3) formaldehyde 6 hours/day, 5 days/week for 24 months. This exposure period was followed by up to 6 months of nonexposure to evaluate recovery. Interim sacrifices were conducted at 6, 12, 18, 24, 27, and 30 months (due to unscheduled deaths, no male mice were sacrificed at 18 or 27 months). Exposure generation was accomplished by sublimation of paraformaldehyde, and exposures were conducted in whole-body chambers. Detailed sectioning and examination of the nasal passages were conducted at each interim sacrifice, beginning at 12 months, and for all unscheduled deaths. Gross organ pathology was noted for all animals and complete histopathologic examination was conducted on all animals in the control and high-exposure groups. There were no differences in survival in any exposure group compared with controls. Generally, poor survival in all groups of male mice was attributed to fighting and infections of the urogenital tract associated with group housing; 78, 77, 81, and 82 unscheduled deaths were recorded before 24 months in the 0, 2.0, 5.6, and 14.3 ppm treatment groups, respectively (n = 119, 120, 120, and 119 males, respectively). After the interim sacrifices (6 and 12 months) only 17−22 male mice survived to the 24-month scheduled sacrifice. Female mice had much

greater survival with only 30, 34, 19, and 34 unscheduled deaths prior to the 24-month sacrifice. The authors did not note the effects of RB in mice, although the RD_{50} for a 10-minute exposure for male B6C3F1 mice has been reported at 4.9 ppm and 4.4 ppm (Steinhagen and Barrow, 1984; Chang et al., 1981).

The first examination of the nasal cavities was conducted at the 12-month interim sacrifice. Inflammation in the nasal turbinates was evident in mice in the 2 and 6 ppm treatment groups (14/20 and 18/20, respectively), including adenitis of the nasal lacrimal duct, lacrimal duct, and vomeronasal gland. Inflammation was not present in mice exposed at 15 ppm, although serous rhinitis was seen in 4 of 20 animals. At 18 months, mice exposed at 2 and 6 ppm no longer exhibited adenitis in the nasoturbinates. Epithelial dysplasia was evident in 4 of 20 mice at 6 ppm exposure. Mice in the high-exposure group had significantly greater nasal pathology; epithelial dysplasia and squamous metaplasia were reported in 18/19 and 17/19 female mice, respectively, exposed to 15 ppm. After 24 months, squamous epithelial hyperplasia of the nasolacrimal duct (29/45) and atrophy of the olfactory epithelium (18/45) were also noted in animals from the high-exposure group (male and female) (Battelle Columbus Laboratories, 1981). Similar pathology was reported in only a small fraction of mice exposed at 2 and 6 ppm (5/48 and 11/60, respectively).

Three months after cessation of exposure, only nine female mice were available for sacrifice, but within this small sample the data suggested recovery of nasal lesions: epithelial dysplasia (4/9), squamous metaplasia (2/9), atrophy of the olfactory epithelium (1/9), and squamous epithelial hyperplasia of the nasolacrimal duct (1/9) (Battelle Columbus Laboratories, 1981).

Of the 17 male mice that survived to 24 months in the 14.3 ppm exposure group, 2 had SCC in the nasal cavity ($p < 0.05$). Of the two tumor-bearing mice, one exhibited significant epithelial pathology, including rhinitis, dysplasia, squamous metaplasia, and hyperplasia. Squamous metaplasia of the nasolacrimal duct was the only related pathology reported for the second mouse. No SCCs were found in female mice, although 48 mice survived to 24 months. The authors reported no other formaldehyde-related tumors. However, comparisons were based on summary tables by organ. Although lymphomas were analyzed by organ and site (e.g., increase in salivary gland lymphoma considered separately from mandibular lymphoma), later reanalysis of lymphoma in female mice, based on tumor-bearing animals (TBAs), does indicate an association with formaldehyde exposure.

4.2.2.5.2. ***Rats.***

Holmström et al. (1989a) evaluated coexposure of inhaled formaldehyde with wood dust in 16 female Sprague-Dawley rats/group. Rats were exposed in whole-body chambers for 6 hours/day, 5 days/week for 104 weeks to formaldehyde alone at 12.4 ± 1.1 ppm (15.21 ± 1.35 mg/m^3), wood dust alone (25 mg/m^3), or both wood dust (25 mg/m^3) and formaldehyde (12.7 ± 1.0 ppm) or to room air as the control. The wood dust was generated from grinding of beech. Microscopic measurements of the wood particles indicated that approximately 70% had a geometric diameter of about 10 μm, while 10−20% were about 5 μm or less. Animals were sacrificed at 104 weeks and histopathology was performed on five transverse sections of the nasal cavity (see Figure 4-6) and the lungs (not otherwise specified).

There were no differences in mortality among the groups at any time during the study period. Rats exposed to formaldehyde were reported to have exhibited yellow discoloration of the fur, and many displayed eye irritation. Formaldehyde exposure, with and without wood dust, induced squamous metaplasia, keratinization, and dysplasia of the nasal epithelium (see Table 4-37).

Table 4-37. Histopathologic findings and severity scores in the naso- and maxilloturbinates of female Sprague-Dawley rats exposed to inhaled formaldehyde and wood dust for 104 weeks

Treatment	Pronounced squamous metaplasia	Pronounced squamous metaplasia with keratinization	Pronounced squamous metaplasia with presence of dysplasia	Sum of rats with pronounced metaplasia and/or dysplasia	CCSCC	Histologic scores at the level of naso- and maxilloturbinates (mean ± SD)
Formaldehyde group (*n* = 16)	7	2	1	10	1	2.25 ± 1.73[a]
Formaldehyde-wood dust group (*n* = 15)	7	1	4	12	0	2.6 ± 1.88[a]
Wood dust group (*n* = 15)	0	0	0	0	0	1.86 ± 0.83[b]
Control group (*n* = 15)	0	0	0	0	0	1.07 ± 0.70

[a]$p < 0.01$.
[b]$p < 0.05$.

Source: Holmström et al. (1989a).

Among the five levels of the nasal cavity that were examined, Holmström et al. (1989a) presented findings for the naso- and maxilloturbinates since formaldehyde-induced tumors had been associated with this level (Morgan et al., 1986a, b). The data also suggested an effect of wood dust on formaldehyde-induced nasal pathology, with a slightly higher histologic score and greater incidence of dysplasia than formaldehyde exposure alone. One SCC (1/16) occurred in the group exposed to formaldehyde only but not in the group exposed to formaldehyde and wood dust. Microscopic examination of the lungs revealed that emphysema (diagnostic criteria not specified) was more prevalent in both groups exposed to wood dust compared with the control group ($p < 0.05$). There was no significant difference in pulmonary epithelial histopathology among the groups.

Tobe et al. (1985) also evaluated F344 rats (32/group) exposed to inhaled formaldehyde for 28 months. Exposures were for 6 hours/day, 5 days/week to formaldehyde concentrations of 0, 0.3, 2, and 14 ppm (0, 0.37, 2.45, and 17.2 mg/m^3). Fourteen of 32 rats (44%) in the high concentration group developed nasal SCCs, compared with none in the other exposed groups and the control group. Tobe et al. (1985) reported increased rhinitis, hyperplasia, and squamous metaplasia of the nasal respiratory epithelium, including in the low-exposure group (0.3 ppm.) However, some level of rhinitis, hyperplasia, and metaplasia were also present in controls. Without a more complete report, it is unknown whether or not the pathology reported at 0.3 ppm was a formaldehyde-related effect.

Kamata et al. (1997) evaluated the effects of inhaled formaldehyde in male F344 (F344/DuCrj) rats (32/group) exposed for 28 months. Formaldehyde exposure was generated by metering 37% formalin (containing 10% methanol) into a sprayer in a glass bottle and diluting with room air. Concentration in the chamber was monitored twice daily by the acetyl acetone method. Exposures were for 6 hours/day, 5 days/week at nominal formaldehyde concentrations of 0, 0.3, 2.0, and 15 ppm (0, 0.37, 2.45, and 18.4 mg/m^3). Actual levels were 0, 0.3 ± 0.07, 2.17 ± 0.32, and 14.85 ± 2.22 ppm (mean ± SD). Rats in the 0 ppm group were given methanol to inhale at the same concentration (4.2 ppm) as the 15 ppm group. A room control no-exposure group was also included in the study. All animals were observed for clinical signs once a day during the study. Body weights and food consumption were recorded weekly. Five animals per group, randomly selected at the end of 12, 18, and 24 months, and all surviving animals at 28 months were sacrificed for hematological measurements (Hb, RBCs, PCV, MCV, mean corpuscular hemoglobin [MCH], MCHC, and WBCs), biochemical determinations (TP, ALB, BUN, ALP, AST, ALT, glucose, albumin/globulin ratio, phospholipids, triglycerides, and total cholesterol), and pathological examinations. Wet weights were taken on brain, heart, lungs, liver, kidneys, spleen, testes, and adrenal gland of each rat. Histopathology was performed on all

moribund or dead animals and those at specified sacrifices on all gross lesions and the following tissues: pituitary, thyroid, nasal cavity, trachea, esophagus, stomach, small and large intestines, prostate gland, urinary bladder, muscle, femur, sciatic nerve, spinal cord, and mesenteric lymph nodes. Histopathologic sections of the nose were obtained from five anatomical levels, but these did not correspond to the typical levels taken in other bioassays. Most notably, section level B was anterior and not posterior to the incisor teeth. The incidence data for nasal histopathology were not reported with respect to section level location, with the exception that the nonproliferative lesions and tumors reported were described to occur predominantly at levels B and C.

Yellow discoloration of the coats occurred in animals exposed at the 2 and 15 ppm levels. Significant decreases in body weight and food consumption were observed in the high concentration (15 ppm) group throughout the exposure period, and elevated mortality was noted at 28 months (88.3 versus 31.8% in controls). The first death occurred after 6 versus 18 months in the control group. Other effects noted in the 15 ppm exposure group include decreased triglycerides, reduced liver weight (both relative and absolute), and increased relative adrenal weights.

Treatment-related macroscopic and histopathologic findings were limited to the nasal cavity. Squamous cell metaplasia was reported in all treatment groups: 16% (0.3 ppm), 37.5% (2 ppm), and 91% (15 ppm) of exposed rats. Epithelial hyperplasia was similarly present in 12.5, 22, and 91% of the animals, respectively. Since a no-effect level could not be determined, the authors reported benchmark doses (BMDs) of 0.25 and 0.24 ppm for squamous cell metaplasia and epithelial hyperplasia (10% response.). Additional lesions only occurring in the 15 ppm dose group were papillary hyperplasia (2/32), SCC (13/32), squamous cell papilloma (3/32), and sarcoma (1/32). The majority of the tumors were located at levels B and C of the nasal cavity.

Albert et al. (1982) and Sellakumar et al. (1985) reported on a set of lifetime studies performed in male Sprague-Dawley rats to evaluate the effects of inhaled formaldehyde alone and in combination with hydrochloric acid (HCl). Rats were exposed 6 hours/day, 5 days/week for life. In the first experiment (Albert et al., 1982), 8-week-old male inbred Sprague-Dawley rats (n = 99) were exposed to a mixture of 10 ppm (12.3 mg/m^3) HCl and 14 ppm (17.2 mg/m^3) formaldehyde, and there were two control groups: air-sham and untreated (n = 50). Bis(chloromethyl)ether (BCME), a known animal carcinogen (Albert et al., 1975; Kuschner et al., 1975; Figueroa et al., 1973; Laskin et al., 1971), is formed when formaldehyde and HCl are mixed. BCME concentrations were estimated at about 1 ppb in the formaldehyde-HCl mixed exposures, based on levels in the mixing chamber. Complete necropsies were conducted when animals died naturally or were killed when moribund. Histologic sections were taken from the

nasal cavity, larynx, trachea, pulmonary lobes, liver, bladder, kidney, spleen, and other organs with gross pathologic alterations.

Exposure to the mixed gases (formaldehyde-HCl-BCME) induced nasal lesions, including epithelial hyperplasia (71%), squamous metaplasia (64%), squamous papilloma (3%), and SCC (25%) (Albert et al., 1982). Although a few squamous metaplasias were noted in the larynx, trachea, and bronchi, these lesions were also noted in controls. Mortality in exposed rats was significantly increased over controls and was approximately 30% when the first carcinoma was reported (233 days). Mortality in exposed rats rose quickly to approximately 60% after the first year of exposure. Therefore, the authors used a life-table method to calculate a mortality-corrected cumulative incidence, reporting a corrected cumulative incidence of 77% at 720 days after first exposure.

In the second experiment performed in this laboratory (Sellakumar et al., 1985; Albert et al., 1982), Sprague-Dawley rats were similarly exposed to HCl (10 ppm) alone, formaldehyde alone (15 ppm), or a combination of both. The combination exposure was generated in two different ways to better understand the influence of BCME formation on study results: premixed at high concentrations and gases fed separately into the inlet air supply at the target concentrations. BCME concentration measured by a gas chromatography/mass spectrometry method in the premixed chamber varied between 0.1 and 0.4 ppb. Cage-side observations and necropsy procedures were as described in Albert et al. (1982) with the exception of the histologic preparation of the head. The head was cut transversely into four tissue blocks, and sections were taken from the face of each.

Animals exposed to formaldehyde alone and formaldehyde-HCl (premixed or nonpremixed) showed a marked decrease in body weight after 16 weeks. After 32 weeks rats exposed to the premixed formaldehyde-HCl (with BCME) had higher mortality compared with the other mixed gas exposures ($p < 0.05$). Nasal pathology was similar among rats exposed to formaldehyde alone or the mixed gases (see Table 4-38). Desquamation of respiratory epithelial cells was reported in the respiratory epithelium that covers the nasomaxillary turbinates and the nasal septum (approximately section levels 2 and 3). Olfactory epithelium in the ET frequently showed an inflammatory reaction with seropurulent exudate filling the lumen. Squamous metaplasia and hyperplasia were reported in the larynx and trachea in all treatment groups.

Table 4-38. Histopathologic changes (including tumors) in nasal cavities of male Sprague-Dawley rats exposed to inhaled formaldehyde or HCl alone and in combination for a lifetime

Observation	Premixed HCl-HCHO	Nonpremixed HCl-HCHO	HCHO	HCl	Air	Colony
Number of animals examined	100	100	100	99	99	99
Rhinitis	74	75	74	81	72	70
Epithelial or squamous hyperplasia	54	53	57	62	51	45
Squamous metaplasia	64	68	60	9	5	6
Polyp or papilloma	13	11	10	0	0	0
SCC	45	27	38	0	0	0
Adenocarcinoma	1	2	0	0	0	0
Mixed carcinoma	0	0	1	0	0	0
Fibrosarcoma	1	0	1	0	0	0
Esthesioneuroepithelioma	1	0	0	0	0	0
Larynx						
Hyperplasia	11	22	21	22	2	2
Squamous metaplasia	10	15	4	0	0	0
Trachea						
Hyperplasia	18	32	21	26	6	2
Squamous metaplasia	9	8	7	0	0	0

Source: Sellakumar et al. (1985).

Tumors arose primarily from the nasomaxillary turbinates and nasal septum. The SCCs were predominantly moderate to well differentiated, with excessive amounts of keratin occluding the lumen, killing the animals by asphyxiation. Statistical comparisons by the log rank test (Peto test) showed that tumor incidence was increased in the premixed formaldehyde-HCl combined exposure group over formaldehyde alone or the combined formaldehyde-HCl (not premixed). There were no significant differences in the latency among groups, with the average latency varying from 603 to 645 days. Rats exposed to HCl exposure alone did not develop tumors.

The esthesioneuroepithelioma is a unique tumor type observed with a high incidence in an earlier inhalation study of rats exposed to BCME (Kuschner et al., 1975), suggesting that the higher incidence of nasal tumors observed in the premixed-combined formaldehyde-HCl-exposure group may have been due to BCME (Krimsky, 1986) since this premixed protocol was the one most likely to generate BCME. Sellakumar et al. (1985) refuted this assertion, stating

that this singular tumor occurred in the absence of other changes in the ethmoid region or in the lungs where BCME was also demonstrated to cause tumors. Furthermore, exposure was approximately one-tenth the cumulative dose in the Kuschner et al. (1975) study that was associated with a single similar tumor. Sellakumar et al. (1985) attributed the higher incidence in the premixed-combination group to traces of other alkylating agents (not BCME) that could have been formed. The results demonstrate that animals exposed to either a combination of formaldehyde-HCl or to formaldehyde alone develop nasal tumors, principally SCCs, at about the same frequency, indicating that HCl plays little or no role in the carcinogenicity of inhaled formaldehyde.

In a companion study to the chronic mouse study described above (Kerns et al., 1983; Swenberg et al., 1980), groups of F344 rats (approximately 120/sex/concentration) were exposed to 0, 2.0, 5.6, or 14.3 ppm (0, 2.45, 6.87, or 17.5 mg/m^3) formaldehyde 6 hours/day, 5 days/week for 24 months. This exposure period was followed by up to 6 months of nonexposure to evaluate recovery. Interim sacrifices were conducted at 6, 12, 18, 24, 27, and 30 months. Study parameters and methods were as described above.

Formaldehyde exposure increased mortality of both male and female rats in all treatment groups ($p < 0.05$ for 6 and 15 ppm groups). Severe treatment-related mortality was seen at the highest exposure group beginning at 12 months with only 30% surviving to the 24 months. There were no alterations in clinical chemistry, neurofunctional, or ophthalmological measurements considered to be related to formaldehyde exposure. A concentration-dependent increase in yellow discoloration of the hair coat was observed. This discoloration dissipated over the 3-month postexposure period. Rats in the highest-concentration group were dyspneic ($p < 0.01$) and emaciated ($p < 0.05$) and had many facial swellings that on closer examination were revealed to be carcinomas protruding through the nasal cavity. Neoplastic lesions in the URT were first observed clinically at day 358 in females and day 432 in males. Macroscopically, these lesions originated in the anterior portion of the nasal cavity and, in a few instances, extended into the ETs.

Figure 4-13 shows the frequency of squamous metaplasia by location in the noses of rats sacrificed at various time points along the 2-year exposure period. Histopathologic lesions were confined to the nasal cavity and proximal trachea in concentration-dependent fashion. The morphologic diagnosis of squamous metaplasia was used to designate zones of altered epithelium that were characterized by the presence of a well-differentiated germinal layer (stratum germinativum) and superficial layers of epithelium (stratum spinosum and stratum corneum). Keratin was produced only in areas of squamous metaplasia. Epithelial dysplasia was detected earlier than squamous metaplasia and was characterized by a mucosa that had

undergone a transition from nonciliated simple cuboidal to one that was several cells thick and squamoid with an organization and polarity of the individual cells that had changed from vertical to horizontal with respect to the basement membrane. Similar histomorphologic changes have also been called basal cell hyperplasia and epidermoid metaplasia (e.g., Albert et al. [1982]). Figure 4-13 clearly illustrates that concentration is a dominant determinant of lesion distribution. At low concentrations the lesions occur only in the most anterior region (cross-section level 1). At 5.6 ppm, the squamous metaplasia in levels 1, 2, and 3 was also associated with purulent rhinitis and epithelial dysplasia. At the highest concentration, the lesions progress to the more distal URT, with lesions evident in level 5 and no difference in the incidence at level 1 or 2 across the various sacrifice times. Statistically significant ($p < 0.05$) regression of the lesion was evident at most locations at the 27-month sacrifice (3 months postexposure) (e.g., level 1 in the 2 ppm group, all levels of the 5.6 ppm group, and levels 4 and 5 of the 14.3 ppm group).

Furthermore, progression of lesions distally to the lower respiratory tract (LRT) occurred only in the high concentration group. Tracheal pathology observed at 18 months included multifocal areas of minimal to mild epithelial hyperplasia, epithelial dysplasia, or squamous metaplasia. There were no significant tracheal lesions present in the 0, 2.0, or 5.6 ppm exposure groups, and tracheal lesions were not observed during the postexposure period in the 14.3 ppm exposure group.

Table 4-39 provides the summary data of all neoplastic lesions in the nasal cavity of exposed rats. The adjusted cumulative incidence rates of SCC in male and female rats from the 14.3 ppm exposure group at 24 months were 67 and 87%, respectively. In this group, the formation of zones of squamous metaplasia with zones of squamous epithelial hyperplasia and increased keratin production appeared to precede areas of squamous papillary hyperplasia with foci of cellular atypia. More advanced lesions included carcinoma in situ and invasive SCC of the nasal turbinates. The neoplasia were extremely osteolytic and were associated with excessive keratin production and mild to severe purulent rhinitis. In many animals from the high-exposure group (with or without carcinoma), the excessive accumulation of keratin and inflammatory exudates within the lumen of the URT caused severe dyspnea and death. Polypoid adenomas were also observed in eight rats (four/sex) from the low-exposure group, six male rats from the intermediate-exposure group, and six rats (five males, one female) from the high-exposure group in level 1, 2, or 3. One control male rat had a similar lesion. When adjusted and unadjusted data were analyzed, no significant differences were observed in pair-wise analyses; however, a significant adjusted trend ($p < 0.05$) was reported for male rats. There was no evidence of progression from polypoid adenoma to SCC.

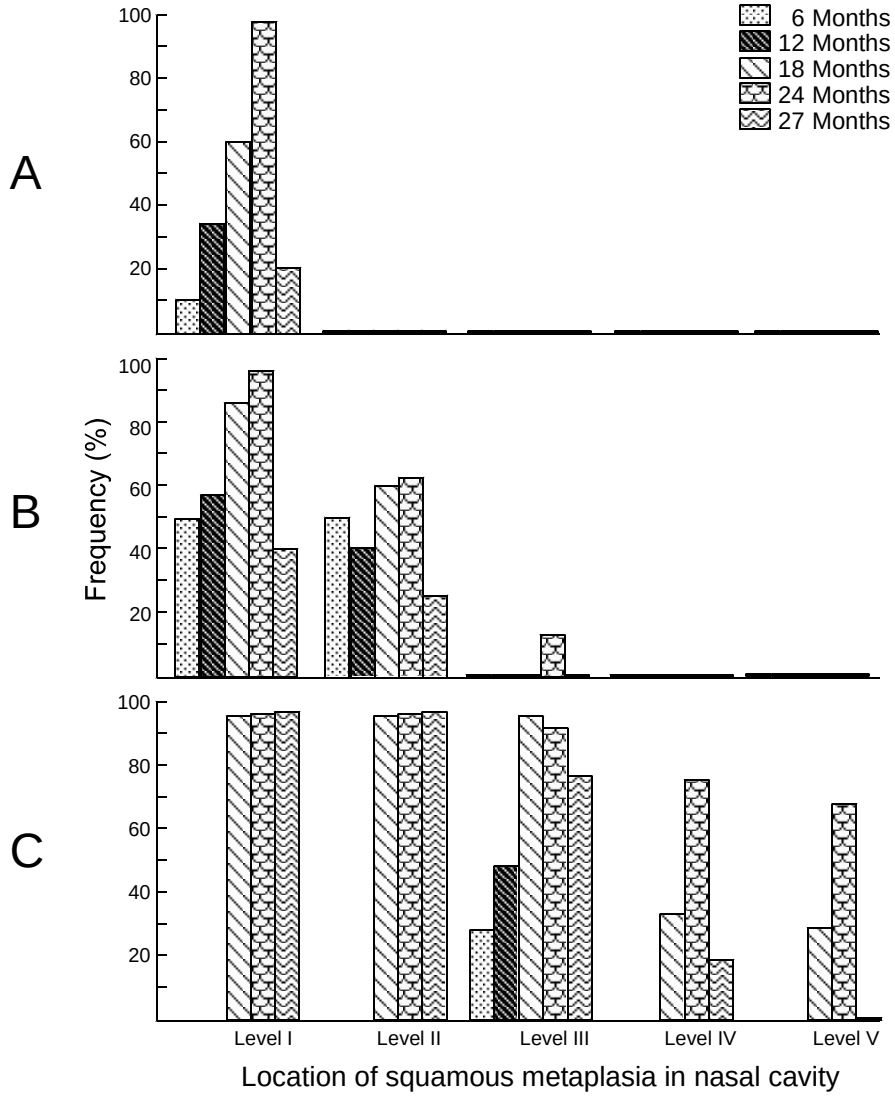

Figure 4-13. Frequency and location by cross-section level of squamous metaplasia in the nasal cavity of F344 rats exposed to formaldehyde via inhalation.

Note: Exposure concentrations were 2.0 ppm (A), 5.6 ppm (B), or 14.3 ppm (C). Nasal cavity levels 2, 3, 4, and 5 were not evaluated at the 6- and 12-month interim sacrifices in the 14.3 ppm exposure group.

Source: Redrawn from Kerns et al. (1983).

Table 4-39. Summary of neoplastic lesions in the nasal cavity of F344 rats exposed to inhaled formaldehyde for 2 years

Formaldehyde (ppm)	Sex	No. of nasal cavities evaluated	SCC	Nasal carcinoma	Undifferentiated carcinoma or sarcoma	Carcino-sarcoma	Polypoid adenoma	Osteo-chondroma
0	M	118	0	0	0	0	1	1
	F	114	0	0	0	0	0	0
2.0	M	118	0	0	0	0	4	0
	F	118	0	0	0	0	4	0
5.6	M	119	1	0	0	0	6	0
	F	116	1	0	0	0	0	0
14.3	M	117	51	1[a]	2[a]	1	4	0
	F	115	52	1	0	0	1	0

[a]One rat in this group also had an SCC.
Source: Kerns et al. (1983).

Morgan et al. (1986b) performed an additional analysis of the slides and tissues from the Kerns et al. (1983) study to more precisely determine the location of each tumor recorded. Additional sections were cut from the existing tissue blocks if a full slide set (i.e., five sections) was unavailable for each animal. For each animal, the location of each tumor was recorded on diagrams of the cross section of the nose, and an attempt to determine the site of origin was made based on the center of the tumor mass. The results for each case were assigned an accuracy rating that was based on the degree of confidence that the pathologist had in the designated site of origin. Results for SCCs are shown in Table 4-40.

Table 4-40. Apparent sites of origin for the SCCs in the nasal cavity of F344 rats exposed to 14.3 ppm of formaldehyde gas in the Kerns et al. (1983) bioassay

Sex	Accuracy rating	Number of animals	Total SCC (%)[a]				
			Area 1[b]	Area 2[b]	Area 3[b]	Area 4[b]	Unable to determine
Male	High	36	56	28	14	3	NA
	Low	25	56	20	8	0	16
Female	High	45	62	27	7	4	NA
	Low	15	47	33	13	0	7
Totals		121	57	26	10	3	4

[a]Rounded to nearest whole number.
[b]Area 1 = lateral aspect of the nasoturbinate and adjacent lateral wall; Area 2 = midventral septum; Area 3 = dorsal septum and roof of dorsal meatus; Area 4 = dorsal and lateral aspect of the maxilloturbinate.
NA = not applicable.
Source: Morgan et al. (1986b).

In the 14.3 ppm exposure group, 98/103 rat noses had adequate numbers and quality of slides for mapping the SCC distribution. Single neoplasia were present in 80 (40/sex), while multiple neoplasia were present in 9 males (21 neoplasia) and 9 females (20 neoplasia). The results were similar for cases with high or low accuracy. For example, more than half (57%) of the SCCs occurred on the lateral side of the nasoturbinate and adjacent lateral wall at the front of the nose (levels 1 and 2); approximately 25% were located on the midventral nasal septum (levels 2 and 3); and about 10% were on the dorsal septum and roof of the dorsal meatus (levels 1, 2, and 3). A small number (3%) were found on the maxilloturbinate (levels 2 and 3), which only involved the medial aspect. All other regions of the nose where SCC was found were considered to be involved as a result of invasion from one or more of the above sites. There were two tumors in the 5.6 ppm group: one male had a single neoplasm on the ventral nasal septum (level 3) while a female had an SCC from the lateral aspect of the maxilloturbinate to the adjacent lateral wall (level 2).

On the basis of the morphology of 19 small neoplasia in this study and in additional work described below (Morgan, 1997; Monticello et al., 1996), it was further concluded that the SCCs arose from the epithelium lining the airway and not from the underlying glandular epithelium. This mapping procedure and that of Monticello et al. (1996) described below were in good concordance and showed a clear site specificity; most of the SCC arose in the anterior lateral meatus (ALM) (57%), which is lined by transitional epithelium, and the midventral nasal septum (26%), which is lined by respiratory epithelium (Morgan, 1997).

The CIIT performed a second bioassay on inhaled formaldehyde in 9-week-old male F344 (CDF[F344]/CrlBr) rats (Monticello et al., 1996). The rats were exposed 6 hours/day, 5 days/week for 24 months to 0, 0.7, 2, 6, 10, and 15 ppm (0, 0.86, 2.45, 7.36, 12.3, and 18.4 mg/m^3) formaldehyde. Study objectives were to repeat the Kerns et al. (1983) bioassay, better defining the concentration response relationship and to seek a correlation between localized data on tumor sites and concomitant cell proliferation assays. Histopathology was performed on six cross-section levels of the nasal cavity on every animal of an unscheduled death and all those of the terminal sacrifice after 24 months. The distribution of lesions for each individual animal was recorded onto epithelial maps of the nasal cavity at 30 selected levels designed to permit accurate localization (Mery et al., 1994). Cell proliferation was measured in a subset of animals (five per treatment group) at 3, 6, 18, and 24 months of exposure in each of the nasal regions to which tumors were mapped (see Table 4-41).

Table 4-41. Incidence and location of nasal squamous cell carcinoma in male F344 rats exposed to inhaled formaldehyde for 2 years

Formaldehyde concentration (ppm)	No. of nasal cavities examined	Nasal location							No. of animals with SCC[a]
		Anterior lateral meatus	Posterior lateral meatus	Anterior mid-septum	Posterior mid-septum	Anterior dorsal septum	Anterior medial maxillo-turbinate	Maxillary sinus	
0	90	0	0	0	0	0	0	0	0
0.7	90	0	0	0	0	0	0	0	0
2	96	0	0	0	0	0	0	0	0
6	90	1	0	0	0	0	0	0	1
10	90	12	2	0	0	0	0	0	20
15	147	17	9	8	1	3	4	0	69

[a]Total number of animals with SCCs, including those too large to allocate and those located in a site not listed in this table.
Source: Monticello et al. (1996).

Yellow discoloration of the fur, a consistent response to formaldehyde in rats, was observed in the rats exposed to 10 and 15 ppm formaldehyde. There were numerous premature deaths in the 15 ppm exposure group, resulting in significantly decreased survival relative to controls (18.8 versus 35.7%; $p < 0.001$). Survival was higher in the three lowest exposure groups and statistically comparable to controls in the 10 ppm exposure group (35.7 versus 31.3%, respectively).

Control animals showed no histopathologic evidence of disease in the nasal passages. Buccal cavity SCC, not associated with the nasal cavity, was present in 2 of 90 control animals. This was considered an incidental finding and within the spontaneous incidence range reported for this strain of rat. Buccal SCCs were observed in three animals at 15 ppm and in one animal at 2 ppm. All other neoplastic responses in the respiratory tract were confined to the nose and considered to have originated from the epithelium lining the nasal airways. The nasal neoplasia included SCCs and polypoid (transitional) adenomas and were similar in morphologic characteristics to those described in the Kerns et al. (1983) chronic bioassay. The incidence of nasal SCCs by location is summarized in Table 4-41, which demonstrates a clear concentration-response relationship. No SCCs occurred in the two lowest exposure groups or in the controls. One nasal rhabdomyosarcoma and two nasal adenocarcinomas were reported in animals in the highest treatment groups.

Regional analysis indicated that the SCCs arose in nasal regions lined with transitional or respiratory epithelium and were most common in the lateral meatus and the midseptum (see Table 4-41). Within the lateral meatus and mid-septum, there was clear evidence of a higher tumor incidence rate in the anterior sample site (p = 0.001 and 0.02, respectively). Smaller numbers of SCCs were observed on the medial aspect of the maxilloturbinate and the dorsal septum and on the posterior lateral wall and lining of the nasopharyngeal meatus (data not shown). No SCCs were observed in the maxillary sinus, with the exception of one animal exposed to 15 ppm that had a small tumor in the wall of the ostium of this sinus. Tumor rates across the seven nasal epithelial sites are presented in Table 4-41. There was an increasing tumor response between the 10 and 15 ppm exposure groups in all sites, except in the ALM. The SCC rates at 10 and 15 ppm were virtually identical (13.3 and 11.6%, respectively), which is probably attributable to the occurrence of many large neoplasia in the lateral meatus site that were not suitable and not counted in the analysis.

The nonlinear tumor response is mirrored by a highly nonlinear response in cell proliferation measured after 3, 6, 12, and 18 months of exposure. Significant treatment-induced responses in cell proliferation indices at these time points were only observed at the two highest exposure concentrations (10 and 15 ppm). Other treatment-induced lesions, predominantly epithelial hypertrophy, hyperplasia, squamous metaplasia, and mixed inflammatory cell infiltrate, were also most severe at these two exposure concentrations. Significant distortion and destruction of the nasoturbinate architecture occurred in many animals exposed to 15 ppm. Nasal turbinate adhesions and olfactory degeneration (usually confined to the walls of the anterior dorsal medial meatus) also occurred in animals exposed to 10 and 15 ppm. Lesions in the 6 ppm exposure group were limited to focal squamous metaplasia in the anterior regions.

As discussed briefly above, small numbers of polypoid adenomas were also induced by formaldehyde exposure and were similar in acinar-like structure and location to those in the Kerns et al. (1983) bioassay. No polypoid adenomas occurred in the control animals or in the 0.7, 2, or 6 ppm exposure groups. A clear concentration response was observed in the 10 and 15 ppm exposure groups. Five of 90 animals (5.6%) in the 10 ppm exposure group and 14 of 147 animals (9.5%) in the 15 ppm exposure group had a polypoid adenoma. Most of these polypoid adenomas (79%) were located in or adjacent to the lateral meatus. The significance of these tumors for risk assessment remains to be determined (Morgan, 1997).

Appelman et al. (1988) studied the effects of bilateral intranasal electrocoagulation damage on susceptibility to inhaled formaldehyde in male SPF Wistar (Cpb: WU) rats. Rats were exposed 6 hours/day, 5 days/week for 13 or 52 weeks to 0, 0.1, 1.0, or 10 ppm (0, 0.12, 1.23, or 12.3 mg/m^3) formaldehyde. These concentrations were chosen because the various

short-term studies performed in the same laboratory (described in Section 4.2.1.2) showed that formaldehyde was noncytotoxic to the nasal mucosa at levels of 0.3, 1.0, and 2.0 ppm, slightly cytotoxic at 3 and 4 ppm, and strongly cytotoxic at 10 and 20 ppm (Zwart et al., 1988; Wilmer et al., 1987; Woutersen et al., 1987). Furthermore, because nasal tumors have only been found at exposure concentrations that also induced severe degenerative, hyperplastic, and metaplastic changes in the nasal epithelium (Griesemer et al., 1985; Squire and Cameron, 1984), Feron et al. (1984) and the investigators at the TNO-CIVO Toxicology and Nutrition Institute postulated that formaldehyde at a subcytotoxic concentration was only a very weak initiator without promoting activity. Appelman et al. (1988) used an electrocoagulation method in this study to evaluate if damage to the mucosa followed by compensatory cell proliferation might render the epithelium vulnerable to subcytotoxic levels of formaldehyde. One-half of the rats used in the study (10/group) were damaged bilaterally and then subjected to the first 6-hour exposure to formaldehyde approximately 20−26 hours after the electrocoagulation procedure. Ten undamaged rats/group were also exposed at each concentration for either 13 or 52 weeks. Histopathologic examination included six standard cross-section levels in the nose; livers of all rats killed at 14 weeks and of all control and 10 ppm exposed rats killed in week 53; larynges, tracheas, and lungs of all rats of the control and 10 ppm exposed rats killed in week 53; and organs and tissues of control and 10 ppm exposed rats with an undamaged nasal mucosa killed in week 53.

Yellow discoloration of the fur occurred in all animals of the two highest exposure groups. Growth retardation was observed in the animals killed with or without damaged noses after 2 weeks of exposure to 10 ppm formaldehyde. No toxicologically significant findings in the body weights or organ weights of any animals in the other exposure groups were observed. No relevant differences between groups were found in any of the hematological or urinary parameters with the exception of frequent oliguria ($p < 0.05$) in the top exposure group without nasal coagulation and killed in week 53. Three-way ANOVA revealed a significant increase in TP content of the liver in rats with damaged noses as compared with rats with undamaged noses, and there was a significant negative correlation between the formaldehyde exposure level and TP in these same rats. Hepatic GSH was positively correlated with both nasal damage and age of the animals. No treatment-related gross findings were observed in animals sacrificed at either 14 or 53 weeks except for yellow discoloration of the fur in rats exposed at the two highest concentrations. No changes observed in the larynx, trachea, lungs, liver, or other tissues evaluated were regarded as related to formaldehyde.

Few nasal lesions were noted in intact rats exposed at 0.1 or 1 ppm for either 13 or 52 weeks (n = 10/group). Focal squamous metaplasia was noted in a single animal exposed at

1 ppm for 13 weeks. Rats exposed at 10 ppm formaldehyde demonstrated clear pathology in the
respiratory epithelium progressing from 13 to 52 weeks, including squamous metaplasia, basal
cell hyperplasia, and focal rhinitis. Additionally, focal nest-like infolds of the epithelium were
present in 4 of 10 rats at 52 weeks, and minor changes to the olfactory epithelium were noted
(thinning/disarrangement and focal basal cell hyperplasia.)

All rats with damaged nasal passages exhibited similar minor pathology of the respiratory epithelium at 13 and 52 weeks (squamous metaplasia, focal basal cell hyperplasia, and focal rhinitis). Formaldehyde-related effects were note at 52 weeks, where the squamous metaplasia of the respiratory epithelium was no longer noted in controls (versus 13 weeks) but was clearly present in all formaldehyde-treatment groups, including progression from focal to diffuse lesions (at 1 and 10 ppm) and keratinization (3/10 and 4/10 at 0.1 ppm and 10 ppm, respectively). The formaldehyde effects on the respiratory epithelium were much more severe in rats with damaged nasal passages, with all animals demonstrating thinning and disarrangement of the olfactory epithelium and 8 of 10 rats exhibiting "loosely arranged submucosal tissue." Squamous metaplasia and focal rhinitis of the olfactory epithelium were seen in less than half of the formaldehyde-treated rats with damage. No changes in the olfactory epithelium due only to electrocoagulation were encountered.

The most notable effects of nasal damage from electrocoagulation were the ones at the highest formaldehyde exposure (10 ppm) on the olfactory epithelium. Damage to the respiratory epithelium also occurred more posteriorly in rats with damaged noses. Since electrocoagulation often induced damage that included partial or complete loss of turbinates and septal perforation, a likely explanation for the posterior distribution of the damage is an abnormal airflow pattern. This gross damage to the nasal structure may have also disrupted normal mucous production and flow. Therefore, formaldehyde-induced pathology appearing deeper in the nasal passages, including the respiratory epithelium, may be due to formaldehyde penetrating more deeply into the nasal passages and resulting in greater tissue doses in these areas.

Woutersen et al. (1989) conducted a lifetime study in parallel to the 1-year study described above for Appelman et al. (1988). Male Wistar rats with nasal damage induced by electrocoagulation (60/group) or without nasal damage (30/group) were exposed 6 hours/day, 5 days/week to the same concentrations as in the previous study (0, 0.1, 1.0, and 10 ppm) for 28 months or for 3 months followed by a 25-month observation period. The general condition and behavior of the animals were checked daily. Body weight, organ weight, and gross pathology were evaluated as described for Appelman et al. (1988). Histopathologic examination was conducted on all animals at the standard six cross sections (see Figure 4-6).

No remarkable findings on behavior were observed except for yellowing of the fur in animals at the two highest concentrations. There were no relevant differences in mortality (data not shown). Growth retardation was observed relative to controls in animals with or without damaged noses exposed to 10 ppm from day 14 onward. Body weights were generally slightly lower in formaldehyde-exposed animals with an intact nasal mucosa and slightly higher in exposed animals with damaged noses than in the corresponding controls.

The effects of formaldehyde exposure on the respiratory and olfactory epithelium after 28 months of exposure were similar to those reported for 52 weeks exposure (Appelman et al., 1988): rhinitis, squamous metaplasia with some keratinization of the respiratory epithelium, and thinning/disarrangement and slight squamous metaplasia of the olfactory epithelium at the 10 ppm exposure. Effects attenuated from the anterior to posterior sections (I−II, III, IV, and V−VI). A low incidence of olfactory epithelium replaced by respiratory epithelium (<10%) and vacuolation and atrophy of olfactory cells (<10%) was reported, this in part may be due to the larger study size (30 rats per group versus 10). Squamous metaplasia in levels I−II of the respiratory epithelium at 10 ppm was the only treatment-related pathology remaining in rats exposed for 3 months followed by a 25-month recovery period.

Similarly, as reported by Appelman et al. (1988), rats with noses damaged by electrocoagulation did demonstrate increased pathology of the respiratory epithelium. Formaldehyde exposure at 10 ppm exacerbated these changes, and effects were noted in more posterior sections than in rats without nasal damage (levels III, IV, and V). Olfactory pathology was also greater in formaldehyde-treated rats: basal cell hyperplasia, replacement of olfactory epithelium by respiratory epithelium (10−20% at level III and <10% at level IV). Although the incidences are low, there is some evidence that effects on the olfactory epithelium may be increased at the lower formaldehyde exposures (0.1 and 1 ppm.) Analysis of the number of animals with olfactory pathology would be helpful to better understand the potential of low-level formaldehyde effects on these less frequent lesions. Interestingly, the recovery of the olfactory and respiratory epithelium seen in rats with undamaged nasal cavities after a 25-month recovery period was not evident in rats with damaged noses. Formaldehyde-exposure effects are only present at the 10 ppm exposure for the respiratory epithelium (squamous metaplasia, basal cell hyperplasia), and the formaldehyde-related effects on the olfactory epithelium (thinning/disarrangement, basal cell hyperplasia, and replacement by respiratory epithelium) are seen at 0.1 and 1.0 ppm as well.

A single SCC, 1 out of 30 rats, was found in each 28-month formaldehyde-treatment group (1/26, 1/28, and 1/26, respectively) but not in any control animals (n = 52). SCCs were also noted in rats with noses damaged by electrocoagulation (1/54, 1/58, 0/56, and 15/58 for

control rats and the formaldehyde-treatment groups, respectively). These data clearly indicate a synergistic effect of high formaldehyde exposure and nasal damage on the formation of SCCs in rats. One adenosquamous carcinoma and one adenocarcinoma were also reported as increasing the frequency to 17/58 for all tumors. Additionally SCC was present in two rats in the 0.1 and 1 ppm 3-month exposure groups with damaged noses only, although only one SCC was reported in the 10 ppm 3-month groups with and without damaged noses. Rats not surviving to 28 months are included in these results, as well as the histopathology reported above. Since no mortality data are reported, it should be noted that the incidence of both nasal lesions and tumors are not controlled for early deaths.

In total, 30 tumors were examined from this study. In general, the tumors (26/30 or 87%) were SCCs, and 69% (18/26) of these clearly originated from the respiratory epithelium lining the septum or nasal turbinates. The eight other SCCs, derived from the epithelium lining the nasolacrimal duct, were seen in connection with severe odontodystrophy and periodontitis or might have originated from the skin or salivary glands. Four remaining rats bearing a nasal tumor developed a small polypoid adenoma located on the nasoturbinate, an adenocarcinoma originating from the olfactory epithelium, an adenosquamous carcinoma of the respiratory epithelium lining the septum or turbinates, or a carcinoma in situ of epithelium in the nasolacrimal duct.

4.2.2.5.3. ***Hamsters.***

Dalbey (1982) examined the effects of inhaled formaldehyde alone for a lifetime or combined with diethylnitrosamine (DEN) in an initiation-promotion study design using male Syrian golden hamsters. For the first experiment, hamsters were exposed at either 0 or 10 ppm (0 or 12.3 mg/m^3) formaldehyde in whole body chambers 5 hours/day, 5 days/week for a lifetime (132 controls, 88 exposed). Histopathologic evaluations were carried out on two transverse sections of the nasal turbinates (otherwise not specified), longitudinal sections of larynx and trachea, and all lung lobes cut along the bronchus prior to embedding. In the formaldehyde-only (10 ppm) experiment, mortality was increased relative to unexposed controls ($p < 0.05$). No tumors and little evidence of toxicity to the nasal epithelium were observed. There was no increase in rhinitis. Epithelial hyperplasia and metaplasia were increased in formaldehyde-treated animals (5% incidence) versus none observed in controls.

The second set of experiments by Dalbey (1982) examined interaction of formaldehyde exposure on tumor formation from DEN administered subcutaneously. The five treatment groups included: (1) controls ($n = 50$); (2) formaldehyde only ($n = 50$); (3) DEN 0.5 mg, once per week for 10 weeks ($n = 100$); (4) formaldehyde exposure for life with DEN injection for the

first 10 weeks given 48 hours after formaldehyde exposure (n = 27); and (5) DEN injection for 10 weeks, followed by formaldehyde exposure for life (n = 23). In all groups hamsters were exposed at 30 ppm formaldehyde 5 hours/day, once a week. Histopathologic examinations were conducted as above.

Although weekly exposures to formaldehyde alone (30 ppm once a week) did not influence mortality, treatment with DEN alone significantly ($p < 0.05$) increased mortality above that of untreated controls, and mortality was further elevated ($p < 0.05$) in the two groups exposed to both DEN and formaldehyde compared with DEN alone. No respiratory tract tumors were observed in untreated animals or those receiving only formaldehyde. DEN treatment alone resulted in a high incidence (77%) of tumors (nasal, larynx, trachea, and lung). Formaldehyde pre- or post-treatment did not further increase the number of TBAs-. All tumors observed were classified as adenomas. Formaldehyde pretreatment nearly doubled the number of tumors per animal in the trachea (but not lung or larynx) ($p < 0.05$). This increase in tumors initiated by DEN given 48 hours after formaldehyde exposure suggests a role of formaldehyde- induced changes in the respiratory tract in tumor promotion (e.g., cell proliferation and inflammation).

4.2.2.5.4. ***Summary.***

Chronic rodent studies of inhalation exposure to formaldehyde provide a consistent picture of the agent's toxicity—especially on the URT—on which most studies focus. All three species tested—hamsters, mice, and rats—had some degree of hyperplastic and metaplastic change in the nasal passages. The pathology defined in acute and subchronic exposures is similarly described in chronic studies, where progression, severity, and presence in more posterior sections of the nose increase with both the concentration and duration of exposure.

Pathology of the respiratory epithelium includes rhinitis, goblet cell hyperplasia, pseudoepithelial cell hyperplasia, squamous metaplasia, and dysplasia (see Table 4-42). At higher exposures and longer durations of exposure, similar effects are seen on the olfactory epithelium, present further into the nasal passages. In addition to hyperplasia and squamous metaplasia, thinning and disarrangement of the olfactory epithelium noted and, in a few cases, cell damage and replacement of olfactory epithelium with respiratory epithelium appear, including loss of sensory cells (Woutersen et al., 1989; Kerns et al., 1983; Battelle Columbus Laboratories, 1981).

Table 4-42. Summary of respiratory tract pathology from chronic inhalation exposures to formaldehyde

Species/strain	No./ group	Treatment[a]	Respiratory effects	Noncancer LOAEL/NOAEL	Reference
Chronic bioassays					
Mice					
C3H mice (sex unstated)	60	0, 41, 82, or 163 ppm 1 hour/day, 3 days/week for up to 35 weeks. Low- and mid-group mice then exposed at either 122 or 244 ppm during weeks 35−70.	**Pathology:** Histologic changes in the tracheobronchial epithelium by exposure, including basal-cell hyperplasia, stratification squamous cell metaplasia, and atypical metaplasia. **Carcinogenicity:** No SCC formation was evident in mice exposed to formaldehyde alone.	LOAEL = 41 ppm No evidence of carcinogenicity	Horton et al. (1963)
Male and female B6C3F1 mice	120/sex	0, 2, 5.6, or 14.3 ppm 6 hours/day, 5 days/week for 24 months. The protocol featured a 6-month recovery period. Interim sacrifices occurred at 6, 12, 18, 24, and 30 months.	**Pathology:** Rhinitis; hyperplasia, dysplasia, and squamous metaplasia of the nasal epithelium; atrophy of the olfactory epithelium; glandular adenitis and nasolacrimal duct hyperplasia and metaplasia. **Carcinogenicity:** Nasal SCC in male mice at 24 months (2/17). No SCC in female mice.	LOAEL = 2 ppm Evidence of carcinogenicity	Swenberg et al. (1980); Kerns et al. (1983); CIIT (1982) ; Battelle Columbus Laboratories (1981)
Rats					
Female Sprague-Dawley rats	16	0 or 12.4 ppm formaldehyde ± wood dust 6 hours/day, 5 days/week for 104 weeks.	**Pathology:** Squamous metaplasia and dysplasia. **Carcinogenicity:** One of 16 rats exposed to formaldehyde alone developed SCCs.	LOAEL = 12.4 ppm Support for carcinogenicity	Holmström et al. (1989a)
Male and female F344 rats	32/sex	0, 0.3, 2, or 14 ppm 6 hours/day, 5 days/week for 28 months.	**Pathology:** Increased rhinitis, hyperplasia, and squamous metaplasia of the nasal respiratory epithelium **Carcinogenicity:** Nasal SCCs in high concentration rats (44%).	LOAEL = 0.3 ppm Support for carcinogenicity	Tobe et al. (1985)

Table 4-42. Summary of respiratory tract pathology from chronic inhalation exposures to formaldehyde (continued)

Species/strain	No./ group	Treatment[a]	Respiratory effects	Noncancer LOAEL/NOAEL	Reference
Male F344 rats	32	0, 0.3, 2, or 15 ppm 6 hours/day, 5 days/week for 28 months.	**Pathology:** Squamous cell metaplasia and epithelial hyperplasia. **Carcinogenicity:** SCC (13/32), squamous cell papilloma (3/32), and sarcoma (1/32).	LOAEL = 0.3 ppm BMD_{10} = 0.24 ppm Evidence of carcinogenicity	Kamata et al. (1997)
Male Sprague-Dawley rats	100	0 or 15 ppm 6 hours/day, 5 days/week for life.	**Pathology:** Squamous metaplasia, epithelial hyperplasia, and polyps/papillomas. **Carcinogenicity:** SCCs formed in the nasomaxillary turbinates and nasal septum (25%).	LOAEL = 15 ppm Evidence of carcinogenicity	Albert et al. (1982); Sellakumar et al. (1985)
Male and female F344 rats	120/sex	0, 2, 5.6, or 14.3 ppm 6 hours/day, 5 days/week for 24 months. The protocol featured a 6-month recovery period. Interim sacrifices occurred at 6, 12, 18, 24, and 30 months.	**Pathology:** Lesions of the nasal cavity were the primary effects, including squamous metaplasia and epithelial dysplasia, hyperkeratosis, goblet cell hyperplasia, and rhinitis. Salivary gland: atrophy, squamous metaplasia, and sialadenitis. **Carcinogenicity:** SCCs were evident in the nasal cavity of high concentration rats, plus some polypoid adenomas.	LOAEL = 2 ppm Evidence of carcinogenicity	Swenberg et al. (1980); Kerns et al. (1983); CIIT (1982); Battelle Columbus Laboratories (1981); Morgan et al. (1986b)
Male F344 rats	90 and 150 controls	0, 0.7, 2, 6, 10, or 15 ppm 6 hours/day, 5 days/week for 24 months.	**Pathology:** Olfactory degeneration, squamous metaplasia, epithelial hypertrophy and hyperplasia, and mixed inflammatory cell infiltrate. **Carcinogenicity:** SCCs and polypoid adenomas in the nasal cavity	LOAEL = 2 ppm Evidence of carcinogenicity	Monticello et al. (1996)

Table 4-42. Summary of respiratory tract pathology from chronic inhalation exposures to formaldehyde (continued)

Species/strain	No./ group	Treatment[a]	Respiratory effects	Noncancer LOAEL/NOAEL	Reference
Male SPF Wistar rats	10	0, 0.1, 1, or 10 ppm 6 hours/day, 5 days/week for 13 or 52 weeks. An electrocoagulation method was applied to damage the noses of ½ of each study group.	**Pathology:** Formaldehyde-induced focal changes to the respiratory and olfactory epithelium, including rhinitis, hyperplasia, and metaplasia (10 ppm). In rats with damaged noses: squamous metaplasia of the respiratory epithelium increased at all formaldehyde exposures. Pathology of the olfactory epithelium increased at the 10 ppm exposure. **Carcinogenicity:** No tumors noted; 1-year study	LOAEL = 0.1 ppm in rats with damaged nasal passages NOAEL = 1 ppm for rats with intact noses	Appelman et al. (1988)
Male Wistar rats	30 (without nasal damage), 60 (with nasal damage)	0, 0.1, 1, and 10 ppm 6 hours/day, 5 days/week for 28 months or for 3 months with a 25-month observation period. An electrocoagulation method was applied to damage the nasal cavity.	**Pathology:** Intact noses: squamous metaplasia in the high concentration group exposed for 28 months and degeneration of the olfactory epithelium. Changes were more severe in animals with damaged noses . **Carcinogenicity:** SCCs developed in 15/60 rats with damaged noses exposed at 10 ppm. In other groups, the incidence of nasal tumors was low irrespective of the state of nasal damage.	NOAEL = 1 ppm Evidence of carcinogenicity	Woutersen et al. (1989)
Hamsters					
Male Syrian golden hamsters	88 treated 132 controls.	0 or 10 ppm formaldehyde 5 hours/day, 5 days/week for life.	**Pathology:** Increased mortality. Epithelial hyperplasia and metaplasia increased in formaldehyde-treated animals (5% incidence) **Carcinogenicity:** No tumors reported.	LOAEL = 10 ppm No evidence of carcinogenicity	Dalbey (1982)

Table 4-42. Summary of respiratory tract pathology from chronic inhalation exposures to formaldehyde (continued)

Species/strain	No./ group	Treatment[a]	Respiratory effects	Noncancer LOAEL/NOAEL	Reference
Male Syrian golden hamsters	50	0 or 30 ppm 5 hours/day, 1 day/week for life ± injections with 0.5 mg DEN.	**Pathology:** Increased mortality in conjunction with DEN—above DEN-only treated animals. Respiratory pathology not reported. **Carcinogenicity:** Only hamsters receiving DEN developed tumors (77%, adenomas). There was an increase in the number of tumors per TBAs in the trachea of animals exposed to formaldehyde 48 hours prior to DEN (but no increase in TBAs).	LOAEL = 30 ppm. Evidence for formaldehyde as a promoter	Dalbey (1982)

Clear species differences in the severity of lesions are present. Although the bioassays in mice, hamsters, and rats do represent similar exposure concentrations and duration of exposure, hamsters exhibit little pathology and rats (three strains tested) exhibit gross toxicity and even increased mortality. Mice similarly exposed exhibit a range of effects on the respiratory epithelium but not near the severity seen in rats. Many factors may contribute to these observed species differences. As Chang and Barrow (1983) reported, the increased RB of mice seems to be protective of POE damage in comparison to that of rats. The reduced ventilation rate and minute volume of rodents in the presence of a reactive gas can reduce the effective delivered dose at the same exposure concentration (Chang and Barrow, 1983). Additionally, as illustrated in the computational fluid dynamic (CFD) modeling (see Section 3.5), there are species differences in nasal architecture that influence areas of formaldehyde absorption or flux into the tissue. Localized differences in mucus flow and production as well as metabolic enzymes have also been posited as having roles in differential toxicity of formaldehyde on the URT (see Chapter 3).

Formaldehyde-induced tumors were present in exposed rats and mice and primarily involved SCCs later in life (Kamata et al., 1997; Tobe et al., 1985; Kerns et al., 1983; Swenberg et al., 1980). Although exposure of male Syrian hamsters to either 10 or 30 ppm did not result in formaldehyde-induced nasal tumors, a classic initiation-promotion assay with DEN-induced tumor formation did indicate that formaldehyde increased the tumor burden per animal, where DEN induced tumors in 77% of the animals (Dalbey, 1982). This study suggests a role for promotion in the observed carcinogenicity of formaldehyde. Less clear are the implications of the synergistic effect of formaldehyde exposures and gross damage to the respiratory epithelium by electrocoagulation on tumor formation (Woutersen et al., 1989).

4.2.2.6. *Summary of Respiratory Pathology and Carcinogenic Potential*

The progressive pathology of the nasal passages from inhalation exposure to formaldehyde is well documented, especially in rodents (rats and mice) (see Tables 4-12, 4-23, 4-36, 4-42). Although there are species differences in tissue dose (see Section 3.4) due to variations in nasal architecture and breathing patterns, the nature and progression of the pathology is fairly well conserved across species, including nonhuman primates. The observed formaldehyde-induced pathology includes disruption of the mucociliary apparatus, rhinitis (**s**erous and purulent), hyperplasia (cell proliferation), metaplasia (transition of cell type), dysplasia (disarrangement of cells), nest-like infolds and invaginations of the epithelium, thinning of the epithelial layer and focal to diffuse lesions, atrophy of the olfactory epithelium,

thickening and keratinization (usually of squamous metaplasia), tumors (adenoma, sarcoma, carcinoma) (see Section 4.2.2).

Progression of lesions can be viewed as progression from the anterior to posterior sections of the nasal cavity or as a progression in severity of lesions at a particular location (e.g., level or region) of the nasal passages. In both cases, progression is evident with increasing exposure concentration and with increasing duration of exposure (Kamata et al., 1997; Monticello et al., 1996; Morgan et al., 1986b; Takahashi et al., 1986; Sellakumar et al., 1985; Kerns et al., 1983; Albert et al., 1982). The data suggest that concentration and duration of exposure do not act in a simply cumulative manner (e.g., C × t). Additionally the influence of concentration, duration, and repeated exposure may be different for various effects. For example, some lesions may be transient (e.g., low-exposure cell proliferation), others may have a threshold and vary little after that (e.g., rhinitis). Additionally, as the nasal epithelium responds with both adaptive and adverse epithelial changes, the absorption of formaldehyde into the tissue at that location may be reduced. As respiratory epithelium transitions to squamous metaplasia, the effective tissue dose of formaldehyde increases posterior to these lesions. As barriers to formaldehyde flux into the tissue develop (e.g., squamous metaplasia, keratinization), formaldehyde penetrates more deeply into the nasal passages (Kimbell et al., 2006). Therefore, although both concentration and duration of exposure do affect the adverse effect, the relationship is difficult to define and in fact may be different for various adverse effects.

Respiratory histopathology has been commonly reported in response to exposure to formaldehyde in rats and mice (Lino dos Santos Franco et al., 2006; Javden and Taher, 2000; Kamata et al., 1996a, b; Cassee and Feron, 1994; Bhalla et al., 1991; Monteiro-Riviere and Popp, 1986; Buckley et al., 1984; Chang et al., 1983), rabbits (Ionescu et al., 1978), hamsters (Schreibner et al., 1979), and rhesus monkeys (Monticello et al., 1989). The histopathologic lesions ranged from inflammation to ulceration, necrosis, and metaplasia that occurred in nasal turbinates, maxilloturbinates, and goblet and microvillus cells (Bhalla et al., 1991). These effects were observed at a variety of doses (e.g., 10 ppm for 4 hours, 3.13 ppm for 6 hours for 1, 2, or 4 days, 6 or 15 ppm). Wilmer et al. (1989, 1987) assessed whether a dose and time-dependent interaction (C × t) is associated with histopathologic lesions. Results indicated that concentration, rather than duration or cumulative exposure, correlates best with severity of lesions (Wilmer et al., 1989, 1987).

Histopathologic lesions and changes to biochemistry have been reported in the lung as well, though these effects were observed following a high dose of formaldehyde. In addition, changes in clinical chemistry, P450 expression and activity in lung tissue, and gene expression that is phenotypically anchored to the observed respiratory pathology have been reported.

Extrapulmonary effects have also been noted, including changes in liver chemistry, relative brain weight, and focal, chronic inflammation in the heart and kidney. Most of these changes occurred at exposures of 20 ppm, and those that occurred at lower formaldehyde exposures (3.7 ppm) could not be strictly correlated with formaldehyde exposure.

Some researchers have reported formaldehyde-induced effects in the pulmonary region in rats, mice, and rabbits. Kamata et al. (1996a) observed reduced lipid content of pulmonary surfactant in rats exposed to 128.4 or 294.5 ppm formaldehyde. Kamata et al. (1996b) reported biochemical changes in lung homogenates and altered lipid content of BAL at 145.6 ppm formaldehyde. Lino dos Santos Franco et al. (2006) observed increased leukocytes (and neutrophils) and degranulated mast cells recovered in BAL fluid (concentration of 1% formalin not provided). In rabbits, Ionescu et al. (1978) observed frank necrosis of lung parenchyma after aerosol inhalation of 3% formalin for 3 hours/day for 50 days (concentration of formaldehyde not provided). These pulmonary effects may be due to frank toxicity resulting from the high dose of formaldehyde used in these studies.

Several recent toxicogenomics studies have assessed gene expression changes in nasal and lung tissue in animals and in humans by using in vivo and in vitro approaches. Hester et al. (2005, 2003) documented changes in gene expression associated with DNA repair and apoptosis in nasal tissue from male rats after a single instillation of formaldehyde. Other gene expression changes were observed in those genes related to xenobiotic metabolism and in cell cycle and repair. These preliminary results provide an initial basis for forming a phenotypically anchored set of gene expression changes associated with exposure to formaldehyde and may assist in determining the underlying MOA, as will be discussed in Section 4.5. Sul et al. (2007) investigated gene expression genes in lung tissue from formaldehyde-exposed rats. Yang et al. (2005) performed a proteomics analysis by using lung tissue extracted from formaldehyde-exposed rats. Two studies used human tracheal cell lines to investigate formaldehyde-induced gene expression changes in vitro (Lee et al., 2008, 2007). However, the relevance of these findings to actual exposures remains unknown. In total, toxicogenomics studies hold promise, but they must be interpreted with caution until results can be replicated and phenotypically linked to observable changes.

Thus, formaldehyde-induced respiratory pathology has been commonly described in the nasal passages and includes cellular proliferation, mucociliary function, and histopathologic lesions. Pulmonary effects have been documented as well but at high doses. The nasal pathology may occur as a result of both concentration and duration components of exposure.

4.2.2.6.1. ***Carcinogenic potential.***

In the respiratory tract, only nasal tumors are considered formaldehyde induced in rodent studies. The majority of studies were conducted using rats (F344, Wistar, or Sprague-Dawley), and all studies of 18 months or greater in mice and rats show evidence of formaldehyde-induced nasal carcinogenicity. The nasal tumors are primarily SCCs, although papillomas, polypoid adenoma, adenocarcinoma, fibrosarcoma, and esthesioneuroepithelioma have been reported (Kamata et al., 1997; Monticello et al., 1996; Morgan et al., 1986a, b; Takahashi et al., 1986; Sellakumar et al., 1985; Kerns et al., 1983; Albert et al., 1982). Although hyperplasia, dysplasia, and squamous metaplasia of the respiratory epithelium have been observed beyond the nasal cavity, other respiratory tract tumors have not been significantly increased by formaldehyde exposure alone.

Increased tumor incidence and decreased latency are correlated with increasing formaldehyde exposure concentration. Reviewing data from the only lifelong inhalation study with multiple exposure groups, SCC is first noted at 8 and 9 months for high exposed (15 ppm) female and male F344 rats autopsied as "early deaths" prior to the 12 month sacrifice, with an incidence of 43% over the course of the study (unadjusted for mortality) (Kerns et al., 1983). In contrast only two SCCs were found in male and female rats sacrificed after 24 months of exposure (incidence of SCC 2.5% at 24 months) (Kerns et al., 1983). In a follow-up study by Monticello et al. (1996), the incidence of SCC in rats exposed at 15 ppm was 47% with the first tumor noted at 12 months. The incidence of SCC in male rats exposed at 10 ppm was 22% with the first SCC noted at 18 months. Moreover, of 90 rats exposed at 6 ppm for 20 months only one SCC was noted. No SCCs were detected in rats exposed at 0.7 or 2 ppm formaldehyde. These incidence rates are not mortality adjusted and include animals from each scheduled sacrifice (3, 6, 12, and 18 months). In a lifelong study of male Sprague-Dawley rats exposed at 15 ppm formaldehyde, the cumulative nasal tumor incidence was calculated as a function of time of exposure (see Figure 4-14) (Sellakumar et al., 1985). After 2 years of exposure, the probability of nasal carcinoma was greater than 60%.

There is some evidence that less-than-lifetime exposure to formaldehyde can induce nasal tumors over an extended observation period. Two studies, both in male Wistar rats, report nasal tumors in response to less-than-lifetime exposures (Woutersen et al., 1989; Feron et al., 1988). A 13-week exposure at 20 ppm resulted in four nasal tumors (three SCCs), a cystic SCC of the nasolacrimal duct, and an epithelial tumor on the mandible, for a total of six tumors observed over 30 months of observation (Feron et al., 1988). No tumors were noted in 13-week controls. A limited number of formaldehyde-related tumors were noted due to 4 or 8 weeks of exposure followed by 30 months of observation. Although the tumor incidence of these less-than-lifetime

exposures is low, this is consistent with the 2-year bioassays in Wistar rats. Wistar rats are more resilient to formaldehyde-induced nasal toxicity than F344 or SD rats (see Section 4.2.1), and only 1 of 26 (4%) Wistar rats exposed at 10 ppm for 28 months developed SCC (Woutersen et al., 1989) versus 22% in F344 rats (Monticello et al., 1996).

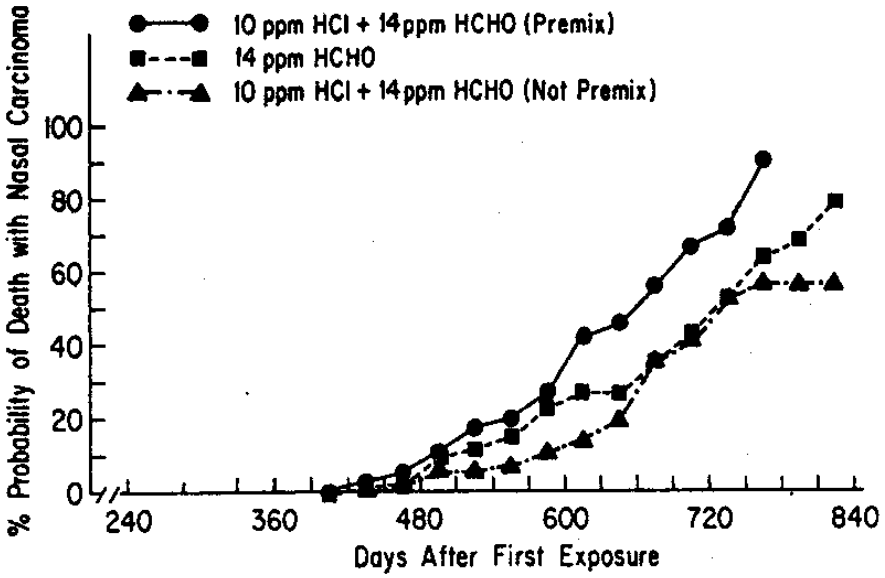

Figure 4-14. Mortality corrected cumulative incidences of nasal carcinomas in the indicated exposure groups.

Source: Sellakumar et al. (1985).

Woutersen et al. (1989) also examined the effect of severe nasal damage from electrocoagulation on formaldehyde-induced SCC in Wistar rats. Nasal tumors were noted in formaldehyde-exposed rats without damaged noses (exposed for only 3 months and observed for 25 months). However, the low incidence of tumors in each treatment group (1/26, 2/60, 2/60, 1/58) indicates these data should be considered suggestive even though no SCCs were noted in control rats with or without damaged noses (n = 83). The studies by Woutersen et al. (1989) did demonstrate a synergistic effect of nasal damage from electrocoagulation and 10 ppm formaldehyde exposure (3 months), where 15/58 rats had SCC versus 1/26 with undamaged noses. The study was originally designed to examine the effect of formaldehyde on the damaged tissue on cancer promotion. However, it is unclear if the synergistic effect of formaldehyde exposure on damaged nasal tissue is an effect of formaldehyde on the damaged cells and joint effects of a mutagen with regenerative proliferation from the nasal damage. It is also possible

the damaged nasal passages may alter airflow in the nasal passages, resulting in significantly different flux of formaldehyde into the tissue.

There is a single inhalation study (Dalbey, 1982) that investigates the role of promotion in formaldehyde-induced cancer. Although hamsters exhibit little to no effects of formaldehyde on the nasal mucosa or other respiratory tract tissues (Rusch et al., 1983a, b; Dalbey, 1982), DEN-induced respiratory adenomas were increased with formaldehyde exposure (10 ppm) 48 hours prior to DEN injection (but not by formaldehyde alone or formaldehyde exposure after DEN injection). The number of tracheal tumors per TBA was doubled by formaldehyde exposure. The study authors note that adenomas should be considered independent tumors and that the increase in tracheal tumors is of biological significance even given the incidence of TBAs (77%, DEN alone), was not further increased by formaldehyde exposure. It is of particular interest that a promotion study in hamsters is positive, since so little nasal pathology occurs with formaldehyde exposure. The absence of significant hyperplasia and tissue damage in these animals suggests that formaldehyde may induce subtle changes in the respiratory tract mucosa that permit formaldehyde to act as a tumor promoter.

4.2.3. Gastrointestinal Tract Pathology

As with inhalation, the POE is thought to be the principal target tissue in response to oral exposure. A concentration-dependent pattern of toxicity longitudinally down the GI tract has been observed upon oral exposure. Some evidence (Til et al., 1989, 1988) suggests that, with regard to oral exposure, duration in addition to concentration is important in the development of toxicity.

Formalin and paraformaldehyde were used to dose animals in oral toxicity studies. Formalin contains 12−15% methanol as a preservative to inhibit the polymerization of formaldehyde and subsequent precipitation as paraformaldehyde (Kiernan, 2000). The presence of methanol in formalin may confound the results of a formaldehyde study. Methanol has been shown to be a developmental and neurologic toxin (e.g., Degitz et al. [2004a, b]; Rogers et al. [2004, 2002]; Weiss et al. [1996]; Sharpe et al. [1982]). Oral dosing with paraformaldehyde is preferred because it allows for the preparation of methanol-free formaldehyde in the laboratory by dissolving paraformaldehyde in slightly basic water.

4.2.3.1. ***Short-Term and Subchronic Ingestion Studies***

Til et al. (1988) evaluated the oral toxicity of formaldehyde and acetaldehyde in a subacute study in Wistar (Cpb:WU; Wistar random) rats. Groups of rats (10/sex/dose) were exposed to paraformaldehyde dissolved in drinking water at 0, 5, 25, and 125 mg/kg-day for 4

weeks. The control group was comprised of 20 rats of each sex. To account for potential effects of decreased water consumption in treated animals, an additional control group of 10 male and 10 female rats was given drinking water in an amount equal to the amount of liquid consumed by the group given the highest dose. Examination of the GI tract was performed in all dose groups and included the tongue, esophagus, and stomach. Histopathology for the other tissues was performed on high-dose and control animals.

The rats appeared to be healthy throughout the study, and no effects on growth occurred despite significant decreases in food and water intake that occurred at the high dose (125 mg/kg-day). Yellow discoloration of the fur occurred in the rats on the high dosage from week 3 onward. There were no significant changes in hematology among the exposed groups except for slight (not statistically different) increases in PCVs in the water-restricted group and in high-dose males. The high-dose groups of the formaldehyde exposed and in the water-restricted controls had slightly increased urine density, but again this was not statistically significant. Plasma TP and ALB levels were decreased in the males of the highest dose group. No changes in organ weights occurred except for relative kidney weights that were slightly increased in the females of the high-dose group. Gross pathological findings were restricted to the GI tract and revealed a thickening of the limiting ridge of the forestomach in all animals exposed at the highest dose that was accompanied by a yellowish discoloration of the mucosa. These latter changes were not observed in the acetaldehyde-exposed animals. Treatment-related histopathologic changes were seen in the GI tract only. Slight (8/20) or moderate (12/20) focal hyperkeratosis of the forestomach and slight focal atrophic gastritis occurred in animals of the high-dose groups only (see Table 4-43). One female had moderate focal papillomatous hyperplasia. No histopathologic changes were observed in any animals of the lower-dose groups. The study established a LOAEL and NOAEL for epithelial changes in the GI tract of male and female Wistar rats exposed to formaldehyde in drinking water at 125 mg/kg-day and 25 mg/kg-day, respectively.

Johannsen et al. (1986) performed a subchronic study by using rats exposed to paraformaldehyde dissolved in drinking water and dogs exposed to paraformaldehyde in the diet. Groups of albino Sprague-Dawley rats (15/sex) were administered the equivalent of 0, 50, 100, or 150 mg/kg-day in their drinking water for 91 consecutive days. Pure-bred beagle dogs (four/sex/group) were fed a diet with added aqueous formaldehyde to approximate 0, 50, 75, or 100 mg/kg-day. Dogs were observed daily and rats at frequent intervals for behavioral reactions. Body weights and food and water intake were recorded on a weekly basis in both species. Hematology (HCT, Hb, total and differential leukocyte counts), clinical chemistry (blood sugar, BUN, ALP, AST and ALT in dogs only), and urine analyses (color, appearance, pH, specific

Table 4-43. Summary of lesions observed in the gastrointestinal tracts of Wistar rats after drinking-water exposure to formaldehyde for 4 weeks

Type of lesion	Formaldehyde (mg/kg-day)			
	0	5	25	125
	Number of male rats examined			
	20	10	10	10
Focal hyperkeratosis of forestomach				
Very slight	3	0	0	0
Slight	1	0	0	4
Moderate	0	0	0	6
Focal gastritis				
Slight	0	0	0	2
Moderate	0	0	0	1
Dilated fundic glands (single or a few)	0	0	0	0
Submucosal mononuclear cell infiltrate	0	0	0	1
Type of lesion	**Number of female rats examined**			
	20	**10**	**10**	**10**
Focal hyperkeratosis of forestomach				
Very slight	6	0	0	2
Slight	0	0	0	2
Moderate	0	0	0	6
Focal gastritis				
Very slight	0	0	0	1
Slight	0	0	0	1
Moderate	0	0	0	1
Focal papillomatous hyperplasia	0	0	0	1
Polymorphonuclear leukocytic infiltration	0	0	0	1

Source: Til et al. (1988).

gravity, sugar, protein, and microscopic elements) were evaluated in 10 male and 10 female rats selected from each test group and in all dogs. Organ weights were recorded for the adrenals, gonads, hearts, kidneys, livers, lungs, and thyroids in each species. Histopathology was performed on a set of over 20 or 30 tissues and organs from rats or dogs, respectively, in the high-dose and control groups only.

No deaths or abnormal reactions were observed in either species. Significant reductions in weight gain were observed in dogs of both sexes at 100 mg/kg-day, in rats of both sexes at 150 mg/kg-day, and in male rats at 100 mg/kg-day of formaldehyde. There was a dose-related decrease in liquid consumption of both sexes in rats given formaldehyde, but there was no overall difference in mean food intake or feed efficiency, so the reductions in body weight gain were considered to be systemic effects. Dogs administered formaldehyde had reduced food consumption and feed efficiency at all doses tested. No significant effects on hematology, clinical chemistry, or urine analyses were observed in either species. No effects in either species were reported on organ weights. The GI mucosa in both species was reported to appear normal with no indication of irritation. This study suggests a NOAEL of 150 mg/kg-day in Sprague-Dawley rats and of 100 mg/kg-day in beagle dogs for formaldehyde in drinking water. Differences in the results for the rats with those reported in other studies (Til et al., 1989, 1988; Tobe et al., 1989) may be due to strain differences or duration of the exposure. The dog may be a more sensitive species than the rat based on these results and on those of 2-week pilot studies.

4.2.3.2. *Chronic Ingestion Studies*

The same laboratory that tested formaldehyde and acetaldehyde in a 4-week study (Til et al., 1988) performed a chronic bioassay with formaldehyde in drinking water. Til et al. (1989) administered paraformaldehyde dissolved in drinking water to Wistar rats (Cpb: WU; Wistar random) (70/sex/dose). Interim sacrifices (10/sex/dose) were performed at 12 and 18 months. Formaldehyde was administered in drinking water to provide target doses of 0, 5, 25, and 125 mg/kg-day. The mean formaldehyde doses administered were 0, 1.2, 15, or 82 mg/kg-day for males and 0, 1.8, 21, or 109 mg/kg-day for females. Concentrations were adjusted weekly for the first 12 weeks based on dose estimates derived from body weight and liquid consumption data. Such adjustments were made every 4 weeks from weeks 12 to 52 and kept constant. Fresh solutions of the test concentrations were prepared weekly and stored at 15ºC.

Endpoints examined included daily observations for condition and behavior, body weight at weekly intervals for the first 12 weeks and then every 4 weeks thereafter, liquid intake weekly, and food intake weekly for the first 12 weeks and then every 2 weeks for the remainder of the study. Samples of blood were taken for hematological and clinical chemistry analyses on weeks 26 and 103. Analysis of blood glucose and urine pH, density, and volume was performed on samples at weeks 27, 52, 78, and 104. Pooled urine samples were also evaluated for glucose, occult blood, ketones, urobilinogen, and bilirubin in samples at weeks 27 and 104. Weights of all major organs were recorded at interim sacrifices and at term. Gross and histopathologic examinations were carried out on all major tissues of the rats in the high-dose and control

groups. The livers, lungs, stomach, and noses were examined in all rats. Additionally, the adrenals, kidneys, spleens, testes, thyroids, ovaries, pituitaries, and mammary glands (for females) were examined in all sacrificed animals at weeks 53 and 79 and at term.

The general health and behavior of the rats were not affected in any of the formaldehyde-exposed groups. Slight yellowing of the fur did occur in the animals exposed at the mid and high doses from week 3 onward. The mean body weights were decreased in the males from week 1 and in the females from week 24 onward. At the high dose, liquid consumption was significantly decreased in both sexes, and food intake was significantly decreased in the males. There were no toxicologically significant effects on hematological, urinary, or clinical chemistry parameters. Decreases in absolute heart, liver, and testis (males) weights were attributed to lower body weights. Relative kidney weights were increased in females of the high-dose group, and relative brain weights were increased in both sexes of the high-dose group. Relative testis weight was increased in males. Treatment-related changes in gross pathology were restricted to the forestomach. Histopathologic examinations at the two interim sacrifices and final sacrifice revealed GI tract changes. Renal changes were observed in the high-dose group at final sacrifice. There was no indication of treatment-related effects in other tissues.

As shown in Table 4-44, significant histopathology in the GI tract was limited to the forestomach and stomach of rats in the high-dose groups. Some progression with duration of exposure may have occurred by week 105 because GI lesions were observed in the lower dose groups at this time point, whereas none were observed in these groups at interim sacrifices. The histopathologic changes included papillary epithelial hyperplasia in the forestomach that was frequently accompanied by hyperkeratosis on the limiting ridge or its vicinity. The mucosa showed an irregular layer of hyperplastic basal cells, but no atypical nuclei or other subcellular structures were observed. Chronic atrophic gastritis occurred to varying degrees in the stomachs of all high-dose rats. In some cases the inflammatory process involved the entire mucosa and was seen to extend to the whole muscularis mucosae and met the criteria for ulceration.

Histologic examination also showed that the incidence and degree of renal papillary necrosis was increased in animals of the high-dose groups at the terminal sacrifice. This change was located at the tip of the papilla and was characterized by patchy necrosis of interstitial cells, capillaries, and loops of Henle. There was no evidence of a dose-related response in chronic nephropathy. The incidence of chronic nephropathy was lower in the males of the high-dose group than in controls. In females, the incidence was slightly higher in the test groups than in controls but only achieved statistical significance at the lowest dose. It is likely that the decrease in liquid intake incurred in the high-dose groups contributed to the increased incidence and

Table 4-44. Incidence of lesions observed in the gastrointestinal tracts of Wistar rats after drinking-water exposure to formaldehyde for 2 years

	Incidence of lesions with formaldehyde dose (mg/kg-day)[a]							
	Males				Females			
	0	1.2	15	82	0	1.8	21	109
Week 53								
Number of rats examined[b]	**9**	**10**	**10**	**10**	**10**	**10**	**10**	**9**
Forestomach								
Focal papillary epithelial hyperplasia	0	0	0	7	0	0	0	5
Glandular stomach								
Chronic atrophic gastritis	0	0	0	10[c]	0	0	0	9[c]
Focal ulceration	0	0	0	3	0	0	0	1
Focal mononuclear cell infiltrate	1	0	3	0	2	0	0	0
Atypical glandular hyperplasia	0	0	0	0	0	0	0	1
Week 79								
Number of rats examined	**10**	**10**	**10**	**10**	**10**	**9**	**10**	**10**
Forestomach								
Focal papillary epithelial hyperplasia	2	1	1	8	1	0	1	9
Glandular stomach								
Chronic atrophic gastritis	0	0	0	10[c]	0	0	0	10[c]
Focal ulceration	0	0	0	2	0	0	0	0
Focal squamous metaplasia	0	0	0	1	0	0	0	0
Submucosal inflammatory cell infiltrate	1	2	0	0	0	0	0	0
Focal mononuclear cell infiltrate	0	1	0	0	1	1	0	0
Glandular dilation	2	4	4	1	2	2	4	0
Week 105								
Number of rats examined[b]	**47**	**45**	**44**	**47**	**48**	**49**	**47**	**48**
Forestomach								
Focal papillary epithelial hyperplasia	1	2	1	45[c]	1	0	2	45[c]
Focal hyperkeratosis	2	6	4	24[c]	3	5	3	33[c]
Focal ulceration	1	1	1	8	0	0	2	5
Focal acanthosis	1	0	2	1	0	0	0	1
Focal basic cell hyperplasia	0	1	1	0	1	0	0	0
Diverticulum	0	0	1	0	0	0	0	0
Exophytic papilloma	0	1	0	0	1	0	0	0
Glandular stomach								
Chronic atrophic gastritis	0	0	0	46[c]	0	0	0	48[c]
Focal ulceration	0	0	0	11[c]	0	0	0	10[c]
Glandular hyperplasia	0	1	0	20[c]	0	0	0	13[c]
Mineralization	3	2	1	0	0	0	0	0
Focal inflammatory cell infiltrate	5	3	2	0	2	3	1	0

[a]Incidence in rats that died or were killed when moribund during the experiment or were killed at week 53, 79, or 105.
[b]A few rats were lost because of advanced autolysis.
[c]The values differ significantly (Fisher's exact test) from the control value ($p < 0.001$).
Source: Til et al. (1989).

degree of renal papillary necrosis observed in the high-dose animals because dehydration has been shown to enhance its production by various analgesics.

The results of this chronic bioassay indicated that formaldehyde is cytotoxic to the epithelial mucosa of the nonglandular (forestomach) and glandular stomach with a LOAEL of 82 and 109 mg/kg-day and a NOAEL of 15 and 21 mg/kg-day in males and females, respectively. The findings provided no evidence of carcinogenicity in either the GI tract or systemic sites for formaldehyde administered in drinking water to Wistar rats at doses as high as 82 mg/kg-day.

Tobe et al. (1989) performed a chronic toxicity study of Wistar rats (Slc:Wistar) exposed to paraformaldehyde dissolved in drinking water. Groups of 20 male and 20 female rats were given formaldehyde solution in their drinking water at concentrations of 0, 0.02, 0.10, and 0.50% for 24 months. Interim sacrifices of six randomly chosen rats from each group were performed after 12 and 18 months. Based on the estimated average amount of water intake and body weight, the actual doses of formaldehyde in either sex were reported to be 0, 10, 50, and 300 mg/kg-day. Fresh test solutions were prepared twice each week. The rats were observed daily for the entire study. Body weights and water and diet intake were measured once weekly or biweekly. Hematology (RBC, WBC, and Hb) and serum clinical chemistry (TP, ALB, BUN, uric acid, total cholesterol, inorganic phosphorous, ALP, AST, and ALT) were made at each necropsy. Organ weights were measured for the brain, heart, lung, liver, kidney, spleen, adrenal, testis or ovary, pituitary, and thyroid. These organs and the stomach, small and large intestine, pancreas, uterus, lymph nodes, and all tumors were examined histopathologically.

The general condition of animals in the high-dose group was poor with significantly reduced body weight gain as well as intake of water and diet. An increase in mortality was also observed in this group. Some clinical chemistry parameters were altered in this group. No significant changes in absolute or relative organ weights were observed. Mortality was 100% in the high-dose group by 24 months. At the 12-month sacrifice, hyperplasia of the squamous epithelium with or without hyperkeratosis was observed in the forestomach of all high-dose animals (12/12). Basal cell hyperplasia with growth into the submucosa was also observed in most cases (10/12). Erosions and/or ulcers with submucosal inflammatory cell infiltrates were observed in the glandular stomach of most rats (10/12). Regenerative changes of the glandular epithelium (glandular hyperplasia) were noticed in most cases (10/12) along the limiting ridge of the fundic mucosa. No lesions were observed in the glandular stomach at the 50 mg/kg-day dose, and forestomach hyperplasia was observed in only one of six males and in one of eight females at 18 and 24 months. No lesions in either the forestomach or glandular stomach were observed in rats treated at 10 mg/kg-day.

This study corroborates the Til et al. (1989) study and shows that the main targets for formaldehyde toxicity administered by drinking water to rats are the forestomach and glandular stomach. Although the lesions observed at the 50 mg/kg-day were minimal in this study, Tobe et al. (1989) designated the NOAEL at 10 mg/kg-day, further supporting the NOAEL of 15 mg/kg-day from the Til et al. (1989) study.

Takahashi et al. (1986) studied the effects of formaldehyde in an initiation-promotion model of stomach carcinogenesis in male outbred Wistar rats (Shizuoka Laboratory Center, Shizuoka). Rats (n = 17) were given 100 mg/L of *N*-methyl-*N*¹-nitro-*N*-nitrosoguanidine (MNNG) in drinking water and a diet supplemented with 10% sodium chloride (NaCl) for the first 8 weeks as an initiation phase. This was followed by 0.5% formalin (which contains 12−15% methanol) in drinking water for 32 weeks as the promotion phase of the protocol. A comparison group (n = 10) was given stock water and diet without any supplementation for the first 8 weeks followed by 0.5% formalin in drinking water for 32 weeks. Animals were observed daily and weighed once every 4 weeks. Small pieces of the stomach and other tissues in the peritoneal cavity were fixed for histopathologic examination.

Body weight gain was reduced by exposure to MNNG with sodium chloride, and formaldehyde exposure during the promotion phase exacerbated this effect. Histopathologic investigations were restricted to the GI tract. Formaldehyde was shown to statistically increase the incidence of lesions in the forestomach and stomach in the animals initiated with MNNG with NaCl as compared with controls receiving no initiation (see Table 4-45). Increases in papilloma in the forestomach, adenomatous hyperplasia in the fundus, and adenocarcinoma in the pylorus were observed. Histopathology in the animals receiving formaldehyde alone during weeks 9 through 32 showed an increase in forestomach papillomas but with no lesions in the glandular stomach (see Table 4-45). The adenomatous hyperplasia were defined as proliferative, noninvasive mucosal lesions, and the adenocarcinomas were defined as well differentiated and composed of typical glandular structures, demonstrating a tubular pattern and cellular or structural atypism without metastasis. No definition of criteria for papilloma diagnosis was provided. The findings in this study are inconsistent with those of Til et al. (1989), who found no evidence of carcinogenicity in a 2-year bioassay at comparable concentrations (assuming 37% formaldehyde in formalin results in 0.19% formaldehyde in this study). As discussed above, the differences may be due to differences in the strains of rat or in the diagnostic criteria. The lack of more than one test concentration precludes dose-response analysis of this study and provides only a stand-alone LOAEL of 0.2% formaldehyde in drinking water. The lack of consumption data precludes an estimation of dose in mg/kg-day.

Table 4-45. Effect of formaldehyde on gastroduodenal carcinogenesis initiated by MNNG and NaCl in male Wistar rats exposed to formaldehyde (0.5% formalin) in drinking water for 8 weeks

No MNNG initiation prior to 8-week oral exposure to formaldehyde (0.5% formalin in drinking water)							
			Glandular stomach tumors				
			Fundus		Pylorus		Duodenum
	Gastroduodenal carcinoma	Forestomach papillomas	Adenocarcinoma	Adenomatous hyperplasia	Adenocarcinoma	Preneoplastic hyperplasia	Adenocarcinoma
Control	0%	0%	0%	0%	0%	0%	0%
Formaldehyde	0%	80%[a]	0%	0%	0%	0%	0%
MNNG initiation (100 mg/L in drinking water for 8 weeks) prior to 8-week oral exposure to formaldehyde (0.5% formalin in drinking water)							
			Glandular stomach tumors				
			Fundus		Pylorus		Duodenum
	Gastroduodenal carcinoma	Forestomach papillomas	Adenocarcinoma	Adenomatous hyperplasia	Adenocarcinoma	Preneoplastic hyperplasia	Adenocarcinoma
Control	13.3%	0%	0%	0%	3.3%	23.3%	10.0%
Formaldehyde	29.4%	88.2%[a]	0%	88.2%[a]	23.5%[b]	41.2%	5.9%

[a]Significantly different from control animals with MNNG initiation, $p < 0.01$.
[b]Significantly different from control animals with MNNG initiation, $p < 0.05$.

Source: Takahashi et al. (1986).

4.2.3.3. *Summary of Gastrointestinal Effects and Evaluation of Carcinogenic Potential*

Short-term and subchronic exposures to formaldehyde via drinking water for 4 weeks yielded slight to moderate histopathologic lesions (focal hyperkeratosis) at 125 mg/kg-day in male and female Wistar rats, as well as slight focal gastritis and submucosal infiltrate in one to two animals of both sexes (Til et al., 1988). No histopathologic lesions were noted in albino Sprague-Dawley rats or beagle dogs that received oral doses of formaldehyde in drinking water for 91 days (Johannsen et al., 1986). In both studies, decreases in weight gain were noted in exposed animals compared with controls.

As with the respiratory tract, the proximal portion of the GI tract exhibits formaldehyde-induced lesions in the forestomach and glandular stomach (Til et al., 1989; Tobe et al., 1989; Takahashi et al., 1986). In a chronic drinking water study, Til et al. (1989) reported that formaldehyde is cytotoxic to the epithelial mucosa of the nonglandular (forestomach) and glandular stomach with a LOAEL of 82 and 109 mg/kg-day and a NOAEL of 15 and 21 mg/kg-day in males and female Wistar rats, respectively. The findings provided no evidence of carcinogenicity in either the GI tract or systemic sites for formaldehyde administered in drinking water to Wistar rats at doses as high as 82 mg/kg-day. The incidence and degree of renal papillary necrosis was increased in animals of the high-dose groups at the terminal sacrifice (Til et al., 1989). Findings by Tobe et al. (1989) corroborate the Til et al. (1989) study and show that the main targets for formaldehyde toxicity administered by drinking water to rats are the forestomach and glandular stomach.

There is evidence that formaldehyde may act as a tumor promoter by the oral route as well as the inhalation route (discussed above). Takahashi et al. (1986) studied the effects of formaldehyde in an initiation-promotion model of stomach carcinogenesis in male outbred Wistar rats (Shizuoka Laboratory Center, Shizuoka, Japan Takahashi et al. (1986) reported an increase in MNNG-initiated GI cancers with formaldehyde exposure (29.4 versus 13.3% TBA in controls); the greatest difference in tumor-containing versus nontumorigenic mice was associated with adenocarcinoma in the glandular stomach (23.5 versus 3.3% in controls). Additionally, forestomach papillomas and preneoplastic hyperplasia in the glandular stomach were increased with formaldehyde exposure alone.

4.2.4. Immune Function

Leach et al. (1983) documented potential immunomodulatory effects of formaldehyde inhalation exposure. F344 rats were exposed nose only to formaldehyde 6 hours/day, 5 days/week for up to 30 days. The target concentrations for exposure were 0, 3, 16, 61, and 99 ppm formaldehyde (0, 3.7, 19.7, 75.0, and 122 mg/m^3). Body weight and food consumption were recorded, and blood samples for standard hematology and immune assays were collected

(details not given). Immune measures referenced include in vitro lymphocyte transformation, hemagglutination assays, and enumeration of B cells, WBCs, and RBCs. No effects were seen at 3 ppm formaldehyde. However, dose-dependent responses were reported for weight loss, decreased food consumption, increased WBCs, increased segmented neutrophils and nucleated RBCs, and decreased ability to produce antibodies to sheep RBCs. The results of the lymphocyte transformation assay were inconsistent, with a 25−30% reduction in stimulation after exposure to 99 ppm but an initial stimulation seen after 16 and 61 ppm exposures. Further details were not available, making it difficult to determine if these reported immunomodulatory effects may have been, in part or in full, secondary to effects on the URT. Subchronic exposures at 61 and 99 ppm formaldehyde would be expected to result in frank toxic effects in mice (see Section 4.2.1). However, these findings suggest possible immunomodulatory effects due to formaldehyde exposure and require further exploration.

Dean et al. (1984) investigated the effects of formaldehyde exposure on a range of indicators of immune function. Female B6C3F1 mice were exposed to 15 ppm formaldehyde (18.4 mg/m^3) 6 hours/day, 5 days/week for 3 weeks. Three trials were run with a total of 255 formaldehyde-treated mice. Body and organ weights were recorded at sacrifice for control and formaldehyde-exposed mice (10 per group). Measures of host susceptibility, cell-mediated immunity MP function, and antibody reactions were conducted 2 to 6 days after the end of exposure (see Table 4-46). Lymphocyte subsets, spleen cellularity, bone marrow cellularity, and progenitor cell subsets were enumerated. Host susceptibility and delayed type hypersensitivity were measured in vivo. Lymphocyte proliferation, natural killer cell activity, phagocytosis, hydrogen peroxide production, and IgM plaque-forming cells (PFCs) were measured ex vivo after in vivo stimulation in some cases (see Table 4-46).

Body weight, organ weights and cellularity, progenitor cell populations, blood cell counts, and differentials were unchanged in formaldehyde-treated mice (Dean et al., 1984). Circulating blood monocytes were decreased in treated mice, which may be a reflection of the local inflammatory response expected in the nasal epithelium (Dean et al., 1984). However, there was no corresponding decrease in peritoneal MPs. There was a trend, but no statistical significance, for decreased spleen weight, cellularity, and B cell precursors (87, 83, and 78% of controls, respectively). The mean body weight of formaldehyde-treated mice was 21.1 versus 20.9 g in control mice, and thymus and spleen weights were not normalized by body weight.

All indicators of natural killer cell function, cell-mediated immunity, and humoral immunity in formaldehyde-treated mice were unchanged from controls (Dean et al., 1984). Phagocytic capacity of both resident and elicited peritoneal MPs was unchanged by formaldehyde treatment. However, hydrogen peroxide production in elicited peritoneal MPs was significantly

increased in formaldehyde-treated mice, 78 versus 42 nmol/mg protein ($p < 0.05$) (Dean et al., 1984).

Table 4-46. Battery of immune parameters and functional tests assessed in female B6C3F1 mice after a 3 week, 15-ppm formaldehyde exposure (6 hours/day, 5 days/week)

Immune function	Model	Challenge	Metric
Host susceptibility	Tumor resistance	PYB6 sarcoma cells	Subcutaneous injection, followed by skin palpation to track tumor development
	Tumor resistance	16F10 melanoma cells	Lung tumor burden determined by [^{125}I]UdR incorporation
	Bacterial resistance	*Listeria monocytogenes*	Survival after challenge
Cell-mediated immunity	Delayed type hypersensitivity	Keyhole limpet hemocyanin	Radiometric index of delayed hypersensitivity responses
	Lymphocyte proliferation	T-cell mitogen, PHA[a] B-cell mitogen, LPS[b] (ex vivo)	Ex vivo proliferation, 3 days, measured by [^{3}H]-thymidine incorporation
	Lymphocyte subsets	None	Percentage of cells positive for cell surface markers (Thy-1, Mac-1, Lyt-1)
	Natural killer cell activity	Yac-1 target cells (^{51}Cr labeled) (ex vivo)	% cytotoxicity by ^{51}Cr release
MP function (both resident and MVE-1 elicited MP)	Phagocytosis	Sheep RBCs (^{51}Cr labeled) (ex vivo)	^{51}Cr incorporation as a measure of RBCs phagocytized
	Hydrogen peroxide production	Pharmacologic stimulation (ex vivo)	H_2O_2 release in culture
Humoral cell immunity	Antibody PFC responses, IgM PFCs	Sheep RBCs, TVF-LPS, or TNF-Ficoll	Plaques formed
Progenitor cells	Bone marrow cellularity (femur)	None	Cell enumeration by a Coulter counter
	Granulocyte-MP progenitors	None	Cell enumeration by a Coulter counter
	B-cell precursors	None	Clonogenic assay

[a]T-cell mitogen, phytohemagglutinin (PHA-P).
[b]B-cell mitogen, lipopolysaccharide (*Escherichia coli*).
Source: Dean et al. (1984).

As shown in Table 4-47, several indicators of host resistance in the female B6C3F1 mice were increased after formaldehyde exposure (Dean et al., 1984). Tumor mass and pulmonary foci after B16F10 melanoma cell challenge were significantly reduced in formaldehyde-treated

mice, indicating improved tumor immunity ($p < 0.05$). However, following PYB6 sarcoma cell challenge, formaldehyde-treated mice had a 7.1% tumor incidence versus 11.1% in controls, which was not statistically different. Mortality due to *Listeria monocytogenes* (LM) was decreased from 70 to 30% ($p < 0.05$). Because resistance to LM is primarily MP dependent, the authors speculated that this enhanced resistance might be due in part to increased bactericidal activity as was also suggested by increased hydrogen peroxide production ex vivo in elicited peritoneal MPs from female mice (Dean et al., 1984).

Table 4-47. Summary of the effects of formaldehyde inhalation on the mononuclear phagocyte system (MPS) in female B6C3F1 mice after a 3-week, 15 ppm formaldehyde exposure (6 hours/day, 5 days/week)

In vivo indicators of MPS	Metric	Formaldehyde effect
Cellularity	Circulating monocytes	Decreased[a]
	CMF progenitor cells	No change[a]
	Resident peritoneal MP	No change[a,b]
	Elicited peritoneal MP	No change[a,b]
In vivo test of host resistance	LM	Increased resistance[a]
	B16F10 tumor challenge	Increased resistance[a]
	PYB6 tumor challenge	No significant increase[a]
Ex vivo indicators of MPS	**Cell type/activation**	**Formaldehyde effect**
H_2O_2 production	Resident, no PMA[c]	None detected [a,b]
	Resident, with PMA	None detected [a,b]
	Elicited, no PMA[d]	None detected [a,b]
	Elicited, with PMA	Increased [a,b]
Phagocytosis	Resident	No change[a]
	Elicited	No change[a]
Assessment of MP maturation Leucine aminopeptidase content	Resident	Decreased[b]
	Elicited	No change[b]
	Resident	No change[b]
	Elicited	No change[b]
Acid phosphatase content	Resident	No change[b]
Binding of tumor cells	Elicited	No change[b]
	Resident	No change[b]
Lysing of tumor cells	Elicited	Increased at mid-range target-to-effector cell ratio[b]

[a]Dean et al. (1984).
[b]Adams et al. (1987).
[c]Phorbol 12-myristate 13-acetate (PMA).
[d]Peritoneal MPs were elicited with the pyran copolymer Murray Valley encephalitis virus (MVE-2).
Sources: Adams et al. (1987); Dean et al. (1984).

Overall, the observations of increased hydrogen peroxide production and increased host resistance in peritoneal MPs distant from the POE suggest that formaldehyde has an effect on the mononuclear phagocyte system (MPS). The authors postulated that this effect may be indirect, due in part to the tissue inflammatory response in the URT or a direct systemic effect on the MPS by formaldehyde exposure (Dean et al., 1984). Subsequent studies by the same researchers explored the possibility of systemic effects of formaldehyde exposure on MPS function and maturation stage (Adams et al., 1987). Female B6C3F1 mice were exposed to 15 ppm (18.4 mg/m^3) formaldehyde 6 hours/day, 5 days/week for 3 weeks, as before (Adams et al., 1987). Both resident and Murray Valley encephalitis virus (MVE-2)-elicited peritoneal MPs were examined for hydrogen peroxide production, enzymatic activity, phagocytic ability, binding, and lysis of tumor cells (Adams et al., 1987).

Similar to the findings of Dean et al. (1984), formaldehyde treatment increased hydrogen peroxide production almost twofold in MVE-2 elicited peritoneal MPs (Adams et al., 1987). As summarized in Table 4-47, no treatment differences were seen in phagocytic ability in either resident or elicited MPs (Adams et al., 1987). Resident peritoneal MPs from formaldehyde-treated mice were not different in their ability to bind or lyse tumor cells. Although formaldehyde treatment did not increase the ability of elicited MPs to bind tumor cells, lysis of the target cells (P815 tumor cells) was increased from 28 to 37% by formaldehyde treatment but only at the midrange target-to-effector-cell ratio tested in the assay ($p < 0.05$) (Adams et al., 1987). Although this is statistically significant, the authors questioned the biological significance of this result since it was not observed at all three target cell ratios tested. However, an increase in tumor cell lysis in vitro would be consistent with the in vivo increased tumor resistance previously reported (Dean et al., 1984). The in vitro lysis response curve suggests that assay conditions may result in a maximum cytolysis near 40%. If so, any treatment effects on lysis would be difficult to discern at higher effector cell ratios.

Jakab (1992) investigated the effect of formaldehyde exposure on the alveolar MPs and resistance to respiratory infections. The first set of experiments assessed bactericidal activity by directly quantifying the pulmonary bacterial loading after exposure to *Staphylococcus aureus*. White female Swiss mice were exposed to formaldehyde after bacterial infection (regimens A and C), before bacterial infection (regimen B), or before and after infection (regimen D) (see Table 4-48).

Table 4-48. Formaldehyde exposure regimens for determining the effects of formaldehyde exposure on pulmonary *S. aureus* infection

	Preinfection treatment	Postinfection treatment	Results
Regimen A	None	4 hours 0, 1, 5, 10, or 15 ppm[a]	15 ppm, increased bacterial loading
Regimen B	18 hours 0, 0.5, or 1 ppm[b]	None	No effect
Regimen C	None	4 hours 0, 0.5, or 1 ppm	No effect
Regimen D	18 hours 0, 0.5, or 1 ppm	4 hours 0, 0.5, or 1 ppm	1 ppm, increased bacterial loading

[a]0, 1.2, 6.2, 12.3, or 18.5 mg/m^3 formaldehyde.
[b]0, 0.62, or 1.2 mg/m^3 formaldehyde.

Source: Jakab (1992).

For regimen A, mice were exposed to 0, 1, 5, 10, or 15 ppm (0, 1.2, 6.2, 12.3, or 18.5 mg/m^3) formaldehyde. For regimens B−D, mice were exposed to 0, 0.5, or 1 ppm (0, 6.2, or 1.2 mg/m^3) formaldehyde. A 30-minute exposure to an infectious aerosol of *S. aureus* deposited 2×10^5 staphylococci in the lungs. Bacterial loading was determined in homogenized lung tissue by culturing diluted aliquots for an estimate of bacteria present immediately after loading and 4 hours later. Bacterial loading was expressed as a percentage change between control and formaldehyde-exposed animals. Mice exposed to 15 ppm formaldehyde for the 4 hours following bacterial infection (regimen A) had approximately an 8% increase in bacteria, indicating decreased host resistance ($p = 0.006$) (Jakab, 1992) (see Table 4-48). Mice receiving lower concentrations of formaldehyde following bacterial infection did not have increased pulmonary bacterial loading. Preinfection exposure to 0.5 or 1.0 ppm did not change bacterial loading 4 hours after infection (regimen B). However, combining an 18-hour preinfection formaldehyde exposure with a 4-hour postinfection 1 ppm formaldehyde exposure increased pulmonary bacterial loading by approximately 6.5% ($p < 0.05$). This effect was not seen with only a 0.5 ppm pre- and post-treatment regimen. Increased bacterial loading indicates that formaldehyde exposure (regimens A and D) reduced pulmonary bacterial resistance. This is in apparent contradiction to the findings of increased host resistance by Dean et al. (1984). However, there are important differences between the studies. The studies by Jakab (1992) are acute studies examining effects at the respiratory tract where direct effects are possible. Additionally, in some cases, the exposures were concurrent with bacterial infection, and it is

difficult to distinguish the potential for formaldehyde effects directly on the mucociliary apparatus as a barrier to infection.

A second set of experiments in the same report (Jakab, 1992) examined the effects of coexposure to formaldehyde and carbon black on pulmonary infection with *S. aureus*. The particle size distribution of the carbon black aerosol was less than a 5 μm aerodynamic diameter and, therefore, 98% respirable. Female Swiss mice were exposed nose only to formaldehyde and carbon black. Experiments were run at two target concentrations: (1) 2.5 ppm (3.1 mg/m^3) formaldehyde and 3.5 mg/m^3 carbon black or (2) 5 ppm (6.2 mg/m^3) formaldehyde and 10 mg/m^3 carbon black. Coexposure was given either for 4 hours after a 30-minute *S. aureus* infection or 4 hours/day for 4 days as a pretreatment prior to *S. aureus* infection. Bacterial loading was determined 0 and 4 hours after the *S. aureus* infection to assess bacterial survival. Formaldehyde-carbon black coexposure did not alter bacterial survival either as a pretreatment or post-treatment to bacterial exposure. However, this exposure regimen was not run for formaldehyde or carbon black separately, and the 4 hours/day for 4 days pretreatment was not included in the formaldehyde alone experiments (see Table 4-48).

Jakab (1992) also assessed the phagocytic activity of alveolar MPs collected by lavage at various time points after formaldehyde, carbon black, or coexposure. Female Swiss mice were coexposed to 5 ppm (6.2 mg/m^3) formaldehyde and 10 mg/m^3 carbon black 4 hours/day for 4 days. Mice were sacrificed and alveolar MPs harvested 1, 3, 5, 25, and 40 days after exposure. Mice exposed only to formaldehyde or carbon black were sacrificed 3, 10, 25, and 40 days after exposure. Fc-receptor-mediated phagocytosis was assessed ex vivo by using sensitized sheep RBCs. The phagocytic index (PI) was reported as the total number of RBCs in 100 MPs. Neither formaldehyde nor carbon black exposure alone significantly changed the PI (Jakab, 1992). These findings are consistent with the first coexposure experiment, since no changes in PI were seen immediately after exposure. However, coexposure did decrease the PI of alveolar MPs in a time-dependent manner, with maximal decrease to less than 70% of controls by 25 days after exposure (see Figure 4-15). Decreases in the PI reflect changes in both the percentage of phagocytic MPs and the number of RBCs phagocytized (Jakab, 1992). The PI recovered to control levels by 40 days postexposure.

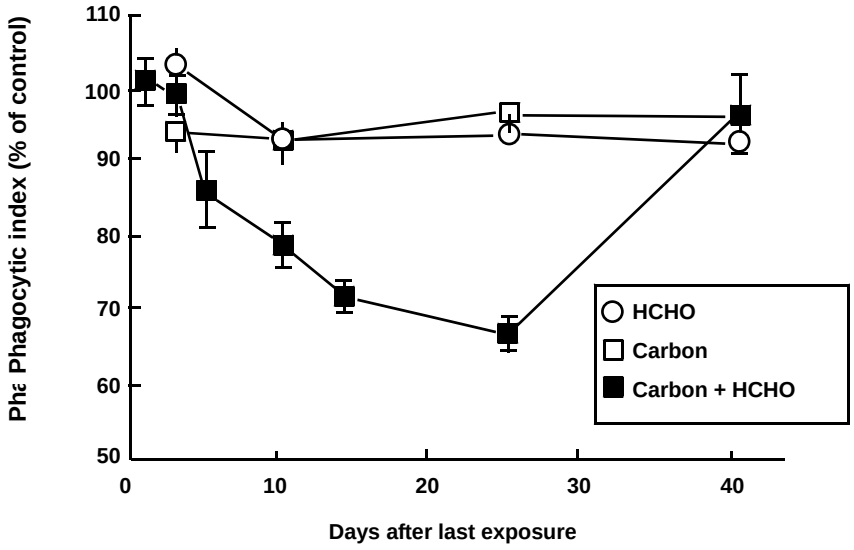

Figure 4-15. Alveolar MP Fc-mediated phagocytosis from mice exposed to 5 ppm formaldehyde, 10 mg/m^3 carbon black, or both.

Note: Exposure was 4 hours/day for 4 days. Each value represents the mean ± SEM of five determinations.

Source: Redrawn from Jakab (1992).

Holmström et al. (1989b) evaluated the effects of long-term formaldehyde exposure on antibody production. Female Sprague-Dawley rats were exposed to 12.6 ppm formaldehyde (15.5 mg/m^3) 6 hours/day, 5 days/week for 22 months. Body weight, tumor incidence, and pathology were reported elsewhere (Holmström et al., 1989b). Rats were given a subcutaneous injection of pneumococcal polysaccharide antigens or tetanus toxoid 21 to 25 days prior to sacrifice. The two vaccines chosen represent T-cell-dependent and T-cell-independent antigens, respectively. Antibody titers (IgG and IgM) were determined prior to vaccination and at sacrifice. Formaldehyde treatment had no effect on antibody titers either before or after vaccination (Holmström et al., 1989b).

Summary of Formaldehyde Effects on Immune Function

Although there were initial reports of systemic immunomodulation attributed to formaldehyde exposure (Leach et al., 1983), formaldehyde effects on measures of humoral and cell-mediated immunity were not confirmed by Dean et al. (1984). The authors did report increased host resistance to both tumor and bacterial tumor challenges after a 3-week exposure to

15 ppm formaldehyde. An increased resistance to these challenges, presented distal to the site of formaldehyde exposure (administered subcutaneously or intravenously), suggests a systemic effect of formaldehyde exposure. In addition, increased host resistance and hydrogen peroxide release from peritoneal MPs were reported and confirmed (Adams et al., 1987; Dean et al., 1984). Chronic inflammation and tissue damage to the respiratory mucosa expected with formaldehyde exposure may result in an up regulation of the MPS and therefore increase host immunity. It is unclear if this response would be specific to formaldehyde or similar to enhancement of immune function seen with chronic inflammation.

Jakab (1992) demonstrated decreased pulmonary resistance to bacterial infection where animals were exposed to 15 ppm formaldehyde immediately after bacterial loading or when they were given an 18-hour pre-exposure to formaldehyde followed by 1 ppm formaldehyde exposure after bacterial loading. The authors speculated that formaldehyde may directly act on pulmonary MPs, reducing their effectiveness. However, Jakab (1992) showed that there was no change in Fc-mediated phagocytosis of alveolar MPs immediately after formaldehyde exposures. Degradation of the protective mucus layer and possible epithelial cell damage may contribute to more effective bacterial infection in the presence of formaldehyde without a direct action on MP function. As mentioned above, degradation of the mucus layer may result in a more potent inoculation and therefore higher bacterial loading.

Although neither formaldehyde nor carbon black alone impacted Fc-mediated phagocytosis of alveolar MPs, Jakab (1992) demonstrated that there was decreased Fc-mediated phagocytosis after formaldehyde and carbon black coexposure. Carbon black may have acted as a carrier for formaldehyde, allowing higher levels of formaldehyde to be delivered more deeply into the lungs than would be seen with formaldehyde alone.

Formaldehyde is known to break down the mucus layer protecting the respiratory tract, allowing exposure of the underlying epithelium (Morgan et al., 1986a, c, d). Additionally, formaldehyde can directly induce tissue inflammation through sensory irritation via substance P from the trigeminal nerve (Fujimaki et al., 2004a). These actions together could contribute to some of the observed effects on immune response attributed to formaldehyde exposures. Degradation of the protective mucus layer would make antigens more available to the immune system. It has been shown that direct application of an antigen to the nasal associated lymph tissue, bypassing the mucus layer, is a more effective delivery of antigen (Hou et al., 2002). Therefore, increased availability of these antigens to the immune system may in part explain observed increased antibody production seen against ovalbumin (OVA) or common dust mite allergen (Der f) during formaldehyde exposure (Sadakane et al., 2002; Riedel et al., 1996; Tarkowski and Gorski, 1995). Neurogenic inflammation may also contribute to more efficient

antigen processing and presentation by activation of resident MPs. These factors are consistent with the observation that formaldehyde exposures do not affect antibody production to antigens administered outside of the respiratory tract, even after chronic exposures (Holmström et al., 1989b).

This effect was initially observed several days after exposure was ended with maximal suppression seen 25 days after a 4-day formaldehyde exposure. The delayed onset of this response, however, suggests an effect beyond the POE effects observed at the time of exposure. Table 4-49 presents a summary overview of the effects of formaldehyde on immune function in laboratory animals.

4.2.5. Hypersensitivity and Atopic Reactions

Adverse reactions in humans exposed to formaldehyde in the workplace and homes have been reported, which are consistent with an allergic response or a chemical sensitivity (see Section 4.1.1 for details). Rashes and skin reactions are reported in some individuals after dermal exposures, and in some cases exacerbation of asthma is reported after inhalation of formaldehyde. However, the reports of human reactions do not allow a clear determination of whether this sensitization is immunogenic or neurogenic in origin. Formaldehyde-induced sensitization may have both neurogenic and immunologic components. Numerous animal studies have been conducted in order to understand the potential for sensitization to formaldehyde. Although hypersensitivity and allergic sensitization are often considered solely immunologic in origin, neurogenic mechanisms may result in bronchial hypersensitivity and increased immunologic sensitization. Therefore, the animal studies regarding formaldehyde-induced sensitization are evaluated discretely in order to examine these etiologic possibilities.

Classically, hypersensitivity is characterized as an immune response to an antigen, resulting in an inflammatory reaction that itself damages the tissues or is otherwise harmful (Kuby, 1991). These reactions may be localized, as in topical dermatitis, or systemic, as in anaphylactic shock from an allergen. Hypersensitivity can be mediated by a humoral immune response or by a cell-mediated immune response. Four classes of hypersensitivity are generally recognized that differ in their immune system components and functions. Although a single agent (e.g., penicillin) may induce all four types of hypersensitivity, it is more usual for an agent to primarily induce one form of hypersensitivity.

Table 4-49. Summary of immune function changes due to inhaled formaldehyde exposure in experimental animals

Species	No./ group	Treatment[a]	Observations	LOAEL/ NOAEL	Reference
F344 rats	8	0, 3, 16, 61, 99 ppm 6 hours/day, 5 days/week for 4 weeks	No effects at 3 ppm. Mixed results at higher doses that were not consistent.	NA[b]	Leach et al. (1983)
B6C3F1 mice (female)	10	15 ppm 6 hours/day, 5 days/week for 3 weeks	Increased H_2O_2 production, and increased host resistance to tumor formation, but other immune parameters unchanged.	LOAEL 15 ppm	Dean et al. (1984)
B6C3F1 mice (female)	Pooled MPs from a number of mice	15 ppm 6 hours/day, 5 days/week for 3 weeks	Increased H_2O_2 production in MVE-2-elicited peritoneal MPs.	LOAEL 15 ppm	Adams et al. (1987)
White Swiss mice (female)	18	0, 1, 5, 10, or 50 ppm for 18 hours before and/or 4 hours after a 30-minute exposure to bacterial infection (*S. aureus*)	Combining an 18-hour pre-exposure to formaldehyde with 4-hour postexposure to formaldehyde increased bacterial loading at 1 ppm by 6.5%.	LOAEL 1 ppm	Jakab (1992)_
White Swiss mice (female)	18	5 ppm (2.6 mg/m^3) formaldehyde and 10 mg/m^3 carbon black 4 hours/day for 4 days	Phagocytic index was decreased by coexposure to formaldehyde and carbon black but not by either insult alone.	NA	Jakab (1992)
Sprague-Dawley rats (female)	5	12.6 ppm 6 hours/day, 5 days/week, 22 months	Formaldehyde treatment had no effect on antibody titers either before or after vaccination with pneumococcal polysaccharide antigen or tetanus toxoid.	NA	Holmström et al. (1989b)

NA = not applicable.

Chemical sensitivity generally implies a neurogenically induced sensitization (Meggs, 1995). A chemical may directly interact with sensory nerves, releasing mediators or neuropeptides such as substance P (a tachykinin) that trigger inflammation, hence called neurogenic inflammation,. Repeated exposure to the same chemical is hypothesized to potentiate neurogenic inflammation (Meggs, 1995). The resulting signs of tissue inflammation may be similar to immunogenic inflammation (occurs by binding of antigen to antibody or leukocyte receptor), but there would be no requirement that the immune system recognize the chemical as an antigen for this type of response. Therefore, a chemical may induce one or more clinical signs of atopic asthma without IgE-mediated type 1 hypersensitivity response. One form of sensitivity directly affects sensory nerve endings, resulting in neurogenic inflammation and is a well-known health effect attributed to formaldehyde. Neurogenic responses may result from the direct and acute interaction of the chemical with sensory nerve ending receptors of the trigeminal nerve that may lead to persistent rhinitis and an asthma-like reactive airway dysfunction syndrome that may develop after short-term human exposures (Brooks et al., 1985). Thus, there is evidence to suggest that neurogenic inflammation may contribute to observed increases in formaldehyde-induced airway hyperresponsiveness and atopic responses. The available animal studies that have investigated formaldehyde-induced airway hyperresponsiveness and atopic responses are summarized below.

4.2.5.1. *Inhalation Studies in Experimental Animals*

This section summarizes animal studies informing the role of formaldehyde-induced chemical sensitization. The symptoms of sensitization (atopy, airway hyperresponsiveness) are frequently associated with immunologic markers (cytokine production, leukocyte infiltration histamine release, and antibody production) but may be mediated by neurogenic sensory irritation, principally by activation of the trigeminal nerve (see Section 4.1.1.1 for a discussion of sensory irritation). The animal studies that illuminate these neurogenic and immunologic responses are discussed outside of the classic neurotoxicology and immunotoxicology study summary sections to allow synthesis of these data.

Sensitization to chemical exposure by inhalation often manifests as an allergic or asthmatic response as characterized by BC or BHR. This sensitization may be a result of immune involvement, as in the case of hypersensitivity, or a neurogenic sensitization, where a chemical may directly stimulate inflammation. Asthma is a specific manifestation of IgE-mediated hypersensitivity, characterized by BHR and airway inflammation, resulting in lower airway obstruction (Fireman, 2003; Kuby, 1991). In asthma, an allergen capable of cross-linking membrane-bound IgE on mast cells initiates immunogenic inflammation resulting in an influx of

eosinophils, neutrophils, and lymphocytes. Mediators of BC, including histamine, eicosanoids, and bradykinin (Kuby, 1991), are released during this process. Prior exposure to the allergen can increase allergen-specific IgE, potentiating the allergic reaction; this is immunogenic sensitization.

Biagini et al. (1989) evaluated the effect of a single pulmonary exposure of formaldehyde on pulmonary mechanics, including BC. The researchers chose cynomolgus monkeys known to be hyperreactive to methacholine (acetyl-β-methacholine chloride), which is a direct-acting stimulant of BC (Cain, 2001). Measures of pulmonary mechanics included pulmonary flow resistance; dynamic compliance; PEFR; FVC; FEV; $FEF_{25-75\%}$, and 50% of VC; and FEFs normalized for VC. Nine cynomolgus monkeys were exposed to increasing levels of methacholine for 1 minute at 10-minute intervals (0, 0.125, 0.5, 2, and 8 mg/mL) as an aerosol (0.065 mL/minute with a mean aerodynamic diameter of 1.0−1.5 μm). Pulmonary mechanics were measured to establish each monkey's response to methacholine. Methacholine challenge, as the positive control, increased pulmonary flow resistance at increasing levels of methacholine (0.125, 0.5, 2, and 8 mg/mL) to 196 ± 16, 285 ± 57, 317 ± 64, and 461 ± 120% of baseline levels, respectively. After a 2-week recovery period, each methacholine-sensitized monkey was exposed to 2.5 ppm formaldehyde (generated from formalin, 15% methanol) for 10 minutes. Measures of pulmonary function were performed at 2, 5, and 10 minutes after exposure.

Formaldehyde exposure increased pulmonary flow resistance from 11.3 ± 1.4 cm H_2O prior to formaldehyde exposure to 16.1 ± 2.1, 16.9 ± 2.8, and 20.0 ± 3.4 cm H_2O at 2, 5, and 10 minutes after 2.5 ppm formaldehyde exposure (with 142, 150, and 177% change, respectively). All other measures of formaldehyde-induced pulmonary mechanics were not significantly different from controls. Increased pulmonary flow resistance, a measure of increased BC, was induced by formaldehyde challenge in previously sensitized mice. However, the differences between methacholine challenge and formaldehyde challenge were not statistically significant. Although both formaldehyde challenge and methacholine challenge increased pulmonary flow resistance, there was no correlation between individual methacholine responsiveness and the magnitude of effect after formaldehyde exposure ($p > 0.1$). Therefore, although formaldehyde exposure stimulated BC similarly to a known direct stimulating agent, formaldehyde may not work through the same site of action as methacholine.

Swiecichowski et al. (1993) assessed pulmonary resistance and airway reactivity due to formaldehyde exposure alone and in response to increasing doses of acetylcholine chloride (a direct-acting BC agent) after formaldehyde exposure in vivo. Male Hartley guinea pigs (eight per group) were exposed at 0.86, 3.4, 9.4, or 31.1 ppm (1.1, 4.2, 11.6, or 38.3 mg/m^3) formaldehyde for 2 hours or at 0.11, 0.31, 0.59, or 1.05 ppm (0.14, 0.38, 0.73, or 1.29 mg/m^3)

formaldehyde for 8 hours. Total pulmonary resistance increased after 2 hours formaldehyde exposure at 9.4 and 31.1 ppm and reached similar peak resistance at the end of the exposure period. This effect was rapidly reversible, with values returning to baseline within 30 minutes after exposure. Although 2-hour exposures at 3 and 1 ppm did not increase pulmonary resistance, 8-hour exposures at 0.3 and 1 ppm did increase pulmonary resistance to similar levels as the 2-hour exposure at 30 ppm. The results indicate that both concentration and exposure time impacted the measured increase in pulmonary resistance. However, a simple multiplicative model (e.g., C × t) does not adequately represent the effects observed. It is noted that an 8-hour exposure at 1 ppm (8 ppm-hours), reached approximately the same pulmonary resistance as 2 hours at 9.4 ppm (19 ppm-hours). This may in part be due to a maximum practical increase in pulmonary resistance in the animals. Conversely, there was no effect at 3 ppm for 2 hours (6 ppm-hours), although significant increase in pulmonary resistance was recorded after an 8-hour exposure at 0.3 ppm (2.4 ppm-hours). Formaldehyde does not appear to exert its effects via a classic C × t paradigm. Exposure concentration, however, did seem to impact recovery time.

In addition, specific pulmonary resistance and airway reactivity to increasing doses of intravenous acetylcholine chloride, a direct respiratory stimulant, were measured immediately after formaldehyde exposure for up to 60 minutes. Formaldehyde-induced airway hyperreactivity was defined as a decrease in the level of acetylcholine chloride needed to produce twice the basal specific resistance (effective dose $[ED]_{200}$). The dose of acetylcholine chloride required to double the specific pulmonary resistance (ED_{200}) and airway reactivity was decreased in animals exposed for 2 hours to formaldehyde. When the duration was extended to 8 hours of formaldehyde exposure, the effective dose of formaldehyde required to elicit a doubled pulmonary resistance (ED_{200}) in the presence of acetylcholine chloride was decreased to 1.07 ppm. Lower ED_{200}s were recorded in formaldehyde-treated animals. This indicates that less acetylcholine was needed to produce BC when formaldehyde was present. Thus, formaldehyde can exacerbate BHR. Additionally the formaldehyde-induced effect increased with duration of exposure, indicating that time as well as exposure concentration are factors in the magnitude of the response. Directly induced increases in airway hyperreactivity peaked 1 hour after exposure and persisted 6 hours after exposure.

In a second set of experiments, male Hartley guinea pigs were treated for 8 hours at 3.4 ppm (4.2 mg/m^3) in order to measure airway hyperreactivity ex vivo (Swiecichowski et al., 1993). After formaldehyde exposure, tracheae were excised and mounted in tissue baths, where tracheal contraction was measured in response to direct application of acetylcholine and then carbachol. Tracheae from similarly exposed guinea pigs were fixed and sectioned for histologic

examination and were assessed for signs of inflammation. Formaldehyde exposure did not increase ex vivo tracheal constriction and suggests that changes in airway reactivity were produced due to both local humoral changes and neural reflexes. However, no changes in epithelial cell morphology or influx of inflammatory cells were recorded even up to 4 days after formaldehyde exposure ended. The authors speculated on possible MOAs for BHR, such as the role of an irritant receptor or altered epithelial cell biochemistry. It may be that the window of acute inflammation occurred early in the exposure protocol and was resolved by the time of first measurement, after 8 hours of exposure. The absence of inflammatory markers may argue against a classic type 1 sensitivity.

The binding of an allergen to receptor bound IgE triggers degranulation of mast cells and basophils, releasing mediators of type 1 hypersensitivity, including the histamine responsible for BC. Brown Norway (BN) rats are known for their high capacity for IgE production and airway hyperresponsiveness in response to allergens or other chemicals; they have often been used as a model of allergic respiratory disease. Ohtsuka et al. (1997) compared the effects of formaldehyde exposure on the nasal epithelium of F344 and BN rats. If the formaldehyde-induced inflammatory response in the nasal epithelium is IgE mediated, BN rats would be expected to display more severe effects of formaldehyde exposure than F344 rats. Both strains of age- and sex-matched rats were exposed to formaldehyde aerosol for 3 hours/day, 5 days/week for 2 weeks. The aerosol was generated from a 1% formaldehyde solution by a two-fluid atomizer, and formaldehyde level was maintained at 2 mg (1% sol.)/L (approximately 16 ppm or 20 mg/m^3), by adjusting the flow rate for formaldehyde solution to the atomizer. During the course of exposure, the following clinical signs were monitored: abnormal respiration, stridor wheezing, nasal discharge, and sneezing. Rats were weighed weekly. Two days postexposure, rats were sacrificed and tissues from the head, trachea, and lungs were fixed and sectioned. Transverse sections were taken at the following palatal landmarks from three animals: level 1 (lateral edge of incisor teeth), level 2 (between incisive papilla and the first palatal ridge), and level 3 (on the second upper molar). The nasal septa of the remaining two animals were revealed for examination by electron microscopy.

Formaldehyde-treated F344 rats showed less body weight gain over the 2-week treatment, resulting in lower body weight at week 1 and week 2 than F344 controls ($p < 0.05$ and 0.01). Body weights of formaldehyde-treated BN rats were unchanged from BN controls. The authors observed fewer clinical signs of respiratory irritation in the formaldehyde-exposed BN rats compared with formaldehyde-exposed F344 rats, such as abnormal respiration (three versus five) and nasal discharge (three versus five). Histologic analysis of lung and trachea tissues revealed no distinct signs of inflammation in either strain. Formaldehyde exposure induced cell

damage in URT tissues. Epithelial cell damage was milder and impacted a smaller portion of the URT in BN rats compared with F344 rats. Squamous metaplasia were present in the respiratory epithelium (levels 1 and 2) in both strains in formaldehyde-treated rats. However, a distinct keratinized layer was noted in level 1 epithelium of F344 rats, and the extent of lesions in level 2 respiratory epithelium was much greater than that seen in BN rats. Additionally, the olfactory epithelium (level 2) in formaldehyde-exposed F344 rats exhibited degeneration, necrosis, and desquamation not seen in BN rats. Mild squamous metaplasia was noted in level 3 of the respiratory epithelium in the treated F344 rats but not the treated BN rats. No pulmonary function measurements were taken, and, thus, no direct comparison in BHR or BC between BN and F344 rats in response to formaldehyde can be made. It appears that BN rats are more resistant to formaldehyde-induced cell damage than are F344 rats, despite the fact that BN rats are known to be IgE responders. These results suggest that IgE responsiveness may be protective of formaldehyde-induced cell damage, or IgE may not play a role at all. The authors note that their earlier research indicated the BN rats have well-developed submucosal glands and speculate that greater mucus flow may be partly responsible for the greater resistance of BN rats to the histologic signs of formaldehyde toxicity.

In a subsequent study in the same laboratory, Ohtsuka et al. (2003) compared histology and cytokine profiles in the nasal mucosa of formaldehyde-treated F344 and BN rats. Formaldehyde aerosol was generated as above and rats (nine per group) were exposed 3 hours/day for 5 days to approximately 16 ppm of formaldehyde (20 mg/m^3). Clinical signs were recorded daily, and monitored respiratory parameters included abnormal respiration, stridor wheezing, nasal discharge, and sneezing. Tissue sections of the nose (five rats per group) were prepared for light microscopy as above: transverse sections at levels 1, 2, and 3. Th-1 cytokines (IFN-γ, IL-2) and Th2 cytokines (IL-4 and IL-5) were determined from the whole nasal mucosa in four rats of each treatment group.

As expected, lesions and neutrophilic infiltration were more severe in F344 formaldehyde-exposed rats compared with treated BN rats. In addition, lesions were observed in all three levels of epithelium examined in F344 rats and impacted both respiratory and olfactory epithelium. Mucosal lesions in formaldehyde-treated BN rats impacted the respiratory epithelium of levels 1 and 2 only. Changes in formaldehyde-induced cytokine mRNA expression were modest in both strains. Th-1-related cytokines (IFN-γ, Il-2) in formaldehyde-treated BN rats were significantly decreased compared with control BN rats. A similar, although not statistically significant, decrease in Th-2 cytokines (IL-4, IL-5) was observed in formaldehyde-treated BN rats compared with unexposed BN rats. There were no treatment differences in either Th-1 or Th-2 cytokine expression in formaldehyde-treated F344 rats

compared with unexposed F344 rats. The modest changes in cytokine profile reported in formaldehyde-treated BN rats were not consistent with type 1 hypersensitivity since type 1 hypersensitivity reactions generally result in increased Th-2 cytokines. The mRNA expression results were not corroborated with protein levels and may not have been captured at their peak expression levels.

Lee et al. (1984) evaluated the potential for formaldehyde to act as a sensitizing agent through different routes of exposure in guinea pigs. The inhalation studies will be highlighted here. Dermal exposure and associated contact sensitivity results will be discussed in the dermal exposure section (see Section 4.2.5.2). Three groups of male English smooth-haired guinea pigs (four/group) were exposed via inhalation to either 6 or 10 ppm (7.4 or 12.3 mg/m^3) formaldehyde 6 hours/day for 5 consecutive days. Depending on the group, animals were then subjected to bronchial provocation challenge with 2 or 4 ppm formaldehyde on day 7 or days 7, 22, and 29 after exposure (see Table 4-50 for clarification).

Table 4-50. Study design for guinea pigs exposed to formaldehyde through different routes of exposure: inhalation, dermal, and injection

	Formaldehyde exposure	Bronchial provocation challenge	Skin test	Blood drawn for antibody titer
Group I—Inhalation	6 ppm formaldehyde[a], days 1−5	Day 7 2 ppm formaldehyde[a] for 1 hour	Day 9	Day 14
Group II—Inhalation	10 ppm formaldehyde, days 1−5	Day 7 2 ppm formaldehyde for 1 hour	Day 9	Day 14
Group III—Inhalation	10 ppm formaldehyde, days 1−5	Days 7, 22, and 29 4 ppm formaldehyde for 4 hours	Day 31	Day 14
Group IV—Dermal	100 μL formalin, days 1 and 3	Day 22 2 ppm formaldehyde for 1 hour 4 ppm formaldehyde for 4 hours	Day 7	Day 14
Group V—Injection	37 mg formaldehyde with Freund's adjuvant	Day 19 2 ppm formaldehyde	Day 7	Day 14

Source: Lee et al. (1984).

Dermal and injection groups are shown for comparison. Pulmonary hypersensitivity was assessed by measuring respiratory rate and tidal volume in response to exposure to 2 ppm formaldehyde challenge for 1 hour, 2 days postexposure for all three groups, and additional measurements were taken 22 and 29 days postexposure for group III. Blood was drawn to characterize IgE antibodies to formaldehyde in a passive cutaneous anaphylaxis (PCA) assay.

Respiratory rate was measured following initial formaldehyde exposure and again after bronchial challenge with formaldehyde.

Respiratory rate (exhibited as a pause during expiration) was depressed by 45% following exposure to 10 ppm formaldehyde during the first hour of exposure. During the first hour of exposure, decreased respiratory rate was accompanied by a pause during expiration that has been categorized as RB and indicated sensory irritation. The decreased respiratory rate is consistent with URT sensory irritation and induction of the trigeminal (neurogenic) reflex (Lee et al., 1984). After the first hour of exposure, decreased respiratory rate was characterized by a pause between breaths, which is similar to the breathing pattern seen in mice exposed to formaldehyde via tracheal cannula (Alarie, 1981). This suggests a separate effect of formaldehyde on the LRT after deep penetration of formaldehyde and suggests pulmonary irritation (Lee et al., 1984).

However, subsequent bronchial provocation challenge with either 2 or 4 ppm formaldehyde for either 1 or 4 hours failed to elicit immediate or delayed-onset respiratory sensitization (see Table 4-51). Respiratory rates were reported as being within ±20% of prechallenge levels (data not shown) and did not reflect statistical significance (Lee et al., 1984). Moreover, increased respiratory sensitivity was not observed in animals that had received an emulsification of formaldehyde and Freund's complete adjuvant by injection. Only two to four animals given formaldehyde injections in the presence of Freund's complete adjuvant developed a low titer of antibodies to formaldehyde (Lee et al., 1984).

Table 4-51. Sensitization response of guinea pigs exposed to formaldehyde through inhalation, topical application, or footpad injection

Exposure route	Pulmonary sensitization	Dermal sensitization	Antibody production
Inhalation			
6 ppm (Group I)	0/4	0/4	0/4
10 ppm (Group II)	0/4	0/4	0/4
10 ppm (Group III)	0/4	2/4	0/4
Topical	0/8	8/8	0/8
Injection	0/4	4/4	2/4

Source: Lee et al. (1984).

Thus, inhalation exposure to 6 or 10 ppm formaldehyde (8 hours/day for 5 days) followed by bronchial challenge with 2 or 4 ppm formaldehyde failed to result in respiratory sensitivity defined as greater than 20% change in respiratory rate. Second, for animals that received an injection of formaldehyde with Freud's adjuvant, it was not effective in inducing pulmonary

sensitivity. While neither inhaled formaldehyde challenge nor injected formaldehyde and Freud's adjuvant emulsion were effective in producing pulmonary sensitivity, this study relied on increased respiratory rate as an indication of hyperresponsiveness and may not be an accurate measure of hyperresponsiveness. Thus, overall, conclusions are uninformative due to study design flaws.

Riedel et al. (1996) tested the effects of formaldehyde inhalation on the development of sensitization to a known allergen. Female Perlbright-white Dunkin-Hartley guinea pigs (12 per group) were exposed to 0, 0.13, or 0.25 ppm formaldehyde 8 hours/day for 5 consecutive days. On day 5, the animals were sensitized to the common model allergen, OVA, in a 3-minute, head-only exposure to an aerosol of a 5% OVA solution. A booster sensitization with OVA occurred on day 19. A compressor nebulizer with an output rate of 0.75 mL/minute generated the aerosol. Particle size ranged from 0.5 to 5.0 μm. On day 26, bronchial provocation testing was conducted with 1% OVA challenge (aerosol). Blood samples were taken and anti-OVA IgG antibodies were quantified by ELISA. Significant airway obstruction was defined as an increase in compressed air in the lung that cannot be expired. Three guinea pigs were exposed to formaldehyde (0.20 ppm) or clean air for 5 days. Immediately after exposure, lung and tracheal tissues were fixed for histologic and morphometric evaluation. Wall thickness of bronchial and alveolar septa was measured systematically with a microscope-digitizing-table set.

Significant airway obstruction as measured by compressed air was seen in 3 of 12 controls, 8 of 12 0.13 ppm-exposed, and 10 of 12 0.25 ppm-treated animals after OVA challenge. The average airway obstruction was increased after 0.25 ppm (mean = 0.35 mL, $p < 0.01$) but not after 0.13 ppm formaldehyde exposure. However, individual response to OVA sensitization was highly varied, and animals exhibiting a 10-fold increase in obstruction (measured as compressed air) were seen in both treatment groups (0.13 and 0.25 ppm). Even at the lower exposure (0.13 ppm), biologically significant responses were seen in individuals (see Figure 4-16).

Specific anti-OVA antibodies (IgG1 class) were not detected in animals prior to sensitization or in control-treated animals after sensitization . Measurable anti-OVA antibodies were elevated in 3 of 12 (at 0.13 ppm) and 6 of 12 (at 0.25 ppm) formaldehyde-treated guinea pigs after sensitization (see Figure 4-17). The average anti-OVA titer for the high-dose group was significantly higher than for controls ($p < 0.05$). The individual responses at the 0.13 ppm exposure level indicate that, although the average group OVA titer may not have reached statistical significance, there was a measureable biological response in three individuals. These results indicate that formaldehyde exposure can sensitize previously naïve (nonsensitized) animals to OVA.

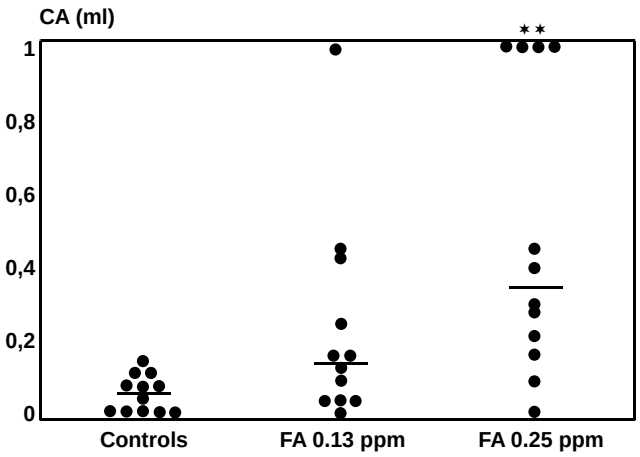

Figure 4-16. Compressed air in milliliters as parameter for airway obstruction following formaldehyde exposure in guinea pigs after OVA sensitization and OVA challenge.

Note: CA = compressed air; FA = formaldehyde; — = median; ** = $p < 0.01$.

Source: Redrawn from Riedel et al. (1996).

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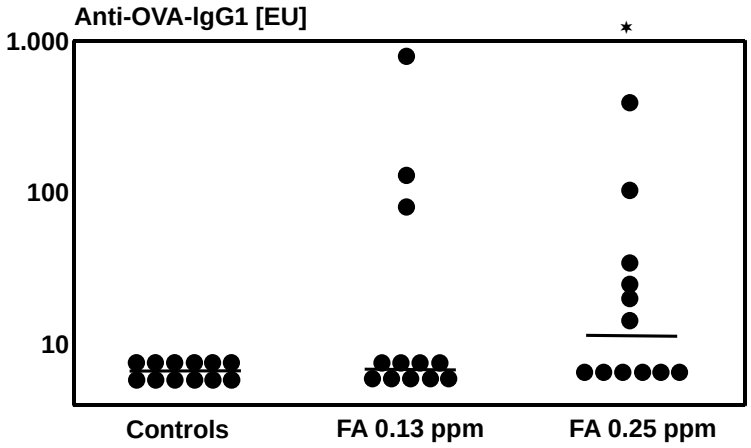


Figure 4-17. OVA-specific IgG1 (1B) in formaldehyde-treated sensitized guinea pigs prior to OVA challenge.

Note: EU = experimental units; FA = formaldehyde; — = median; ** = $p < 0.01$.

Source: Redrawn from Riedel et al. (1996).

The only significant, treatment-related histologic change was bronchial edema, with thickening of the bronchial wall in formaldehyde-exposed animals compared with nontreated animals subjected to OVA sensitization and subsequent OVA challenge. Bronchial walls were measured as 40.9 ± 2.5 versus 28.2 ± 1.2 μm. No signs of inflammation in the bronchial mucosa were seen with this edema.

Tarkowski and Gorski (1995) exposed female Balb/C mice to 0 or 6.63 ppm (0 or 2 mg/m^3) formaldehyde for either 6 hours/day for 10 days or 6 hours/day once a week for 7 weeks. All mice were sensitized intranasally to OVA for 10 days or once a week for 7 weeks. IgE anti-OVA titers were determined from sera collected from four mice every 8 days (1 day after OVA booster) by PCA. A parallel experiment to compare the role of the route of administration was conducted with I.P. rather than intranasal sensitization (1 μg OVA once every 7 days).

OVA titers increased similarly in control mice and mice exposed to formaldehyde once a week (see Figure 4-18). In contrast, mice exposed to formaldehyde 6 hours/day for 10 consecutive days at the beginning of the experiment had increased anti-OVA beginning after the fourth OVA sensitization, which continued to increase through seven doses of OVA to a peak of 70 PCA units ($p < 0.01$) (see Figure 4-18). Anti-OVA IgE titers were significantly different between formaldehyde-treated and nonexposed mice.

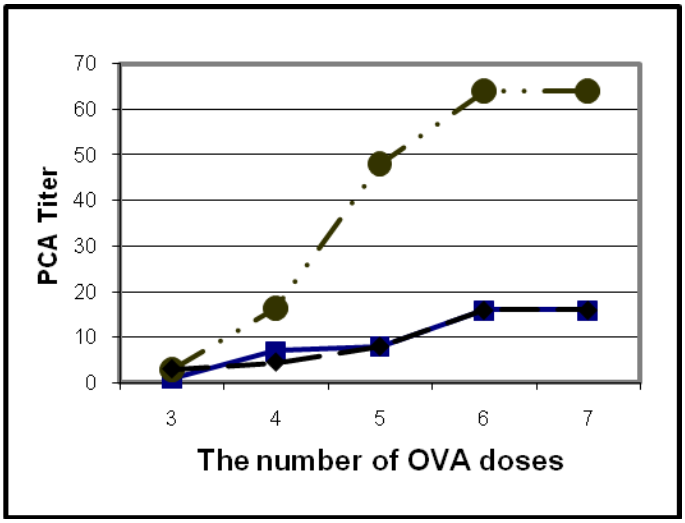


Figure 4-18. Anti-OVA titers in female Balb/C mice exposed to 6.63 ppm formaldehyde for 10 consecutive days or once a week for 7 weeks.

Note: ■ = control mice; ◆ = formaldehyde once a week × 7; ● = formaldehyde 10 days.
Source: Redrawn from Tarkowski and Gorski (1995).

Intraperitoneal sensitization to OVA was much more effective than intranasal sensitization, resulting in titers as high as 1,000 after 4 weeks. However, there were no differences between controls and animals treated with formaldehyde via the I.P. route of exposure. Thus, formaldehyde administered intranasally 6 hours/day for 10 days may facilitate the sensitization to allergens. These changes were not observed when formaldehyde was administered intranasally once a week for 10 weeks or via I.P. injection (Tarkowski and Gorski, 1995). The authors speculate that formaldehyde may increase permeability of respiratory epithelium and destruction of immunologic barriers. Thus the respiratory tract may become vulnerable to inhaled allergens after formaldehyde exposure (Tarkowski and Gorski, 1995).

Ito et al. (1996) conducted three experiments to examine the effects of acute formaldehyde exposure on bronchoconstriction and the mediators of vascular permeability. Male Wistar rats (five to eight per group) were exposed to 0, 2, 5, 15, or 45 ppm (0, 2.5, 6.2, 18.5, or 55.4 mg/m^3) formaldehyde for 10 minutes. Baseline pulmonary insufflation and blood pressure were determined prior to formaldehyde exposure and monitored throughout the experiment. Vascular leakage was measured by injection of Evans blue dye prior to the experiment and determining extravasation 5 minutes postexposure. Briefly, lungs were perfused with 0.9% saline through an aortic cannula. The lower portion of the trachea and main bronchi were removed, and the Evans blue dye remaining was determined and expressed as ng dye/g tissue. A second experiment was conducted to determine if dye leakage continued to increase after exposure. Seven rats were exposed to 15 ppm formaldehyde for 10 minutes, as above. Evans blue dye was injected 5 minutes postexposure, and tissues were perfused and excised 15 minutes later. The final experiment was conducted to determine the effect of certain receptor agonists on the formaldehyde-induced microvascular leakage. Ten groups of Wistar rats (four to seven per group) were exposed to 15 ppm formaldehyde and injected with Evans blue dye, as before. However, each receptor agonist under test or saline sham was injected 4−5 minutes prior to the 10-minute formaldehyde exposure. Agonists tested included tachykinin NK_1 receptor antagonist (CP-99,994) at 1, 3, or 6 mg/kg; a bradykinin B_2 receptor antagonist (HOE 140) at 0.65 mg/kg; and a histamine H_1 receptor antagonist (ketotifen) at 1 mg/kg.

Formaldehyde exposure did not change pulmonary insufflation pressure or blood pressure. Formaldehyde increased vascular permeability in a concentration-dependent manner in both the trachea and main bronchi for the first 5 minutes after exposure, as measured by Evans blue dye extravasation (Ito et al., 1996) (see Figure 4-19). Vascular permeability was not increased by formaldehyde exposure from 5 to 15 minutes postexposure (experiment 2). Administration of a selective NK_1 receptor antagonist (CP-99,994) inhibited the formaldehyde-

induced vascular permeability, reducing Evans dye extravasation to control levels at the 3 and 6 mg/kg doses (see Figure 4-20).

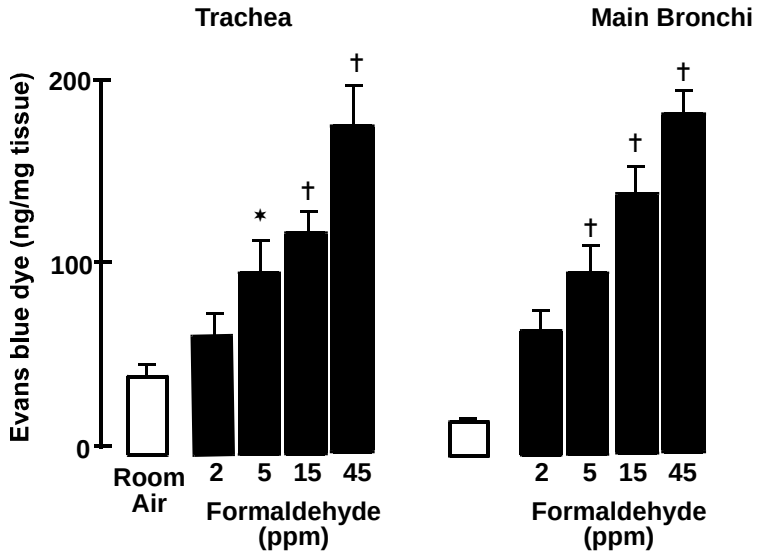


Figure 4-19. Vascular permeability in the trachea and bronchi of male Wistar rats after 10 minutes of formaldehyde inhalation.

Note: Vascular permeability was tested by an increase in Evans blue dye extravasation in the tissue. Solid bars: formaldehyde; open bars: room air, $n = 7$. Values are the means ± SEM of five to seven animals. $^{*}p < 0.05$ and $^{\dagger}p < 0.01$ versus room-air-exposed group (Williams' test).

Source: Redrawn from Ito et al. (1996).

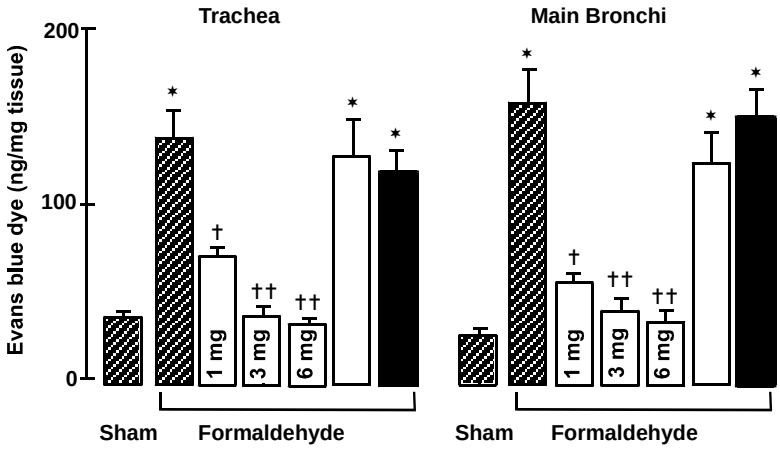


Figure 4-20. Effect of select receptor antagonists on formaldehyde-induced vascular permeability in the trachea and bronchi male of Wistar rats.

Note: Vascular permeability was tested by an increase in Evans blue dye extravasation. Rats were treated i.v. with 1, 3, or 6 mg/kg CP-99,996 (open bars), 0.65 mg/kg HOE 140 (hatched bars), 1 mg/kg ketotifen (solid bars), or vehicle (shaded bars) before formaldehyde challenge. Sham: animals were exposed to the sham gas for 15 ppm formaldehyde (10 minutes) after pretreatment with 0.9% saline (0.5 mL/kg i.v.). Data are the means ± SEM of six to seven rats/group. *$p < 0.05$ versus sham-stimulated group (unpaired Student's *t* test or Welch's test). †$p < 0.05$. ††$p < 0.01$ versus 0.9% saline-pretreated, formaldehyde-exposed control group (Williams' test).

Source: Redrawn from Ito et al. (1996).

Neither the bradykinin B_2 nor histamine H_1 receptor agonists affected formaldehyde-induced vascular permeability (Ito et al., 1996). Therefore, the immediate effect of formaldehyde exposure on vascular permeability is mediated, at least in part, through the NK_1 receptor but does not seem to require the B_2 or H_1 receptors. This implies a role for tachykinins in formaldehyde-induced vascular permeability. These findings suggest a neurogenic inflammatory response because the tachykinins are released from sensory nerve endings in the trachea and bronchi, whereas bradykinin is released from mast cells.

Sadakane et al. (2002) investigated the effects of formaldehyde exposure on airway inflammation caused by Der f. Two groups of male outbred ICR mice (18/group) were exposed to an aerosol of 0.5% formaldehyde solution produced by an ultrasonic nebulizer for 15 minutes, once a week for 4 weeks. Two groups were similarly treated but exposed to saline aerosol only. Details of the aerosol generation and resulting magnitude of exposure were not given. One group each of control and formaldehyde-exposed mice was sensitized to Der f by an injection 1

day prior to formaldehyde exposure (1.5 mg/animal). The same groups were challenged with intratracheal instillation of Der f (10 μg/animal) after 4 weeks. Three days after allergen challenge, mice were sacrificed and blood plasma and lung tissue were collected. Blood plasma was analyzed for Der f-specific immunoglobulins (IgG1 and IgE). Lungs from nine mice in each treatment group were homogenized, and Th1 cytokine IL-2, Th2 cytokines IL-4 and IL-5, granulocyte macrophage-colony-stimulating factor (GM-CSF), and the "chemokine regulated upon activation, normal T-cell expressed and secreted" (RANTES) protein levels were quantified in the supernatant via ELISA. Lungs from nine mice in each group were fixed, sectioned, and stained to evaluate eosinophil infiltration, lymphocyte infiltration, goblet cell proliferation, and localization of RANTES in the airway epithelium.

Der f-specific IgG1 was present in blood plasma of sensitized mice but was unchanged by formaldehyde exposure (Sadakane et al., 2002). IgE was too low to titer. IL-2 and GM-CSF were undetected in lung homogenate supernatant, and Il-4 was unchanged by sensitization or formaldehyde exposure. However, RANTES was increased by both formaldehyde exposure and allergen sensitization and challenge (see Table 4-52). These increases were more pronounced but less than additive for formaldehyde-exposed, allergen-sensitized mice. IL-5 was increased by allergen but unaffected by formaldehyde exposure only. However, formaldehyde exposure potentiated the IL-5 increase seen with allergen challenge.

Table 4-52. Cytokine and chemokine levels in lung tissue homogenate supernatants in formaldehyde-exposed male ICR mice with and without Der f sensitization

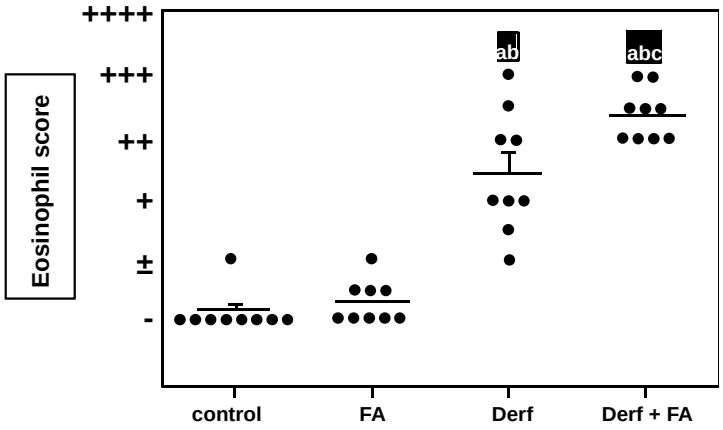
Group	Formaldehyde	Der f	GM-CSF	IL-2	IL-4	IL-5	RANTES
1	-	-	ND[a]	ND	68.1 ± .9	4.4 ± 0.3	200.1 ± 19.7
2	+	-	ND	ND	59.5 ± 4.3	4.1 ± 0.2	390.6 ± 37.4[b]
3	-	+	ND	ND	70.7 ± 4.9	13.6 ± 1.6 [c,e]	479.6 ± 80.0[c]
4	+	+	ND	ND	62.3 ± 5.8	21.6 ± 2.7 [c,e,f]	593.3 ± 58.2[c,d]

[a]None detected.
[b]$p < 0.05$ from control.
[c]$p < 0.001$ from control.
[d]$p < 0.05$ from Group 2.
[e]$p < 0.001$ from Group 2.
[f]$p < 0.001$ from Group 3.

Source: Sadakane et al. (2002).

Der f sensitization and challenge increased eosinophil infiltration into the interstitium around the bronchi and bronchioles as well as goblet cell proliferation in the bronchial epithelium (see Figure 4-21). Formaldehyde exposure exacerbated the eosinophilic and goblet cell responses to a challenge dose of Der f ($p < 0.05$) (Sadakane et al., 2002). Formaldehyde-induced eosinophilic infiltration in the absence of sensitization and challenge was not different from nontreated, nonsensitized mice.

Panel A



Panel B

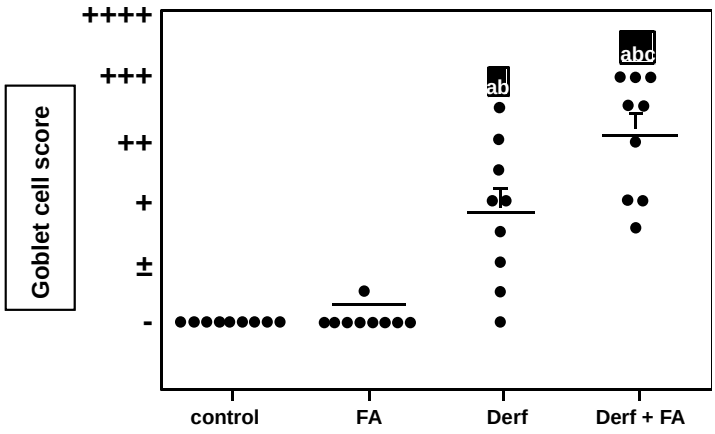


Figure 4-21. The effects of formaldehyde inhalation exposures on eosinophil infiltration (Panel A) and goblet cell proliferation (Panel B) after Der f challenge in the nasal mucosa of male ICR mice after sensitization and challenge.

Note: [a]$p < 0.001$ compared with control group; [b]$p < 0.001$ compared with formaldehyde group; [c]$p < 0.05$ compared with Der f group.

Source: Redrawn from Sadakane et al. (2002).

These results suggest that formaldehyde exposure may aggravate eosinophilic infiltration and goblet cell proliferation that accompanies allergic responses. This response is associated with an increase in IL-5, an eosinophilic attractant, and an increase in RANTES, which recruits eosinophils by chemotaxis in formaldehyde-exposed and Der f challenged animals, although the effect was not statistically significantly elevated compared with Der f challenge-induced levels of IL-5 and RANTES alone.

Fujimaki et al. (2004a) investigated the long-term effects of low-dose formaldehyde exposure on immunologic and neurological inflammation. Female C3H/He mice were exposed to 0, 0.082, 0.393, or 1.87 ppm (0, 0.1, 0.48, or 2.3 mg/m^3) formaldehyde 16 hours/day, 5 days/week for 12 weeks. Six mice at each exposure level were given injections of OVA plus adjuvant before the initial exposure and in weeks 3, 6, 9, and 11 of the experiment. Five mice at each formaldehyde-exposure level did not receive OVA injections. One day after the last exposure, mice were weighed and blood, BAL, spleen, and thymus were collected from each animal. After weighing, spleens were disaggregated and spleen cells harvested for cell culture. Immunophenotype of the spleen cells was determined by flow cytometry (CD4, CD8, CD3, and CD19 positive cells). Lymphocyte proliferation in response to lipopolysaccharide (LPS), phytohemagglutinin A (PHA), or OVA was determined after 72 hours in culture. Splenocytes were cultured for 48 hours in the presence of LPS, PHA, and OVA (immunized mice only), and supernatants were collected for cytokine analysis (IL-4, IL-5, and IFN-γ). Splenocytes were cultured for 24 hours in the presence or absence of OVA to assess chemokine production (MCP-1 and MIP1-α). Anti-OVA IgE, IgG_1, IgG_2, and IgG_3 were quantified in blood plasma.

Body and thymus weights were unchanged by formaldehyde exposure or OVA injection (Fujimaki et al., 2004a), while, in nonimmunized mice, spleen weights were reduced by formaldehyde exposure from 152 mg in controls to 128, 118, and 121 mg in mice exposed to 0.08, 0.4, and 1.8 ppm formaldehyde, respectively. Spleen weights tended to increase in groups exposed to 400 and 2,000 ppb formaldehyde compared with controls in OVA-immunized mice (control: 117.8 mg compared with 400 ppb: 168.6 mg and control: 121.0 mg compared with 2,000 ppb:153.2 mg, respectively) but were not statistically significant.

To gain insight on the overall pulmonary inflammatory response of mice exposed to formaldehyde in both immunized and nonimmunized mice, the total number and differential count of MPs, neutrophils, lymphocytes, and eosinophils in BAL were counted and were found to be unchanged by formaldehyde in nonimmunized mice. By contrast, in immunized mice exposed to 1.8 ppm formaldehyde, the total number of BAL cells, MPs, and eosinophils were significantly increased compared with nonimmunized controls (9.65 versus 2.84, 7.22 versus 2.74, and 2.0 versus 0.02 $\times 10^4$ cells, respectively).

To further assess the pulmonary inflammatory response, protein levels of inflammatory cytokines were determined by ELISA in BAL fluid. Levels of IL-1β in BAL of immunized mice were decreased by formaldehyde exposure ($p < 0.05$ at 1.8 ppm formaldehyde), but IL-1β levels after formaldehyde exposure were not different from controls in nonimmunized mice (Fujimaki et al., 2004a). All other cytokines or chemokines were either unchanged (TNF-α, IL-6, and GM-CSF) or not detected (eotaxin, MIP-1α, and MCP-1).

Various neuropeptides, such as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and substance P are released from vagal nerve endings and mediate a neurogenic inflammatory response. Levels of BDNF, NGF, and substance P were assessed in BAL fluid and/or in plasma. BDNF was not detected in BAL or in plasma. NGF levels in immunized mice were significantly higher than in nonimmunized mice in both BAL fluid and in plasma. NGF levels in immunized mice were significantly attenuated by 0.08 and 0.4 ppm formaldehyde exposure (see Figure 4-22) in both BAL fluid and in plasma. Plasma level of substance P (a mediator of neurogenic inflammation) was increased by formaldehyde exposures in nonimmunized mice (see Figure 4-23) in both BAL fluid and plasma. This increase appears to be dose-dependent and reaches statistical significance at 2,000 ppb formaldehyde exposure in nonimmunized mice compared with nonimmunized controls. Similar to NGF, levels of substance P increased in OVA-immunized mice compared with nonimmunized mice in both BAL fluid and plasma. Similar to NGF, levels of substance P in OVA-immunized mice were attenuated by formaldehyde exposure at 80 ppb.

Fujimaki et al. (2004a) further investigated the effect of low-level formaldehyde exposure from both immunized and nonimmunized mice on the systemic immune response. Spleens were removed from formaldehyde-exposed mice and were cultured in the presence of LPS or PHA (for nonimmunized samples) or OVA (for immunized samples). The secretory ability of immunized and nonimmunized spleen cells was assessed by measuring IFN-γ release by ELISA. Formaldehyde exposure (1.8 ppm) increased IFN-γ fourfold in LPS-stimulated cultured spleen cells from nonimmunized mice. No other cytokine or chemokine was changed by formaldehyde exposure in cultured spleen cells from nonimmunized mice. In OVA-immunized mice, formaldehyde had no significant effect on cytokines from stimulated spleen cells. OVA in vitro stimulation significantly increased the chemokines MIP-1 and MCP-1 for control and formaldehyde-treated OVA-immunized mice. The OVA-stimulated release of MCP-1 in vitro was enhanced by formaldehyde exposure in a concentration-dependent manner, increasing threefold and fourfold at 0.40 and 1.8 ppm, respectively. Increases in MCP-1 correlate with reported increases in the associated cytokine, RANTES, which recruits eosinophils by chemotaxis (Sadakane et al., 2002). These formaldehyde-induced increases in cytokine levels

contribute to pulmonary inflammation. The inflammatory response is not mediated by lymphocytes, since lymphocyte subsets and in vitro cell proliferation were unchanged by OVA immunization or formaldehyde treatment (Fujimaki et al., 2004a).

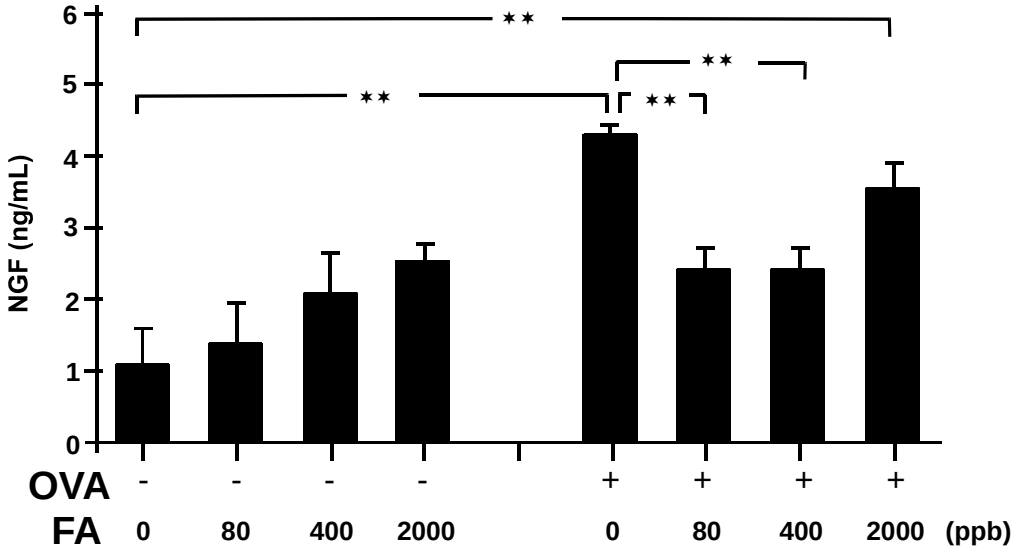


Figure 4-22. NGF in BAL fluid from formaldehyde-exposed female C3H/He mice with and without OVA sensitization.

Note: The day after the final formaldehyde inhalation, BAL fluid was collected from formaldehyde-exposed, nonimmunized and formaldehyde-exposed, OVA-immunized mice, and the production of NGF was determined by ELISA. Data are mean ± SEM from five to six animals. $^{**}p < 0.01$.

Source: Redrawn from Fujimaki et al. (2004a).

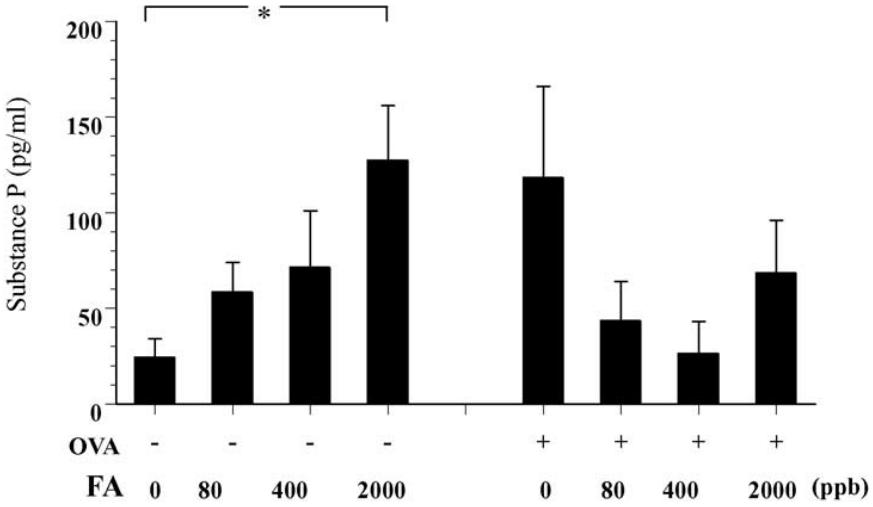


Figure 4-23. Plasma substance P levels in formaldehyde-exposed female C3H/He mice with and without OVA sensitization.

Note: The day after the final formaldehyde inhalation, plasma samples were collected from formaldehyde-exposed, nonimmunized, and formaldehyde-exposed OVA-immunized mice, and the levels of substance P were determined by ELISA. Data are mean ± SEM from five to six animals. $*p < 0.05$. FA = formaldehyde.

Source: Fujimaki et al. (2004a) as amended in Fujimaki et al. (2005; errata).

Anti-OVA (IgE and IgG_{2a}) levels in plasma were unchanged by formaldehyde exposure. Anti-OVA IgG_1 was reduced in immunized mice exposed to 400 ppb formaldehyde compared with nonexposed animals. However, this effect did not persist as dose increased. Anti-OVA IgG_3 was depressed in immunized mice exposed to 0.08 and 0.4 ppm formaldehyde (Fujimaki et al., 2004a). Formaldehyde exposure did not induce an inflammatory response in lung or tracheal epithelium in sections viewed by light microscopy (Fujimaki et al., 2004a). Although there was a mild infiltration of mast cells into the epithelium of OVA-immunized mice, there were no effects of formaldehyde treatment on mast cell infiltration.

A recent study by Lino dos Santos Franco et al. (2009) exposed male Wistar rats for 3 days, 90-minutes/day, to 1% formaldehyde (by weight; exact doses not reported) by inhalation. Of these, one group was sensitized I.P. to OVA (10 μg), a common allergen, immediately following formaldehyde exposure, and subsequently challenged with OVA 2 weeks later. Other rats were sensitized and challenged but were not exposed to formaldehyde. PCA reaction as well as BAL analysis and whole blood analysis were conducted. Immunohistochemical analysis of

platelet endothelial cell adhesion molecule-1 (PECAM-1) expression, an inflammatory mediator, in lung tissue was also measured. When formaldehyde exposure was followed by OVA sensitization and challenge, decreased lung inflammation was reported compared with the group that was OVA-sensitized but had not been exposed to formaldehyde. Reduced lung mast cell degranulation was also reported in the formaldehyde/OVA group compared with the nonexposed OVA group. Total circulating leukocytes, total bone marrow cells, and lung protein expression levels of PECAM-1 were also significantly decreased in formaldehyde/OVA rats compared with non-formaldehyde exposed OVA rats. The reduction in inflammatory parameters in response to formaldehyde may be attributed to different study designs, since in this study animals were sensitized after exposure rather than prior to exposure. The results suggest that formaldehyde may functionally alter the activity of certain cells, like mast cells, that may downgrade an appropriate immune response to antigen and might serve to threaten lung homeostasis. Due to the unique experimental design of this study, it cannot be directly compared with Sadakane et al. (2002) or Fujimaki et al. (2004a). In addition, this study did not intend to measure whether formaldehyde can exacerbate an asthmatic response but rather set out to identify whether formaldehyde could affect immune homeostasis.

In summary, studies suggest that formaldehyde exposure may induce a predominantly neurogenic inflammatory response via release of neuropeptide, such as NGF and substance P from vagal nerve endings. Formaldehyde does not appear to potentiate a systemic immune response. However, localized pulmonary inflammation can be potentiated by formaldehyde exposure, as indicated by the increased presence of eosinophils and certain proinflammatory cytokines (IFN-γ). This response does not appear to be mediated by classic immunogenic mechanisms since studies have failed to report elevated levels of anti-formaldehyde-specific IgE. Several studies have shown that exposure to formaldehyde can facilitate allergic sensitization in previously naïve animals, and it is thought that this effect may occur due to formaldehyde's ability to increase microvascular leakage in the nasal epithelium and by causing damage to the nasal barrier (Ito et al., 1996). Sadakane et al. (2002) demonstrated that formaldehyde exposure can also exacerbate allergic responses by enhancing the response to challenge allergen. Thus, formaldehyde may exacerbate allergic responsiveness by aggravating the sensitization response in previously naïve animals by altering the permeability of the mucosal barrier in nasal compartments. Neurogenically derived inflammation, including stimulation of the trigeminal nerve and release of braykinin, suggests that the MOA for sensitization may ultimately have its roots in neurogenic inflammation rather than an immunogenic response. In addition, using a different protocol, Lino dos Santos Franco et al. (2009) suggest that formaldehyde exposure can adversely affect lung homeostasis by reducing the activity of important inflammatory mediators

(mast cells, circulating leukocytes, PECAM-1 expression) when it occurs prior to sensitization, thus downgrading an appropriate immune response.

4.2.5.2. *Dermal Sensitization*

Wahlberg (1993) used Hartley strain guinea pigs as test animals to determine the skin irritancy of a suite of industrial chemicals, including formaldehyde. Aqueous solutions of the compound in a 0.1 mL volume were applied to the shaved flanks of guinea pigs and gently rubbed into the skin with a cotton-tipped applicator. Sites were left open and the treatments repeated once daily for 10 days. A number of indices of acute skin irritation were monitored, including erythema via visual scoring and edema and skin-fold thickness using Harpenden calipers. Varying concentrations of formaldehyde (up to a 10% solution) induced a dose-dependent increase in skin-fold thickness. Responses also showed shorter latencies at the higher concentrations. For example, erythema was first observed on day 2 when 10% formaldehyde was applied, day 5 (for 3%), and day 6 (for 1%).

Lee et al (1984) investigated the role of different routes of exposure in formaldehyde-induced allergic sensitization. Two sets of four male English smooth-haired guinea pigs received topical applications of 100 μL 37% w/v formalin distributed over two shaved, depilated dorsal sites two times over the course of 2 days at different sites. The total dose was calculated as 74 μg/animal. In addition, eight animals received a single topical application onto a 15 mm area of the dorsal surface. The applied dose of 25 μL formaldehyde was dissolved in saline. Two other groups of guinea pigs were exposed to either 6 ppm (6 hours/day for 5 days) or 10 ppm (6 hours/day for 5 days) formaldehyde by inhalation. A third group of guinea pigs was exposed to 10 ppm formaldehyde for 8 hours/day for 5 consecutive days by inhalation. All animals were evaluated for contact sensitivity by topical application of 20 mL formaldehyde diluted with saline and distributed in a 15 mm area on the backs of the shaved guinea pigs (Lee et al., 1984). Sites were visually inspected for erythema at 1, 6, 24, and 48 hours following the topical application, and reactions were scored. No erythema was observed in control animals. None of the guinea pigs in the 6 hours/day inhalation groups (6 and 10 ppm formaldehyde) developed skin sensitivity tested on day 9 (4 days after the initial exposure regimen ended). Two of four guinea pigs exposed to 10 ppm formaldehyde for 8 hours/day for 5 consecutive days developed mild skin sensitization tested on day 31. Contact sensitivity increased in a dose-dependent fashion in groups of animals that had been sensitized via the dermal route. Thus, dermal exposure resulted in contact sensitivity. Inhalation exposure did not consistently produce contact sensitivity.

Arts et al. (1997) used a local lymph node assay (LLNA) and the induction of IgE to monitor the sensitization of female Wistar rats (low IgE-responders) and BN rats (high IgE responders). For the LLNA assay, animals were sensitized by the application of varying concentrations of formaldehyde in raffinated olive oil on the dorsum of both ears on days 0, 1, and 2. Control animals were treated with raffinated olive oil alone. Animals received an I.P. injection of BrdU on day 5 and were subsequently sacrificed. Ear-draining lymph nodes were collected, fixed, and sectioned, and the mitotic activity was monitored following successive incubation of the sections in anti-BrdU, biotin-labeled rabbit anti-mouse antibody, peroxidase-conjugated streptavidin, and 3,3-diaminobenzidine tetrahydrochloride. For serum IgE responses, 150 µL of different concentrations of formaldehyde were applied to the shaved flanks of rats on day 1, then 75 µL of the same chemical at 50% of the initial concentration were applied to the dorsum of each ear on day 7. The amount of IgE in the blood was measured using ELISA but appeared to be little affected by formaldehyde treatment in either species of rat. However, the ear-draining lymph nodes of both strains of rat showed a comparative increase in weight in response to formaldehyde, and proliferation (BrdU positive) of paracortical cells was observed in response to increasing doses of the compound. This response was most notable in BN rats treated with 10% formaldehyde. Arts et al. (1997) concluded that the irritant and sensitizing properties of formaldehyde may act through non-IgE-immune mechanisms.

Hilton et al. (1998) used the LLNA assay in female CBA/Ca ($H\text{-}2^k$ haplotype) mice to compare the skin sensitizing potencies of formaldehyde and glutaraldehyde. The comparison was set on a quantitative basis by determining the concentration of each compound necessary to induce a threefold increase in lymph node cell proliferative activity (effective concentration [EC_3]). While both aldehydes induced a dose-dependent proliferative response, the incorporation of [^{3}H]-methylthymidine was far greater in animals exposed to glutaraldehyde versus formaldehyde (with EC_3 values of 0.002−0.006 mol/L for glutaraldehyde versus 0.11−0.18 mol/L for formaldehyde). These data indicate the potential of both chemicals to induce skin sensitization, although the potency of glutaraldehyde was far greater than that of formaldehyde.

Xu et al. (2002) evaluated the extent to which the expression of some cytokines may change as a result of cutaneous exposure to formaldehyde in mice. Female Balb/C mice were skin painted with three topical applications of 100 µL of 17.5% formaldehyde or distilled water with a 1-day interval between each application. Spleen and draining lymph nodes were harvested on days 3, 5, 7, 9, or 12 after the last skin painting. In some animals, contact hypersensitivity was induced by applying 2% formaldehyde to both sides of mouse ears on day 3 following the last skin painting. For this endpoint, the percent increase in thickness of the ears

was monitored. For the cytokines, mRNA expression levels of IL-2, IL-4, IL-5, IL-10, IL-12, IL-13, IL-15, IL-18, and INF-γ were determined semiquantitatively by measuring the amount of individual mRNAs following amplification with the reverse transcriptase (RT)-PCR. The relative amounts of cytokine mRNAs were calculated as the ratio of cytokine mRNA to that of glyceraldehyde-3-phosphate dehydrogenase, as revealed in specific bands on an agarose gel.

Cutaneous formaldehyde treatment was associated with the long-lasting expression of IL-4 and IFN-γ mRNAs in mouse spleen and draining lymph nodes and with IL-15 mRNA only in mouse spleen. Only IL-13 mRNAs displayed a transient increase in expression in both spleen and draining lymph nodes. Levels of IL-2, IL-12, and IL-15 were increased in the mouse spleen but not the lymph nodes. The mouse ear swelling test gave positive correlations with enhanced expression of mRNA for IL-4 and IFN-γ (see Table 4-53).

Table 4-53. Correlation coefficients among ear swelling responses and skin mRNA levels in contact hypersensitivity to formaldehyde in mice

Variables	Correlation coefficients		
	IL-2	IL-4	IFN-γ
Ear swelling	0.50	0.74[a]	0.67[a]
IL-2	–	0.39	0.60
IL-4	–	–	0.79[a]

[a]Statistically significant ($p < 0.05$).

Source: Xu et al. (2002).

4.2.5.3. *Summary of Sensitization Studies*

Several animal studies report increased airway resistance and BC due to inhalation exposures to formaldehyde (Nielsen et al., 1999; Swiecichowski et al., 1993; Biagini et al., 1989; Amdur, 1960). Changes in pulmonary resistance were observed as early as 10 minutes after exposure (Biagini et al., 1989), and reported effect levels ranged from 0.3−13 ppm. Other pulmonary effects were reported in conjunction with BHR, such as increased tracheal reactivity and decreased pulmonary elasticity (Swiecichowski et al., 1993; Amdur, 1960). Although BHR is a common result of Type I hypersensitivity reaction to an allergen, the observation of BHR alone is not sufficient to demonstrate that an agent induces Type 1 hypersensitivity.

BHR may be directly induced both pharmacologically and neurogenically (Joos, 2003; Cain, 2001; Meggs, 1995). There is little evidence that formaldehyde itself is an allergen recognized by the immune system, especially via inhalation (Lee et al., 1984). Although

formaldehyde exposure has been reported to alter cytokine levels and immunoglobulins in some experimental systems, these immunomodulatory effects do not support a type 1 hypersensitivity. IgE was unchanged (Fujimaki et al., 2004a; Lee et al., 1984), and cytokine profiles were not consistent with the Th-2 cytokines expected in IgE mediated hypersensitivity (Fujimaki et al., 2004a; Ohtsuka et al., 2003).

Formaldehyde-induced dermal sensitization show parallel results. The physical signs of irritation and sensitization are consistently shown (e.g., rashes, edema). Some involvement of the immune response has been demonstrated with positive LLNA assays, indicating proliferation of lymphocytes in lymph nodes draining the affected area (Hilton et al., 1998; Arts et al., 1997). Increased expression of Th-2 cytokines in the lymph nodes of mice given dermal applications of formaldehyde does indicate an immune component to the observed sensitization. However, the response does not seem to be mediated by IgE (Arts et al., 1997; Lee et al., 1984).

Ito et al. (1996) reported that a tachykinin NK_1 receptor, but not the histamine H_1 or bradykinin B_2 receptors, is involved in formaldehyde-induced vascular permeability. Neuropeptides NGF and substance P were affected in BAL and stimulated splenocytes from formaldehyde-exposed mice, with greater effects seen in OVA-immunized mice. Tachykinins (e.g., substance P and neurokinin A) are produced by nerve cells and can directly stimulate bronchoconstriction (Van Schoor et al., 2000). Substance P is also a mediator of neurogenic inflammation. Therefore, although formaldehyde may induce some of the symptoms of type 1 hypersensitivity, these symptoms are more likely neurogenic than immunogenic in origin.

In contrast, formaldehyde enhances immunogenic hypersensitivity of known allergens (Sadakane et al., 2002; Riedel et al., 1996; Tarkowski and Gorski, 1995). This potentiation varied based on sensitization protocols (respiratory tract versus systemic, frequency and timing of immunization, allergen, etc.) and formaldehyde exposure regimens (concentration, continuous versus intermittent exposures). Taken as a whole, the results support the finding that formaldehyde exposure can aggravate a type 1 hypersensitivity response (see Table 4-54).

Table 4-54: Summary of sensitization and atopy studies by inhalation or dermal sensitization due to formaldehyde in experimental animals

Species	No./ group	Treatment[a]	Observations	LOAEL/ NOAEL	Reference
Inhalation studies					
Cynomolgus monkeys	9	Methacholine-sensitive monkeys exposed to 2.5 ppm formaldehyde for 10 minutes	Formaldehyde increased pulmonary resistance after 2, 5, and 10 minutes.	LOAEL 2.5 ppm	Biagini et al. (1989)
Hartley guinea pigs (male)	8	0.86, 3.4, 9.4, 31.1 ppm formaldehyde for 2 hours or 0.11, 0.31, 0.59, 1.05 ppm for 8 hours	Total pulmonary resistance increased after 2 hours exposure at 9.4 and 31.1 ppm. Effect was reversible and returned to baseline within 30 minutes. Total pulmonary resistance was increased after 8 hours exposure at 0.3 and 1 ppm. Amount of acetylcholine needed to achieve doubled pulmonary resistance was decreased in animals after 2 hours exposure.	NA	Swiecichowski et al. (1993)
Hartley guinea pigs (male)	5−7	3.4 ppm for 8 hours	No changes in ex vivo tracheal constriction or inflammation.	NA	Swiecichowski et al. (1993)
F344 rats and BN rats	5	16 ppm 3 hours/day, 5 days	Modest changes in inflammatory cytokine expression, but respiratory and olfactory epithelial lesions were more severe in F344 rats than in BN rats.	NA	Ohtsuka et al. (2003)
English smooth-haired guinea pigs	4	6, 10 ppm, 6 hours/day, 5 days, combined with provocation challenge (2 or 4 ppm on day 7, or days 7, 22, and 29)	Inhalation challenge with 6 or 10 ppm followed by bronchial challenge failed to increase respiratory sensitivity	NA	Lee et al. (1984)
Perlbright-white, Duncan-Hartley guinea pigs (female)	12	0, 0.13, 0.25 ppm 8 hours/day, 5 days. The animals were sensitized to OVA (3 minutes exposure to 5% OVA aerosol)	Anti-OVA titer was significantly elevated over controls in animals exposed to 0.25 ppm formaldehyde and showed that formaldehyde may sensitize previously naïve animals to OVA.	NA	Riedel et al. (1996)
Balb/C mice (female)	4	0, 6.63 ppm 6 hours/day for 10 days or 6 hours/day once/week for 7 weeks. All mice were sensitized to OVA	Formaldehyde administered intranasally for 6 hours/day for 10 days may facilitate sensitization to allergens since anti-OVA titers were elevated over control animals. However, the length and duration of exposure appears to affect development of sensitization.	NA	Tarkowski and Gorski (1995)

Table 4-54: Summary of sensitization and atopy studies by inhalation or dermal sensitization due to formaldehyde in experimental animals (continued)

Species	No./ group	Treatment[a]	Observations	LOAEL/ NOAEL	Reference
Wistar rats (male)	5−8	0, 2, 5, 15, 45 ppm for 10 minutes	Pulmonary insufflation or blood pressure were not altered. Vascular permeability increased in concentration-dependent manner and could be reduced by adding a NK1 selective antagonist.	NA	Ito et al. (1996)
Outbred ICR mice (male)	18	0.5% formaldehyde for 15 minutes, once/week for 4 weeks. Both control and exposed groups were exposed to Der f by I.P. injection 1 day before formaldehyde and then challenged with Der f after 4 weeks.	More pronounced RANTES production in formaldehyde-treated and sensitized rats than in sensitized rats that had not been exposed to formaldehyde. Formaldehyde also potentiated IL-5 production associated with sensitization.	NA	Sadakane et al. (2002)
C3H/HeJ mice (female)	6	0, 0.082, 0.393, 1.87 ppm 16 hours/day, 5 day/week, 12 weeks. Mice also given OVA plus adjuvant before exposure, and again 3, 6, 9, 11 weeks after exposure. Some formaldehyde mice did not receive any OVA	Substance P and NGF were increased dose dependently in formaldehyde-treated, nonimmunized mice but were attenuated in formaldehyde-treated immunized mice compared with nonexposed, immunized controls.	LOAEL 0.082 ppm	Fujimaki et al. (2004a)
Wistar rats (male)	NA	1% Formaldehyde by weight for 90 minutes for 3 days. One group was sensitized to OVA after to formaldehyde exposure and then challenged with OVA afterwards. Others were sensitized and challenged but not exposed to formaldehyde.	Total circulating leukocytes, bone marrow cells, and lung protein PECAM expression were significantly decreased in formaldehyde/OVA rats compared with OVA rats.	NA	Lino dos Santos Franco et al. (2009)

Table 4-54: Summary of sensitization and atopy studies by inhalation or dermal sensitization due to formaldehyde in experimental animals (continued)

Species	No./ group	Treatment[a]	Observations	LOAEL/ NOAEL	Reference
Dermal sensitization					
Hartley guinea pigs	5	Skin painted once/day for 10 days with 0.1 mL of 1, 3, or 10% formaldehyde	Varying concentrations (up to 10%) induced dose-dependent increase in skin-fold thickness. Erythema seen earlier at higher doses (2 days at 10% formaldehyde vs 5 days at 3% or 6 days at 1%).		Wahlberg et al. (1993)
English smooth-haired guinea pigs	4	Group 1: skin painted, 100 µL 37% formalin twice over 2 days, Group 2: single topical application of 25 µL formaldehyde Group 3: 10 ppm 6 hours/day for 5 days by inhalation	Two of four guinea pigs from group 3 had mild skin sensitization after day 31. Contact sensitivity developed in a dose-dependent manner in the dermal groups (group 1 and 2).		Lee et al. (1984)
Wistar and BN rats (female)	4	Application of formaldehyde to ears on days 0, 1, 2, followed by an I.P. injection of BrdU.	Ear-draining lymph nodes increased in weight in response to formaldehyde, reflected in increased number of BrdU-stained cells, most notably in BN rats (high IgE responders) treated with 10% formaldehyde		Arts et al. (1997)
CBA/Ca mice	NA	Compared glutaraldehyde to formaldehyde to induce a local lymph node assay	Glutaraldehyde and formaldehyde induced a dose-dependent proliferative response that was greater in glutaraldehyde-treated animals		Hilton et al. (1998)
Balb/c mice (female)	3−5	Skin painted with 100 µL of 17.5% formaldehyde every other day for days 3, 5, 7, 9, 12	Cutaneous treatment associated with long-lasting expression of various cytokines from draining lymph nodes and spleen.	NA	Xu et al. (2002)

NA = not applicable.

4.2.6. Neurological and Neurobehavioral Function

4.2.6.1. *Inhalation Exposure*

There are a number of published reports examining the effects of formaldehyde exposure on nervous system structure and function. The reports evaluating behavioral effects fall into three main categories: (1) behavioral responses evaluated during or immediately following formaldehyde exposures, which may include effects due to the potential irritant properties of the chemical, (2) acute or short-term exposures followed by behavioral assessments conducted 2−24 hours after termination of formaldehyde exposure, which reflect sustained effects of chemical exposure independent of its irritant properties, and (3) repeated exposures to formaldehyde followed by neurological assessments performed throughout the treatment period or several days to weeks after termination of treatment. In addition to reports evaluating changes in behavior, there are several reports evaluating neuropathological effects or changes in brain chemistry.

4.2.6.1.1. *Behavioral response.*

4.2.6.1.1.1. *Clinical signs.*

Several studies that were focused on general toxicity or carcinogenicity of formaldehyde also assessed clinical signs in exposed animals, which may be related to adverse effects on the nervous system. Procedural details for the assessments, or specific data regarding findings, were not provided. Signs recorded included uncoordinated locomotion and climbing of cage walls at 20 ppm formaldehyde in rats (Woutersen et al., 1987); restlessness at 15 ppm formaldehyde in rats (Morgan et al., 1986a); dyspnea, listlessness, and hunched posture at 20 ppm and ataxia at 40 ppm in mice (Maronpot et al., 1986); and dyspnea in rats at 14.3 ppm formaldehyde (Kerns et al., 1983). Given the lack of information regarding procedures used for these evaluations and the limited reporting of results, the utility of these data is limited.

4.2.6.1.1.2. *Irritant threshold detection.*

Wood and Coleman (1995) evaluated the irritant properties of acute formaldehyde exposure in mice. Adult male Swiss mice (eight/group) were initially trained to terminate a 60-second exposure to an irritant gas (ammonia, 1,000 ppm) by poking their noses into a conical sensor five times to produce a 60-second facial shower of clean air. Each test session consisted of 25 exposure trials. Following training, response to formaldehyde was evaluated, using the same testing scenario. Each day mice had a morning exposure session to ammonia and an afternoon session to formaldehyde. Formaldehyde concentrations tested were different each day, in sequence from 0, 1, 1.8, 3, 5.6, and 10 ppm (0, 1.23, 2.21, 3.68, 6.87, and 12.3 mg/m^3) and then stepping back again from 10 to 0 ppm. Half of the animals were tested in an ascending

order of formaldehyde concentrations, the other half in a descending order. The frequency of terminating exposure, error rate, and the time lapse to termination were recorded. The concentration at which 50% of the formaldehyde deliveries would be expected to be terminated was estimated (AC_{50}) by simple linear regression or by analysis of covariance on the logit transform of percentage terminated as a function of log concentration.

All mice were trained successfully to terminate 100% of ammonia exposures, but varied responses were observed with formaldehyde exposure. In general, time taken to terminate formaldehyde exposure decreased significantly with increasing formaldehyde concentration. Mice terminated more exposures to 1 ppm formaldehyde than to air alone ($p < 0.0005$), and the error rate, generally below 40%, did not significantly differ with formaldehyde concentration tested. Each animal had two test sessions with each formaldehyde concentration (once during the ascending sequence and again during the descending sequence); both the time to termination ($p < 0.0012$) and AC_{50} were decreased in the second series of tests. One method of estimation by the authors yielded an AC_{50} of 3.63 ppm for the first series of tests versus 1.88 ppm for the second series. A two-way repeated measures ANOVA with replication and concentration as within variables was highly significant ($p < 0.00005$). These studies indicate mice are sensitive to the irritant properties of formaldehyde at exposure concentrations as low as 1 ppm, and animals reacted more swiftly and with greater accuracy to terminate formaldehyde exposure as the concentration increased. However, a wide variety of responses was noted on an individual animal basis. Two of the eight mice terminated 90% of the trials during 1 ppm exposures and 80−100% of trials at all other tested formaldehyde concentrations. One mouse terminated fewer than 10% of the formaldehyde exposure trials (1−10 ppm) during the testing regimen but had a 92% response rate to 20 ppm formaldehyde. The remaining five mice responded with increasing termination frequency as formaldehyde concentration increased from 1 to 10 ppm, with an AC_{50} of 2.72 ppm (3.34 mg/m^3).

4.2.6.1.2. *Motor activity and habituation.*

Malek et al. (2003a) examined open field behavior of rats after acute formaldehyde exposures. Male and female LEW.1K rats (15/sex/group) were exposed to 0, 1, 2.5, or 5 ppm (0, 1.23, 3.08, or 6.15 mg/m^3) formaldehyde for 2 hours. Formaldehyde was vaporized from aqueous solutions directly below the exposure chamber. Formaldehyde levels were checked 16 times throughout the 2-hour exposure periods. Mean formaldehyde levels of 1.01 ± 0.29 ppm, 2.51 ppm (SD is missing), and 5.0 ± 0.27 ppm were achieved. Locomotor activity was assessed for 3 minutes in an open field 2 hours after termination of formaldehyde exposure and again 24 hours later, using an automated device to count the number of squares crossed. Other behaviors

were noted, including grooming (face cleaning, fur licking, and scratching), rearing, sniffing (air and floor), wall climbing, and defecation.

The authors reported no signs of irritation or changes in activity or food or water intake during exposure. In general, sniffing was increased after formaldehyde exposure and movement was decreased (crossed quadrants and climbing) in both male and female rats ($p < 0.05$). Significant reductions in horizontal movements (crossed quadrants) were observed at all dose levels and were characterized by a U-shaped dose response (see Figure 4-24). The lowest dose tested (1 ppm) demonstrated a higher level of activity suppression than the two higher doses, but all groups were still suppressed relative to controls. Although female rats displayed a greater level of activity overall, a similar U-shaped dose-response pattern was also observed.

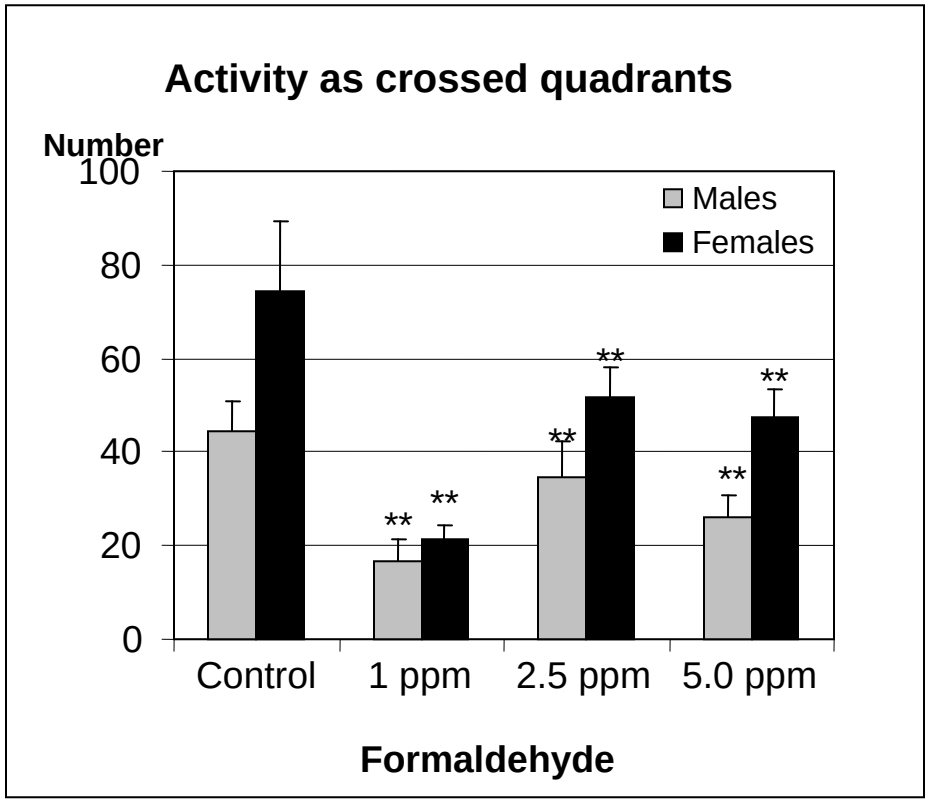


Figure 4-24. Motor activity in male and female rats 2 hours after exposure to formaldehyde expressed as mean number of crossed quadrants ± SEM. Greater reductions were observed in the lowest dose group, a pattern that was evident in both genders. ** = different from control, $p < 0.005$.

Source: Drawn from data reported by Malek et al. (2003b).

Activity in the same apparatus was reassessed 24 hours later. As expected, controls demonstrated habituation to the test apparatus, exhibiting only 20% of the motor activity observed on day 1 (see Figure 4-25). In contrast, formaldehyde-treated animals failed to demonstrate the same degree of habituation. Activity levels for males observed on day 2 were 60−80% of the activity levels seen on day 1 ($p < 0.005$). Formaldehyde-treated females also failed to habituate and actually demonstrated increases in activity on day 2 relative to day 1 at all formaldehyde exposure levels ($p < 0.005$).

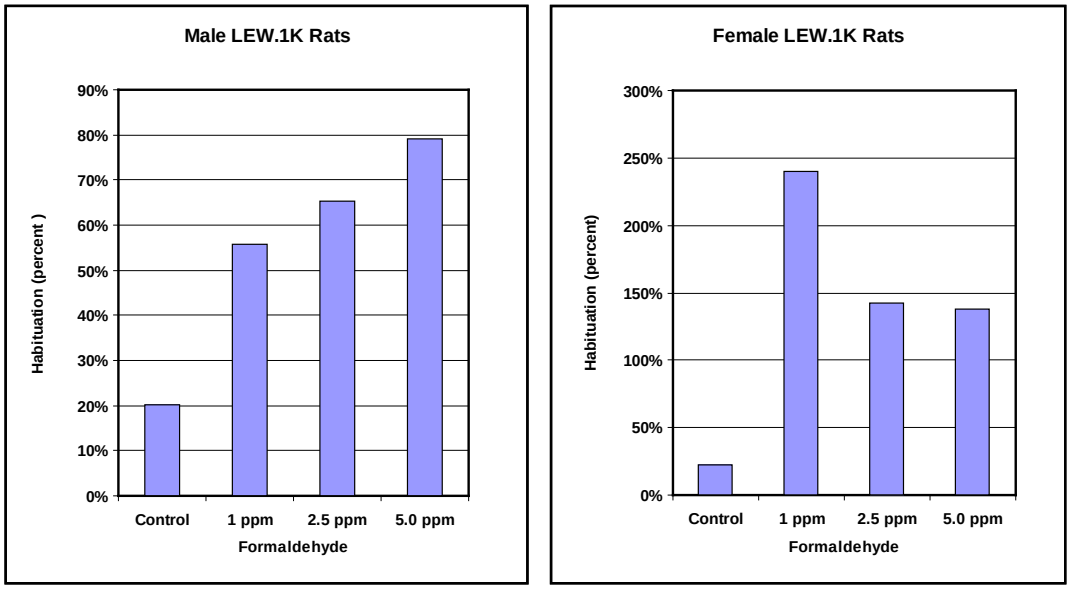


Figure 4-25. Habituation of motor activity was observed in control rats during the second observation period (day 2, 24 hours after formaldehyde exposure).

Note: Habituation is shown here as the percent decrease in number of crossings between sessions from day 1 to day 2. The degree of habituation was reduced in male rats exposed to formaldehyde (left panel) since their activity was closer to 100% of that seen on day 1. Females (right panel) had increased activity on day 2 (greater than 100% of activity on day 1), which is a sensitization rather than habituation.

Source: Drawn from data reported by Malek et al. (2003a).

A follow-up study by Malek et al. (2003b) further expanded the dose-response analysis for acute formaldehyde exposure. As described above, male and female LEW.1K rats (10 per sex per group) were exposed at 0, 0.1, 0.5, or 5 ppm (0, 0.123, 0.615, or 6.15 mg/m^3) formaldehyde for 2 hours. Formaldehyde levels were checked nine times per hour during the

exposure periods, and mean values were found to be 0.13 ± 0.04, 0.48 ± 0.05, and 5.18 ± 0.66 ppm. Open field behavior was evaluated for each animal 2 hours after formaldehyde exposure. The number of crossed quadrants for both controls and a 5 ppm group were generally comparable with those observed in the first study, although female values were somewhat lower. Horizontal movement was decreased by formaldehyde exposure in a dose-dependent manner with significant reductions in motor activity as low as 0.1 ppm in males and 0.5 ppm in females (see Figure 4-26). The consistency of the findings across studies and between genders provides greater confidence in the effects of low-level formaldehyde exposure on this standard test of neurotoxicity.

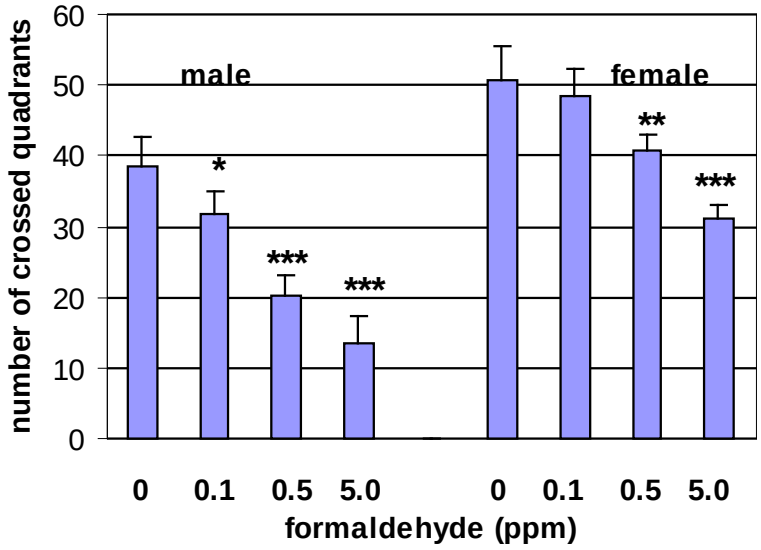


Figure 4-26. Motor activity was reduced in male and female LEW.1K rats 2 hours after termination of 10-minute formaldehyde exposure.

Note: Values are means ± SDs. * = different from control, $p < 0.05$. ** = different from controls, $p < 0.01$. *** = different from controls, $p < 0.001$.

Source: Drawn from data reported in Malek et al. (2003c).

Malek et al. (2004) also assessed the capacity of formaldehyde to induce persistent behavioral deficits in mice. Groups of 20 male AB mice received a single 2-hour exposure to 0, 1.1, 2.3, or 5.2 ppm (0, 1.3, 2.8, or 6.4 mg/m^3) formaldehyde prior to being tested 2 and 24 hours after exposure for a series of behavioral responses, including ambulation (crossed squares), grooming, sniffing, rearing, wall climbing, and defecation. Even though there were no clinical signs of toxicity in any of the exposed groups, a number of behavioral anomalies were apparent

in response to formaldehyde exposure, some of which persisted for at least 24 hours, as indicated in Tables 4-55 and 4-56.

Table 4-55. Fluctuation of behavioral responses when male AB mice inhaled formaldehyde in a single 2-hour exposure: effects after 2 hours

Open field parameter	Formaldehyde concentration (ppm)[a]			
	0	1.1	2.3	5.2
No. of crossed inner squares	34.10 ± 7.51	25.30 ± 5.03[b]	21.20 ± 3.41[b]	16.10 ± 5.37[b]
No. of crossed peripheral squares	56.65 ± 9.68	59.55 ± 9.75	49.70 ± 13.24	29.15 ± 7.47[b]
Total no. of crossed squares	90.75 ± 11.08	84.85 ± 9.96	71.10 ± 13.91[b]	44.20 ± 7.42[b]
Air sniffing	19.35 ± 2.5	21.50 ± 4.26	16.35 ± 3.84[c]	8.10 ± 1.77[b]
Floor sniffing	20.95 ± 3.72	26.50 ± 4.64[b]	21.35 ± 4.77	22.80 ± 4.02
Grooming	7.95 ± 2.26	7.10 ± 3.19	7.05 ± 2.48	6.55 ± 2.06
Rearing	17.85 ± 2.56	13.90 ± 3.19[b]	11.30 ± 2.30[b]	9.95 ± 1.61[b]
Wall climbing	13.20 ± 3.09	14.55 ± 2.74	13.95 ± 2.31	13.95 ± 1.82
No. of excreted fecal boli	0.65 ± 0.81	0.75 ± 0.85	0.80 ± 0.77	0.90 ± 1.12

[a]Values are means ± SDs.
[b]Statistical significance of differences from controls ($p < 0.005$).
[c]Statistical significance of differences from controls ($p < 0.05$).
Source: Malek et al. (2004).

Table 4-56. Fluctuation of behavioral responses when male AB mice inhaled formaldehyde in a single 2-hour exposure: effects after 24 hours

Open field parameter	Formaldehyde concentration (ppm)[a]			
	0	1.1	2.3	5.2
No. of crossed inner squares	10.40 ± 2.35	9.55 ±1.73	9.10 ± 1.25	9.70 ± 1.13
No. of crossed peripheral squares	42.80 ± 9.27	44.85 ± 14.60	44.95 ± 16.56	41.10 ± 9.08
Total no. of crossed squares	53.20 ± 8.67	54.40 ± 14.77	54.05 ± 15.81	50.80 ± 9.15
Air sniffing	13.65 ± 2.81	13.30 ± 3.21	12.65 ± 2.70	12.30 ± 4.14
Floor sniffing	21.55 ± 3.47	15.85 ± 3.94[b]	13.25 ± 4.17[b]	17.65 ± 3.13[b]
Grooming	8.35 ± 2.56	13.95 ± 2.21[b]	10.20 ± 3.33[c]	11.90 ± 3.26[b]
Rearing	18.30 ± 4.23	12.40 ± 2.23[b]	12.25 ± 2.17[b]	12.00 ± 3.32[b]
Wall climbing	9.25 ± 2.38	8.70 ± 1.98	8.20 ± 2.14	9.90 ± 2.27
No. of excreted fecal boli	0.80 ± 0.83	1.20 ± 0.83	1.60 ± 0.94[c]	1.20 ± 0.89

[a]Values are means ± SDs.
[b]Statistical significance of differences from controls ($p < 0.005$).
[c]Statistical significance of differences from controls ($p < 0.05$).
Source: Malek et al. (2004).

Usanmaz et al. (2002) assessed spontaneous locomotor activity (SLMA) in Balb/c mice (4−14 per group, sex unspecified) after both acute and subchronic formaldehyde exposures. Prior to the acute exposure, mice were acclimated to the exposure chamber for 4 days but exposed only to clean air. On the fifth day, mice (six/group, sex unspecified) were exposed for 3 hours at 0, 1.8, 3.2, 4.5, 6.4, 9.7, or 14.8 ppm (0, 2.2, 3.9, 5.5, 7.9, 11.9, or 18.2 mg/m^3) formaldehyde. Mice were removed from the exposure chamber, and SLMA behavior was evaluated by direct observation for 5 minutes. In addition to horizontal and vertical movement, wet dog shake (WDS) behavior was noted. In a separate trial, Balb/c mice (six/group, sex unspecified) were exposed to 8.2 ppm formaldehyde for 1 week, 2 ppm formaldehyde for 2 weeks, or 3.3 ppm formaldehyde for 3 weeks (3 hours/day, 5 days/week) compared with controls exposed only to air. SLMA behavior was observed for 5 minutes after the last exposure. Mice exposed to 8.2 ppm formaldehyde for 1 week, 3.3 ppm formaldehyde for 2 weeks, and 2 ppm formaldehyde for 3 weeks lost weight over the course of the treatment ($p < 0.05$). All other treatment groups had weight gain similar to control mice.

As shown in Figure 4-27, acute 3-hour formaldehyde exposures resulted in a dose-dependent decrease in SLMA. Decreases in horizontal activity were significant for the three highest dose groups (6.4, 9.7, and 14.8 ppm), and decreases in vertical activity were significant for all six formaldehyde treatment groups. SLMA was similarly decreased following subchronic exposures (data not shown here). Although the experimental protocol included longer exposures and a slightly longer observation period (5 versus 3 minutes) than in Malek et al. (2003a, b), the results are consistent, indicating decreased activity in formaldehyde-exposed animals several hours after exposure was ended.

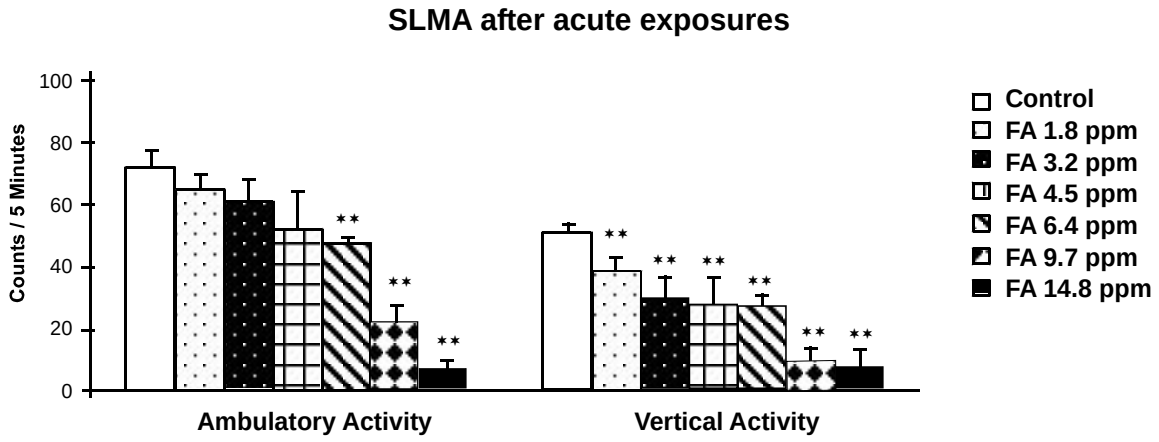


Figure 4-27. The effects of the acute formaldehyde exposures on the ambulatory and vertical components of SLMA.
Note: FA = formaldehyde exposure concentration. ** = $p < 0.01$ from controls.
Source: Usanmaz et al. (2001).

Usanmaz et al. (2002) noted an increase in WDS, after the acute exposures, as a possible preconvulsive effect. However, the mice were only observed for 5 minutes, and it is unclear how the researchers distinguished between a WDS due to an irritating odor and a preconvulsive movement. No other study has noted convulsive effects from formaldehyde exposure in any species. A second set of trials was reported in the same paper that sought to evaluate formaldehyde effects on CNS excitability. Balb/c mice (six/group, sex unspecified) were exposed to 0, 1.8, 6.4, or 14.8 ppm (0, 2.2, 7.9, or 18.2 mg/m^3) formaldehyde for 3 hours. Subchronic exposures were at 2 ppm (2.5 mg/m^3) formaldehyde for 3 weeks or 3.3 ppm (4.1 mg/m^3) formaldehyde for 2 weeks. Seizures were induced by I.P. injection of pentilenetetrazole (PTZ), and the incidence, severity, and course of induced seizures were recorded. The PTZ injection induced seizures in 83, 88, and 91% of controls, with 16, 38, and 67% mortality in controls in the three trials. Mortality was highly variable in treatment groups as well. The authors report that PTZ-induced seizures were decreased in incidence by acute formaldehyde exposure in a dose-dependent fashion with only 33% of mice exposed to 14.8 ppm formaldehyde experiencing seizures versus 91% in control mice ($p < 0.05$ at the highest dose only). However, the methodology for observing and scoring seizures is unclear. Additionally, there was high mortality and high variability of results for the three similarly treated control groups. Therefore, it is difficult to assess data quality and interpret these findings.

Boja et al. (1985) exposed male Sprague-Dawley rats to air or to formaldehyde at 5, 10, or 20 ppm for 3 hours on 2 consecutive days. On the second day, half the rats received the same exposure as the previous day, while half the rats were switched (e.g., half those rats receiving air the first day received formaldehyde the second day, and half those receiving formaldehyde the first day received air the second day), for a total of four possible exposure combinations. During the exposure period, activity levels were monitored by observation, once per minute for the first hour and once every 5 minutes for the second hour. At the end of the second exposure session, rats were sacrificed and brains removed for neurochemical analysis (see Section 4.2.6.1.5).

Behavioral results were described in detail only for control and 5 ppm groups. During the first exposure session, activity levels of formaldehyde-exposed animals were significantly decreased (approximately 50% of control levels). On the second day of exposure, those animals previously exposed to formaldehyde exhibited partial recovery, those experiencing their first formaldehyde exposure behaved similarly to those initially exposed on the first day, and those animals exposed to formaldehyde for a second time had a greater decrease in activity than during the first exposure (to approximately 30% of control levels). The authors stated that a similar effect was seen in animals exposed at 10 ppm but that results at 20 ppm were not interpretable

(data were not presented). Overall, the decreased activity seen in this study is consistent with effects seen by other authors.

Senichenkova (1991) exposed pregnant female rats to 0 or 0.5 mg/m^3 (0 or 403 ppb) formaldehyde on gestation days (GDs) 1−19 for 4 hours/day. Reproductive aspects of this study will be discussed in the reproduction section; however, results from behavioral assessments conducted on the neonates are discussed here. The author stated that maturation of motor reflexes (assessed as surface righting and pendular reflex), open field behavior, and maze learning ability were assessed. Detailed descriptions of procedures and results were not provided for all assessments, but it was stated that motor reflex development did not differ in treated and control animals. Open field motor activity assessments in 40-day-old (juvenile) offspring revealed an increase in squares visited and an increased frequency of rearing on the second and third days of testing, indicating a lack of habituation in the offspring of formaldehyde-treated dams; similar levels of activity by both measures were found on the first test day. Counts of defecation and urination were increased on all 3 days of testing. Increased exploratory behavior, described as increased impulses, was also noted in a learning task (not otherwise described), but the author stated that learning rate and ability of the formaldehyde-treated group was not different from controls (no data were provided).

Mobility and neuromuscular excitability (not otherwise described) in offspring of female white rats were also evaluated by Sheveleva (1971). Dams were exposed to 0.005 or 0.0005 mg/L (approximately 4,000 or 400 ppb, respectively) formaldehyde on GDs 1−19. Spontaneous mobility (over 15 minutes) and neuromuscular excitability were evaluated in offspring at 1 or 2 months of age (other results from this study are discussed under developmental toxicity, above). At 1 month, spontaneous mobility was reduced at the low dose in males (52% of control levels; $p < 0.01$) but not at the high dose, and at both doses in females (to 64 and 56% of control levels at the mid dose and high dose, respectively; $p < 0.02$). At two months, there was a dose-related increase in activity for both sexes, statistically significant ($p < 0.001$) in high-dose females only (391% of control levels).

4.2.6.1.3. *Learning and memory.*

The effects of repeated formaldehyde exposures on learning were investigated by Malek et al. (2003c), using a labyrinth swim maze. In this task, animals are required to make a series of consecutive right or left turns to gain access to an escape platform (Malek et al., 2003c). Adult male and female LEW.1K rats (15/sex/group) were exposed to 0, 0.1, 0.5, or 5.4 ppm (0, 0.123, 0.615, or 6.64 mg/m^3) formaldehyde 2 hours/day for 10 consecutive days. Formaldehyde levels were checked eight times throughout the 2-hour exposure periods. Mean formaldehyde levels of

0.1 ± 0.02, 0.5 ± 0.1, and 5.4 ± 0.65 ppm were achieved. Body weight was measured on days 1, 5, and 10 of the experiment. Two days prior to beginning the formaldehyde exposures, all subjects were given an acclimation trial in which they were individually placed into the water-filled basin at the start position and allowed to navigate to the escape platform with manual assistance to learn the correct route. Thereafter, the water labyrinth test was run on each day of formaldehyde treatment, 2 hours after completion of each daily exposure. Time taken to complete the test and errors made were recorded for each rat (see Table 4-57). An error was defined as swimming toward the start position or circling in the same position without moving forward toward the escape platform. Rats were sacrificed at the end of the experiment, and tissues were taken from the lung, heart, thymus, kidney, liver, pancreas, skeletal muscle, and spleen. Tissues were fixed and prepared for histologic examination by light microscope. No differences were noted in food consumption or body weight gain for either male or female rats (Malek et al., 2003c). No treatment-related differences in organ pathology were reported (with the possible exception of focal microatelectasis (lung collapse at the microscopic level) seen in two to three animals in each formaldehyde-exposed group but not control animals).

Table 4-57. Effects of formaldehyde exposure on completion of the labyrinth test by male and female LEW.1K rats

Male rats	Swimming time (sec)			Error rate (mean)		
	Day 1	Day 6	Day 10	Day 1	Day 6	Day 10
Control	105	12.2	6.33	7.4	0.5	0.0
0.1 ppm[a]	100	12.9	6.07	7.7	5.0[c]	3.2[c]
0.5 ppm	97	16.7[c]	7.60[b]	7.6	4.4[c]	1.8[c]
5.4 ppm	105	25.7[c]	10.9[c]	7.7	5.0[c]	2.8[c]
Female rats	**Swimming time (sec)**			**Error rate (mean)**		
	Day 1	**Day 6**	**Day 10**	**Day 1**	**Day 6**	**Day 10**
Control	103	12.5	6.47	7.9	0	0.0
0.1 ppm	96	12.3	7.53	7.1	5.2[c]	3.0[c]
0.5 ppm	97	14.6[c]	7.60[b]	8.0	4.6[c]	2.2[c]
5.4 ppm	98	23.5[c]	9.73[c]	7.9	5.2[c]	2.6[c]

[a]Rats were exposed to formaldehyde for 2 hours/day, for 10 consecutive days.
[b]Different from control, $p < 0.05$.
[c]Different from control, $p < 0.005$.

Source: Malek et al. (2003c).

A clear learning curve was evident in control animals, with rats completing the task in less time and with fewer errors over days (see Table 4-57). Although the number of errors decreased with increasing experience in all groups, error rates in formaldehyde-exposed rats at all doses were consistently higher than those observed in controls, starting on day 3 (see Figure 4-28). All control animals performed without errors by day 6, whereas all treated animals were still making two to three errors on day 10, the final day of testing. Time required (latency) to complete the maze was also reduced over days. Although this measure of performance was not as sensitive as error rate, formaldehyde-induced deficits were still evident in the 0.5 and 5.4 ppm exposure groups of both sexes.

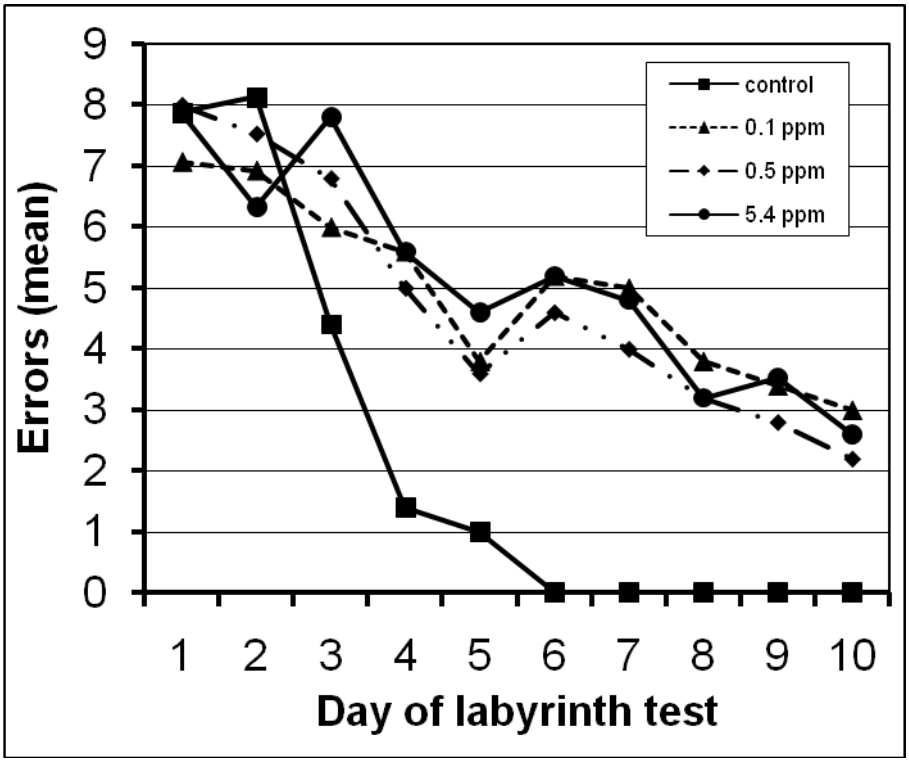


Figure 4-28. Effects of formaldehyde exposure on the error rate of female LEW.1K rats performing the water labyrinth learning test.

Source: Drawn from data reported in Malek et al. (2003a).

Impaired performance on formaldehyde-treated subjects cannot be attributed to alterations in swimming ability, since latencies to complete the maze were identical for 0 and 0.1 ppm groups, yet acquisition of the task was still impaired in the 0.1 ppm group based on number of errors committed (see Figure 4-28). This study reports an adverse effect level of

0.1 ppm for increased error rate in the labyrinth water test, and all dose groups were equally impaired across a broad range of exposures, 0.1−5.4 ppm. An independent estimate of swimming speed was not included, so motor competency could not be directly evaluated. However, comparable latency scores and error rates at the beginning of testing across all groups and latency scores that track together over days suggest that impaired swimming ability does account for the observed differences in latency, which are most likely reflective of the increased number of errors in treated animals (errors usually increase the distance traveled and thus time required for completion of the trial).

Pitten et al. (2000) evaluated the effects of very brief formaldehyde exposures (10 minutes) but prolonged duration (90 days) on previously learned performance in a land version of the labyrinth maze. Adult male and female Wistar rats (13/group) were trained on the task for 14 days, two trials/day. Animals were required to make a series of five left or right turns from the entrance of the maze to retrieve a piece of cheese placed in the goal box at the opposite end. Animals were guided by the experimenter through the maze during this acclimation phase until all subjects were able to retrieve the food without aid. After animals were trained (but prior to formaldehyde exposure), performance was assessed once daily for 11 days, and the latency to complete the maze as well as the number of errors committed when traversing from the entrance to the goal box was recorded. Animals were then assigned to one of three dose groups (five to eight/sex/group) such that task performance was equivalent across groups prior to commencement of formaldehyde exposures. Animals were exposed to 0 ppm, 2.6 ppm (0.25% formaldehyde solution to yield 3.06 ± 0.77 mg/m^3), or 4.6 ppm (0.70% formaldehyde solution to yield 5.55 ± 1.27 mg/m^3) formaldehyde 10 minutes/day, 7 days/week for 90 days. Animals were assessed for performance in the maze every seventh day, at least 22 hours after the exposure on the previous day. At the end of the 90-day exposure period, monitoring of maze performance continued once every 10 days for an additional 30 days. All rats were sacrificed at the end of the postexposure trials and tissue sections were prepared for histologic examination by light microscopy, including liver, trachea, lung, kidney, heart, spleen, pancreas, testicle, and brain. No treatment-related changes in food or water consumption weight gain or in histologic samples obtained at the termination of the experiment were observed.

Pitten et al. (2000) reported that no gender differences existed as a function of formaldehyde treatment; therefore, data were presented by combining sexes. Control rats showed no change in error rate but a slight decrease in running time through the maze during the course of the experiment. The formaldehyde-exposed groups began with a similar performance level and error rate as controls, but their performance degraded over the course of formaldehyde exposure. By the fourth week of exposure, increased numbers of errors were evident in both

exposed groups relative to controls. This trend reached statistical significance at the 12-week time point, with a greater than twofold increase in number of errors ($p < 0.05$). Formaldehyde-treated rats also tended to have increased run times through the maze ($p = 0.04$), but no difference was seen by formaldehyde concentration. By 4 weeks after termination of exposure, no statistical differences among the three groups were evident, but the tendency for the two exposed groups to make more errors and have longer latencies remained. Since Pitten et al. (2000) tested animals after the task was acquired, these results indicate deficits in the retention of a previously learned task.

Lu et al. (2008) evaluated the effects of formaldehyde on performance of mice in a Morris water maze. Kunming mice (five males/group) were exposed to formaldehyde at 0.2, 1, or 3 mg/m^3 6 hours/day for 7 days (measured concentrations: 0.2 $\pm$ 0.01, 0.99 $\pm$ 0.04, and 3.03 $\pm$ 0.16 mg/m^3). Mice were trained to locate a hidden platform in a large, circular tank (106 cm diameter, 31 cm deep). Each animal received four training trials per day, beginning 30 minutes after the end of exposure. During training, latency to locate the platform was recorded for each trial, with a maximum of 60 seconds, after which the animal was guided to the platform. After the last day of training, an additional trial was conducted with the platform removed (the probe trial); time spent in each maze quadrant was measured to determine the time the animal spent searching for the platform in the correct area of the maze. Performance in the water maze, measured as mean escape latency across the seven training trials, was significantly impaired in the 3 mg/m^3 group. No significant difference was seen at 1 mg/m^3, although there appeared to be an increased latency during the second day of testing. During the probe trial, control animals spent significantly more time in the correct quadrant, but neither formaldehyde-exposed group did so. Results of this study indicate deficits in learning and retention of the Morris water maze following formaldehyde exposure, with greater effects seen in the higher dose group.

Apfelbach and Reibenspies (1991) published a brief report of formaldehyde effects on olfactory learning. Ferrets were exposed to 0.25 ppm (0.31 mg/m^3) formaldehyde gas continuously for 6 months. A Y-shaped maze was used to test odor detection, discrimination between odors, and odor threshold. Ferrets were conditioned to distinguish ethyl acetate (0.1 vol %) from clean air. Untreated ferrets achieved 75% success after an average of 110 trials. However, formaldehyde-treated ferrets required on average 320 trials to reach a 75% success rate. A 90% success rate was achieved by untreated ferrets after 420 trials. However, this level of success was not reached in formaldehyde-treated ferrets.

The same researchers also tested olfactory function in formaldehyde-treated ferrets, as summarized in Section 4.2.7 (Apfelbach et al., 1992). A decrease in olfactory discrimination and

a reduction in the percentage of olfactory cells in the olfactory epithelium were reported after 3−12 months exposure to 0.25 or 0.5 ppm formaldehyde. Decreased olfactory sensitivity in rats exposed to 0.25 or 0.5 ppm formaldehyde has also been reported by the same researchers (Weiler and Apfelbach, 1992; Apfelbach and Weiler, 1991), and Weiler and Apfelbach (1992) reported in an abstract that shifts in olfactory thresholds were greater when exposure was initiated at PND 30 than at adult ages. Given the documented changes in olfactory thresholds, observed changes in olfactory learning would likely be confounded by the potential for decreased olfactory function by formaldehyde exposures, and definitive conclusions regarding formaldehyde effects specific to learning cannot be made based on these studies.

4.2.6.1.4. ***Neurosensitization.***

Sorg et al. (1996) studied the potential for formaldehyde exposure to induce sensitization in the CNS, possibly through the limbic pathways in the brain. The authors hypothesized that multiple chemical sensitivity (MCS) has an onset and progression similar to CNS sensitization and may, therefore, be a similar process. These experiments were conducted to test this hypothesis and to determine whether formaldehyde exposure could be used as a model for MCS. Behavioral sensitization can be initiated by psychostimulants (e.g., cocaine) and manifest as increased locomotor activity upon subsequent challenge with the stimulant.

Sorg et al. (1996) evaluated cross-sensitization of cocaine-induced increases in activity from an initial formaldehyde exposure. Female Sprague-Dawley rats (eight to nine) were exposed to 0 or 11 ppm (0 or 13.5 mg/m^3) formaldehyde 1 hour/day for 7 days. Locomotor activity was measured (by photocell) after saline injection (1 day postexposure) and after cocaine injection (2 days postexposure). A similar protocol was conducted on days 36 and 37 postexposure. Motor activity levels following saline injection were similar for controls and formaldehyde-treated rats. However, formaldehyde exposure initiated sensitization to cocaine as evidenced by a greater increase in locomotor activity in mice treated with formaldehyde followed by cocaine ($p < 0.05$) with an average count of crossed grids greater than 40,000 (2 hours) in treated animals compared with 25,000 (2 hours) in controls. The cross-sensitization was transient, with no treatment effects on cocaine-induced activity either 29 or 37 days postexposure. When examining individual data, the authors suggested that the formaldehyde-treated groups in both cases have a cluster of high responders (HRs), suggesting some animals may have been more sensitive. A second group of similarly treated female rats was pretested for locomotor activity and divided into subgroups of HRs or low responders (LRs). They were then given a panel of neurobehavioral tests: anxiety (elevated plus maze, day 11); memory (passive avoidance training, day 12; passive avoidance test, day 19); and nociceptive test (day 20). Trunk

blood corticosterone levels were determined during stress on day 35 postexposure. No significant treatment differences were found in the passive avoidance test, nociception, or corticosterone levels (basal or stress induced). On the elevated plus maze, a two-way ANOVA indicated no overall formaldehyde treatment effects, but the HR rats had higher open arm time ratios (indicating greater anxiety) regardless of treatment. Within the treatment groups, the difference in behavior between HR and LR subgroups was only significant for the formaldehyde-treated rats ($p < 0.05$). Authors suggested that cross-sensitization to cocaine-induced locomotor activity was caused by enhanced dopamine transmission within the mesolimbic system (ventral tegmental area to nucleus accumbens projection) following repeated formaldehyde exposure. A critical role of the hypothalamic-pituitary-adrenal (HPA) axis has also been implicated in cross-sensitization.

Sorg et al. (1998) and Sorg and Hochstatter (1999) further explored formaldehyde-induced behavioral sensitization using the cocaine model. In contrast to the results following exposure to 11 ppm (Sorg et al., 1996), rats exposed to only 1 ppm for 7 days showed no cross-sensitization to cocaine injection. However, animals exposed to 1 ppm formaldehyde for 4 weeks exhibited increased cocaine-induced vertical activity (with no difference in horizontal activity) for 4−6 weeks after cessation of exposure. Activity levels of formaldehyde-exposed rats were approximately threefold those of control rats 3−4 days postexposure and still 1.5-fold control levels at 4−6 weeks postexposure ($p < 0.05$).

Sorg et al. (2001) examined changes in corticosterone levels in rats with and without formaldehyde treatment. Basal corticosterone levels in trunk blood were established in naïve male Sprague-Dawley rats taken directly from their home cage immediately prior to sacrifice. In an acute trial, male rats were exposed to 0, 0.7, or 2.4 ppm (0, 0.86, or 2.96 mg/m^3) formaldehyde for either 20 or 60 minutes, and trunk blood was collected for corticosterone analysis. Therefore, these rats were challenged with a new environment (the exposure chamber) in the presence or absence of formaldehyde. In a separate trial, basal and challenged corticosterone levels were measured after repeated exposure (1 hour/day, 5 days/week for 2 or 4 weeks). Basal corticosterone levels were measured in trunk blood immediately after removing the animal from its home cage. Challenged corticosterone levels were measured after rats were placed into the exposure chamber for a final 20-minute exposure. Body weight was measured at the beginning of each week of exposure and was unchanged by formaldehyde treatment.

Corticosterone levels were increased over basal levels when rats were placed in the exposure chamber for 20 minutes (see Figure 4-29, panel a) but returned to basal levels after 60 minutes in the exposure chamber (not shown). This response may reflect the stress of the new

environment and acclimatization after 60 minutes in the chamber. Corticosterone levels were the same in the presence or absence of formaldehyde, indicating no treatment effect.

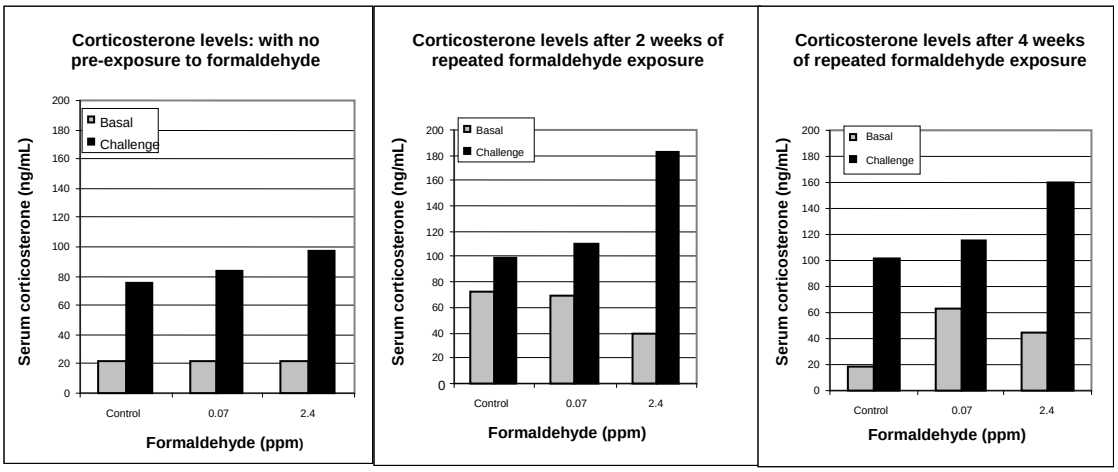


Figure 4-29. Basal and stress-induced trunk blood corticosterone levels in male LEW.1K rats after formaldehyde inhalation exposures.

Note: Panel a: no pretreatment, corticosterone levels after 20-minute formaldehyde exposure. Panels b and c show both basal and induced corticosterone levels after a 2- or 4-week pretreatment to formaldehyde 1 hour/day. Challenge to induce corticosterone was a 20-minute reexposure at the formaldehyde level tested.

Source: Sorg et al. (2001).

Control animals exhibited an increase in basal corticosterone after 2 weeks, which returned to naïve levels after 4 weeks (see Figure 4-29, panels b and c). Formaldehyde-treated rats demonstrated a comparable increase in basal corticosterone levels at 2 weeks, but these levels did not return to naïve levels at 4 weeks as seen with controls. Control and 0.7 ppm exposed rats showed a similar response to challenge (the final 20-minute exposure). However, rats exposed to 2.4 ppm were hyperresponsive, with exaggerated corticosterone levels during this final exposure. Differences in basal corticosterone levels after formaldehyde exposure and the hyperresponsiveness seen in animals exposed at 2.4 ppm provide evidence of formaldehyde-induced perturbations of the HPA axis. Authors suggested that elevated corticosterone levels induced by repeated formaldehyde exposures may contribute to the cross-sensitization to cocaine-induced motor activity.

Formaldehyde-induced changes in the HPA axis may contribute to behavioral effects of formaldehyde exposure reported by Sorg et al. (2004) and Sorg and Hochstatter (1999). The authors also reported an enhanced conditioning to odor in animals previously exposed to repeated formaldehyde. Male and female Sprague-Dawley rats (60−80 days of age) were exposed at 1 ppm (1.23 mg/m^3) formaldehyde 1 hour/day, 5 days/week for 4 weeks (Sorg and Hochstatter, 1999). Two weeks after exposure ended, rats were trained to the conditioned fear task. Rats were conditioned to a fear response by either odor only or odor associated with footpad shock. Orange-oil extract was used as the odor conditioned stimulus (CS). One day after conditioning, rats were reintroduced into the environment without an odor cue, and time spent motionless in the freezing posture (freezing) was observed. On day 2 after conditioning, rats were placed in a novel environment, and time spent in the freezing posture was evaluated in the absence and then the presence of odor. This was repeated on day 12 after conditioning to measure the loss of the freezing response to the conditioned odor.

Both treated and exposed rats showed similar responses on reintroduction into the conditioning environment in the absence of an odor cue on day 1 (Sorg and Hochstatter, 1999). As expected, rats conditioned with a footpad shock demonstrated greater time motionless than odor-trained only rats, and there was no difference between control and formaldehyde-treated rats. However, in the presence of odor on days 2 and 12, formaldehyde-exposed rats who were conditioned with odor associated with foot shock spent significantly more time freezing than odor-only trained rats ($p < 0.05$); control animals on those days showed no difference in time freezing in the presence and absence of odor. The authors concluded that the formaldehyde-treated rats had more difficulty than controls in extinguishing the fear response to the conditioned odor, and speculated that an enhancement of the fear-conditioned response by formaldehyde pretreatment supports the hypothesis that sensitization may include effects through the limbic system of the brain.

In a second experiment, adult male and female Sprague-Dawley rats were exposed at 0 or 2 ppm (2.45 mg/m^3) formaldehyde 1 hour/day, 5 days/week for 4 weeks (Sorg et al., 2004). Two to 3 weeks after exposure ended, rats were trained to the conditioned fear task. Rats were given a foot shock either associated with an odor (paired group) or unassociated with an odor (unpaired group). Orange-oil extract was used as the odor CS. After training, freezing behavior was assessed (1) in the same context in the absence of odor (1 day), (2) in a new context in the presence and absence of the CS (5 consecutive days), and (3) in another novel context in the presence and absence of the CS.

Formaldehyde-exposed male rats demonstrated increased conditioned fear response to an odor CS (orange oil) paired with foot shock with no change in the degree of conditioning to the

context. For female rats, formaldehyde exposure did not affect the percent of time spent freezing, either in the conditioning context or the novel context in the absence of the conditioned odor. In contrast, male rats spent an increased time freezing in a novel context in the presence of odor, indicating a greater conditioned fear response to the olfactory cue ($p < 0.05$). This is in agreement with the previous study where formaldehyde effects were seen in the presence of the conditioning odor but not the environment (Sorg and Hochstatter, 1999). However, in this study female rats did not exhibit a similar enhancement of fear conditioning to the olfactory CS.

The authors suggested that repeated exposure to low levels of formaldehyde acts as a stressor in much the same way as inescapable foot shock, with resulting sensitized responses within the olfactory/limbic pathways (Sorg et al., 2004). This interpretation is consistent with work described above in which augmented basal corticosterone levels following repeated formaldehyde exposures were demonstrated. However, while the fear conditioning in the present study and cross-sensitization to cocaine described above (Sorg and Hochstatter, 1999) occurred 3−4 weeks after termination of exposure to formaldehyde, the duration of corticosterone elevation induced by repeated exposure to formaldehyde has not been determined. It is possible that augmentation of corticosterone levels following formaldehyde exposure results from direct action of formaldehyde on the HPA axis. Experiments designed to compare HPA activation following standard stressors (repeated inescapable foot shock or restraint stress), stress induced by other irritants (chemicals with strong irritant odors but no CNS action), and repeated formaldehyde exposures are necessary to dissociate primary from secondary action of formaldehyde on CNS function in this paradigm. It is also possible that enhanced conditioning to an odor stimulus results from formaldehyde-induced increases in airway irritation, rendering the conditioned odor stimulus a more salient cue, producing a conditioned response that is not extinguished as readily as in air-exposed controls. However, damage of the nasal mucosa and lesions would be expected to be minimal at 1 ppm formaldehyde exposures and most likely resolved 2 weeks after exposure was ended (see Section 4.2.1.2). Therefore, a more salient cue for fear conditioning to odor due to physical irritation is not likely. Alternatively, formaldehyde may act to up regulate olfactory activity, producing a stronger sense of odor during conditioning.

4.2.6.1.5. *Neurochemistry and neuropathology.*

Several studies that were focused on general toxicity or carcinogenicity of formaldehyde also assessed histopathology in exposed animals, including pathological evaluation of the brain. In all cases, details of the pathological evaluation were not provided. Reported results stated that no significant lesions were seen on unspecified tissues (Appelman et al., 1988; Maronpot et al., 1986; Kerns et al., 1983) or that an increase in relative brain weight (data not provided) was

considered of no toxicological significance (Woutersen et al., 1987). The absence of procedural information, or specific reported results, limits the utility of this information.

Boja et al. (1985) measured changes in several neurotransmitters (norepinephrine, dopamine, 5-hydroxytryptamine) and their major metabolites (3,4-dihydroxyphenylacetic acid [DOPAC] and 5-hydroxyindoleacetic acid [5-HIAA]) following one or two 3-hour exposures to formaldehyde at 0, 5, 10, or 20 ppm. Animals were sacrificed immediately following the second exposure, and brains were immediately removed, frozen, and sectioned. Regions of interest were analyzed by high-pressure liquid chromatography with electrochemical detection. Authors stated that neurotransmitter concentrations were measured in multiple brain regions, but results were reported only for the 5 ppm exposure and only for the hypothalamus. No change was seen in concentrations of norepinephrine or 5-hydroxytryptamine for any exposure paradigm. For those animals exposed twice to formaldehyde, there was a slight (statistically significant) increase in dopamine and a larger (approximately fourfold) increase in 5-HIAA. DOPAC was increased (approximately 30%) in animals receiving formaldehyde during the second exposure only.

Recent work by Hayashi et al. (2004) indicates that formaldehyde exposure increases the activity of periglomerular (PG) cells in the main olfactory bulb. Tyrosine hydroxylase activity was measured as a marker for activity of olfactory function. The authors surmised that expression levels of this enzyme are useful markers since it has been reported that the protein is up regulated after sensory stimulation and is down regulated by odor deprivation or when the olfactory epithelium is removed (Cho et al., 1996; Stone et al., 1991; McLean and Shipley, 1988; Baker et al., 1983). Eight-week-old female C3H/HeN mice were exposed at 0, 0.08, 0.4, or 2 ppm (0, 0.1, 0.49, or 2.45 mg/m^3) formaldehyde 16 hours/day for 1 day or 12 weeks (5 days/week). Formaldehyde exposure did not affect body weight. Mice were sacrificed 24 hours after exposure; the brains were removed, fixed, and prepared for sectioning. One side of the olfactory bulb was sliced into 40 µm-thick serial frontal sections and immuno-stained for tyrosine hydroxylase activity. The number of tyrosine hydroxylase-positive PG cells was determined by examining digital photomicrographs of three tissue sections, averaging the counts from 10−15 glomeruli per section.

Neither the size of the olfactory bulb (rostrocaudal, dorsoventral, and mediolateral lengths) nor the total number of PG cells was changed by formaldehyde exposure. The number of tyrosine hydroxylase-positive PG cells per glomerulus was unchanged by a single formaldehyde exposure but increased after 12 weeks of repeated exposures. The increases were similar among treatment groups: 5.54 ± 0.31 at 0.80 ppm, 5.18 ± 0.60 at 0.4 ppm, and 6.0 ± 0.83 at 2 ppm or 196, 167, and 196% of controls, respectively. As an indicator of activity, it is not unexpected that the enzyme was up regulated after repeated exposure to an odorous compound.

Hayashi et al. (2004) hypothesize that the increased tyrosine hydroxylase activity is an indication of increased sensitivity and, therefore, may be a model for MCS. However, it is unknown if the increase in enzyme activity after repeated exposures is transient or could result in sensitization. Tyrosine hydroxylase is the first enzyme in the dopamine synthetic pathway, but the role of dopamine in PG cells is not known. Further research would be needed to understand the potential for formaldehyde to act as a sensitizing agent in this model.

In an abstract, Kakeyama et al. (2004) outline the results of experiments to address the effects of subchronic exposure to low levels of formaldehyde on changes in neurotransmitter-related mRNA expressions in mice forebrains. An unstated number of female C3H/He mice were exposed 16 hours/day, 5 days/week to 400 ppb (0.49 mg/m^3) formaldehyde for 12 weeks. The authors used RT-PCR methodologies to quantify mRNA encoding for the glutamate receptor subunits GluR1 and GluR2, the dopamine receptor D1, and the serotonin receptor 5-HT1A in the neocortex, hippocampus, amygdala, and hypothalamus. Raised levels of mRNA expression were observed for GluR1 in the neocortex and hippocampus; GluR1, GluR2, and the dopamine receptor D1 in the amygdala; and the serotonin receptor 5-HT1A in the hypothalamus. Reduced mRNA expression was observed for GluR2 in the hippocampus and neocortex. When other mice were subjected to a radiofrequency-induced lesion of the hippocampus then exposed to formaldehyde for 12 weeks as before, the altered expression of GluR1 and GluR2 in the neocortex was abolished. However, the increment of mRNA expression of 5-HT1A in the hypothalamus was further enhanced. In demonstrating that formaldehyde affects neocortical GluR1 and GluR2 mRNA expressions through a hippocampal function, Kakeyama et al. (2004) concluded that subchronic exposure to low concentrations of formaldehyde can affect neural transmission in the forebrain.

Fujimaki et al. (2004b) examined the effects of formaldehyde on NGF in the brain and hippocampus. Ten female C3H/HeN mice/group were exposed to 0, 80, 400, or 2,000 ppb (0, 0.1, 0.5, or 2.45 mg/m^3) formaldehyde 16 hours/day, 5 days/week for 12 weeks. Some groups of mice received the same treatment after I.P. injection of 10 μg of OVA and 2 mg alum prior to the commencement of formaldehyde exposure. For this subgroup, booster injections of OVA were administered on days 21, 42, 63, and 77 during the formaldehyde exposure regimen. Quantitative measures of NGF and BDNF in homogenates of whole brain and hippocampus were obtained by ELISA and mRNA determination. The amount of NGF protein in whole brains remained unchanged in the nonimmunized mice. However, brain NGF levels were significantly increased in OVA-immunized mice exposed to 80 and 400 ppb (but not 2,000 ppb) formaldehyde (see Figure 4-30). This result was confirmed by parallel increases in the concentrations of hippocampal NGF mRNA that were produced in immunized mice exposed to

formaldehyde at the same concentrations. However, there were no comparable increases in the amounts of brain-derived neurotrophic factor in either immunized or nonimmunized mice. In discussing the mechanisms potentially associated with their results, Fujimaki et al. (2004b) considered it likely that low-level exposure to formaldehyde could enhance NGF production through the stimulation of the HPA axis together with immunization.

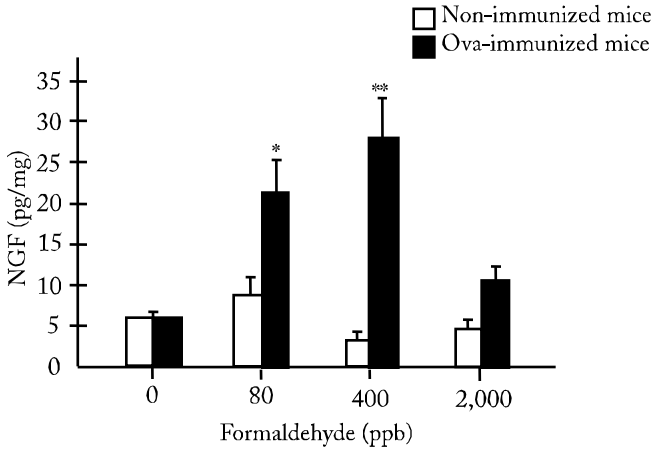


Figure 4-30. NGF production in the brains of formaldehyde-exposed mice.

Note: Female C3H mice were exposed to formaldehyde 16 hours/day, 5 days/week for 12 weeks. NGF in homogenates of whole brain and hippocampus were measured by ELISA. Values are means ± SEM (n = 5−6). * = $p < 0.05$ and ** = $p < 0.01$ versus control mice, as calculated by the authors.

Source: Redrawn from Fujimaki et al. (2004b).

The enhancement of NGF in the brains of immunized mice exposed to formaldehyde gave rise to the suggestion that NGF may promote the survival of hippocampal neurons when challenged with formaldehyde. To examine whether or not apoptosis plays a role in this process, Tsukahara et al. (2006) measured the effects of formaldehyde on apoptotic mechanisms regulating the survival and death of cells and on *N*-methyl-D-aspartate (NMDA) receptors. Female C3H/HeN mice (13/group) were exposed to 0 or 400 (393 ± 34) ppb (0 or 490.8 μg/m^3) formaldehyde 16 hours/day, 5 days/week for 12 weeks. Seven control and formaldehyde-treated mice were immunized with 10 μg OVA plus 2 mg aluminum hydroxide prior to exposure. Subsequently, these mice received OVA via aerosol as a booster during weeks 3, 6, 9, and 11. Hippocampi were dissected from all animals 1 day after the final exposure and homogenized in hypotonic buffer. The 12,000 rpm supernatants were analyzed by Western blotting for the

presence of the proteins BCl-2 (which inhibits apoptosis) and Bax (which opposes BCl-2 action and promotes apoptosis) and the NMDA receptor subtypes 2A and 2B (NR2A and NR2B). Immunohistochemical analysis was also carried out for the presence of active caspase-3, an apoptosis marker.

The levels of NR2A and NR2B were unaffected by exposure to formaldehyde in either immunized or nonimmunized mice. Likewise, the number of caspase-3 immunoreactive cells did not change as a result of formaldehyde exposure. However, when measured amounts of Bcl-2 and Bax were normalized to the amount of β-tubulin, the ratio Bcl-2/Bax was significantly increased in immunized mice exposed to formaldehyde. Nonimmunized mice did not show this apparently compound-related response. Consistent with the concept that the proportions of Bcl-2 and Bax are critical for the regulation of cell survival and death, the authors interpreted their data as an indication that changes to the ratio of Bcl-2/Bax expressions might be an important adaptive response to the effects of formaldehyde, such that the antiapoptotic changes might contribute to the protection of hippocampal neurons from the pernicious effects of formaldehyde exposure itself.

The same research group used the immunized mouse model to determine whether formaldehyde exposure affected mRNA expression of genes related to synaptic plasticity (Ahmed et al., 2007). Ten female C3H/HeN mice were exposed to 0 or 400 ppb formaldehyde 16 hours/day, 5 days/week for 12 weeks. All mice were immunized with 10 µg OVA plus 2 mg aluminum hydroxide prior to initial formaldehyde exposure then treated in weeks 3, 6, 9, and 11 with aerosolized OVA as a booster. Five treated and control animals were I.P. injected with 1 mg/kg MK-801, a noncompetitive NMDA receptor agonist before the last formaldehyde exposure. At term, hippocampi were dissected and frozen at −80^{o}C until processing. At that point, total mRNA was extracted and first strand cDNA was synthesized by using reverse transcriptase. Expression levels of various proteins/receptors, including NMDA NR2A and NR2B receptor subunits, dopamine D1 and D2 receptors, cyclic AMP responsive element-binding proteins (CREB-1 and CREB-2), and the transcription factors FosB and ΔFosB were determined by using the PCR. The expression level of each mRNA species was expressed relative to the sample's content of 18S rRNA. The total protein lysate was also assayed for pCREB by Western blotting.

In the first of a sequence of histograms, Ahmed et al. (2007) demonstrated a significant increase in mRNA expression of NR2A as a result of formaldehyde exposure. However, this effect was abolished in animals treated with MK-801. A similar trend in the mRNA expression of NR2B in response to formaldehyde exposure did not achieve statistical significance. MK-801 treatment significantly reduced receptor in mRNA expression in the presence of formaldehyde.

The authors provided data showing an increased expression of dopamine D1 and D2 receptor mRNA response to formaldehyde, in both cases abolished by treatment with MK-801. The expression of CREB-1 mRNA also conformed to the pattern of being increased as a result of formaldehyde exposure but abolished by MK-801. However, the expression of CREB-2 and FosB/ΔFosB was unaffected by formaldehyde. When normalized to the amount of β-tubulin, there were no significant effects of formaldehyde exposure and MK-801 treatment on the protein levels of pCREB. Finally, there was no significant difference in the expression of transient receptor potential vanilloid receptor (TRPV1) between control and formaldehyde-exposed mice, and MK-801 itself did not significantly alter the mRNA level of TRPV1. In seeking to explain their results, the authors speculated that low-level exposure of immunized mice to formaldehyde had an effect on hippocampal synaptic plasticity at the mRNA level, as evidenced by the enhancement of mRNA for NR2A, the dopamine D1 and D2 receptors, and CREB1, with up regulation compensating for the sustained levels of enhanced protein expression under low-level formaldehyde exposure. The interpretation of these changes in NR2A mRNA, in the context of the results of Tsukahara et al. (2006), showing no change in NR2A and NR2B protein expression, was not discussed.

4.2.6.1.6. ***Neurogenesis.***

Two papers have examined the effects of subacute exposure to formaldehyde on the overall size (volume) of discrete cellular areas of the hippocampus in neonatal rats. The researchers also used an optical fractionator counting method to derive a plausible estimate of cell number. Aslan et al. (2006) studied the effects of formaldehyde exposure on the number and volume of granular cells in the hippocampal dentate gyrus. Sarsilmaz et al. (2007) examined the impact of postnatal formaldehyde exposure on brain hemisphere volume and on the size and cell number of pyramidal cells in the cornu ammonis region of the hippocampus. The in-life phase was the same in each study, featuring the exposure of 10 neonatal male Wistar rats/group to 0, 6, and 12 ppm (0, 7.36, and 14.7 mg/m^3) formaldehyde 6 hours/day, 5 days/week for 30 days. Five rats/group were sacrificed at that point (PND 30), while the rest were maintained without further treatment until PND 90.

For both pyramidal and granular areas, a much lower number of cells was seen on PND 90 versus PND 30 ($p < 0.001$). This response was evident irrespective of the amount of exposure to formaldehyde and is consistent with normal brain development. Compound-specific effects of formaldehyde on the volume and number of granular and pyramidal cells varied by dose and over the two time points. There was a small increase in the volume of the granular cell layer of the dentate gyrus in rats sacrificed on PND 30 ($p < 0.001$) in response to increasing formaldehyde concentration (Aslan et al., 2006), with no significant change in neuron number;

the increased volume (now accompanied by an increase in neuron number) was still evident at the low-exposure level on PND 90 ($p < 0.01$) but not at the high dose. Brain hemisphere volume was decreased at both concentrations on PND 30 ($p < 0.01$) but was increased at both concentrations ($p < 0.01$, with a larger magnitude of effect at 6 ppm) on PND 90 (Sarsilmaz et al., 2007). In the hippocampal cornu ammonis region, the volume of the pyramidal cell layer on PND 30 was increased in low-dose animals ($p < 0.001$) but decreased in high dose animals ($p < 0.001$) as compared with control values; neither group was significantly different from controls on PND 90. There was a dose-related decrease in total neuron number in the cornu ammonis on PND 30 ($p < 0.01$ for both doses); on PND 90 the decrease in neuron number remained statistically significant in both treatment groups ($p < 0.01$), but there was no longer any difference in the magnitude of the effect between doses (Sarsilmaz et al., 2007).

In a third study from the same laboratory, Songur et al. (2008) used the same exposure paradigm to evaluate changes in oxidant and antioxidant systems in the cerebellum of perinatally exposed rats. Exposure was carried out as in Aslan et al. (2006) and Sarsilmaz et al. (2007), described above. On PND 30 or 90, cerebellums from seven male rats per group were evaluated for levels of malondialdehyde (MDA), NO, superoxide dismutase activity (SOD), and glutathione peroxidase (GPX) activity. Dose-related increases in NO (approximately 20−80%), MDA (100−160%), and GPX (25−60%) and dose-related decreases in SOD (20−30%) were seen on PND 30. In general, the magnitude of change from control levels was maintained on PND 90, with the exception of MDA levels in 6 ppm animals, which appeared to approach control levels at 90 days. The authors stated that these findings indicated that formaldehyde exposure may cause neurotoxicity via the production of oxidative damage in the brain. Persistence of the effect to the 90-day time point (30 days after cessation of exposure) supports the possibility that formaldehyde may cause long-lasting or permanent changes in the brain following early life exposure. These results are consistent with the earlier studies by Aslan et al. (2006) and Sarsilmaz et al. (2007), finding permanent changes in brain structure (although in a different brain region) following early life exposure.

4.2.6.1.7. *Summary of formaldehyde effects on neurobehavioral and neuropathological measures, following exposure via inhalation.*

As has been demonstrated in mice (Wood and Coleman, 1995), it is possible that rats experience respiratory tract irritation during low-level formaldehyde exposure. Perturbations in nervous system function reported with formaldehyde exposure include reductions in motor activity, lack of habituation, impairment in acquisition of a new learning task, deficits in retention of a previously learned task, increases in corticosterone levels, sensitization to cocaine-induced locomotor activity, and enhanced fear conditioning using an olfactory CS (see

Table 4-58). Many of these effects were observed at exposure levels at or below 1 ppm, and some persisted days to weeks after termination of exposure.

Malek et al. (2004, 2003a, b) detected behavioral changes in rats and mice tested 2 to 24 hours postexposure. The mechanism of these behavioral changes is unknown, and available data do not allow dissociation of direct effects on the nervous system and behavioral responses to the irritant effects of formaldehyde (control experiments [e.g., using a different aversive odor with or without irritant properties] were not included). Given that behavioral changes were observed hours to days after cessation exposure (i.e., beyond the time required for formaldehyde-induced irritation to subside), it is unlikely that these behavioral changes were caused by formaldehyde-induced irritation. Similarly, although it is possible that systemic effects of formaldehyde exposure might cause reduced motor activity during or immediately following exposure, it is unlikely that these effects can account for the differences in responses of male rats 24 hours after exposure (Malek et al., 2003a). Furthermore, a follow-up study demonstrated reduced motor activity in animals 2 hours after a 2-hour exposure to much lower levels of formaldehyde (0.1 ppm), which fall well below the levels identified by Wood and Coleman (1995) as the AC_{50} for formaldehyde in mice (Malek et al., 2003b).

Two studies reported significant reductions in learning or retention following brief periods of repeated exposure to low levels of formaldehyde (Malek et al., 2003c; Pitten et al., 2000) (see Table 4-58). Malek et al. (2003c) reported an increased number of errors in acquiring a water maze task; testing took place daily 2 hours after termination of a 2-hour exposure. The work of Pitten et al. (2000) revealed that brief exposures over many weeks led to increases in errors performing a previously learned task and that the magnitude of the effect increased over the course of the exposure period. Testing occurred remote from the time of exposure (22 hours after the previous exposure), and the deficits appeared to persist for several weeks after exposure terminated, minimizing the possibility that these effects were related to irritant properties of formaldehyde. Although the exposure levels were moderately high (2.6−4.6 ppm) and continued over several months, the duration of a single exposure event was very brief (10 minutes).

Sorg and Hochstatter (1999) and Sorg et al. (2004, 2001) suggest that behavioral sensitization associated with low-level formaldehyde exposure was linked to alterations in HPA control of corticosterone. Cross-sensitization to the locomotor activity-enhancing properties of cocaine and changes in response to a conditioned fear paradigm were observed in animals exposed several weeks earlier to repeated low-level formaldehyde. Direct activation of mesolimbic dopamine pathways or activation of conditioned fear response in the amygdala by formaldehyde could underlie these behavioral effects; these observations were also seen by study authors as consistent with a formaldehyde-induced stress response.

Table 4-58. Summary of neurological and neurobehavioral studies of inhaled formaldehyde in experimental animals

Species	No./ group	Treatment[a]	Observations	LOAEL/ NOAEL	Reference
Irritant detection threshold					
Male Swiss mice	8	0, 1, 1.8, 3, 5.6, or 10 ppm 60-second exposure episode to determine irritant response	*Sensitivity of mice to acute formaldehyde levels* determines the median concentration at which 50% of exposures were terminated by the subject (AC_{50}) decreased upon repeat exposure. AC_{50} = 3.63 for first series, AC_{50} = 1.88 ppm for second series. Time to exposure termination decreased with increasing formaldehyde concentration. Time to termination was decreased in repeat exposures.	NA[b]	Wood and Coleman (1995)
Motor activity and habituation					
Male and female LEW.1K rats	10/sex	0, 1, 2.5, or 5 ppm for 2 hours	*Reduced horizontal activity*: Number of crossed quadrants reduced 2 hours after exposure at all doses for males and females ($p < 0.005$). *Reduced habituation*: Exposed rats exhibited greater activity than controls when reintroduced into the testing environment 24 hours later (males and females, all doses, $p < 0.005$).	LOAEL = 1 ppm 2 hours	Malek et al. (2003a)
Male and female LEW.1K rats	10/sex	0, 0.1, 0.5, or 5 ppm for 2 hours	*Reduced horizontal activity*: Number of crossed quadrants reduced 2 hours after exposure at all doses for males and females ($p < 0.005$ for males at all doses; $p < 0.005$ for females at two higher doses).	LOAEL = 0.1 ppm 2 hours in males	Malek et al. (2003b)
Male AB mice	5−7/sex	0, 1.1, 2.3, or 5.2 ppm for 2 hours	*Reduced horizontal activity*: Number of crossed quadrants reduced 2 hours after exposure at all doses.	LOAEL = 1.1 ppm 2 hours	Malek et al. (2004)
Balb/c mice	6	0, 1.8, 3.2, 4.5, 6.4, 9.7, or 14.8 ppm for 3 hours	*Reduced horizontal and vertical activity*: Dose-dependent decreases in crossed quadrants and rearing. Significant for males at 1.8 ppm and greater ($p < 0.01$). Significant for females at 6.4 ppm or greater ($p < 0.01$).	LOAEL = 1.8 ppm 3 hours in males	Usanmaz et al. (2002)
Balb/c mice	6	3.3 ppm for 2 weeks or 2 ppm for 3 weeks 3 hours/day, 5 days/week	*Reduced horizontal and vertical activity* decreases in crossed quadrants and rearing. 3.3 ppm (2 weeks) and 2 ppm (1 week) ($p < 0.01$, $p < 0.05$).	LOAEL = 2 ppm 3 weeks	Usanmaz et al. (2002)
Sprague-Dawley rats	8	0, 5, 10, or 20 ppm; 3 hours/day for 1 or 2 days	*Reduced activity levels on both days.* Decreases seen at 5 and 10 ppm; data reported only for 5 ppm group	LOAEL = 5 ppm, 3 hours	Boja et al. (1985)

Table 4-58. Summary of neurological and neurobehavioral studies of inhaled formaldehyde in experimental animals (continued)

Species	No./ group	Treatment[a]	Observations	LOAEL/ NOAEL	Reference
Rats		0 or 0.5 mg/m^3 (0.4 ppm) on GDs 1−19, 4 hours/day	*Increased motor activity on the 2nd and 3rd days of testing (reduced habituation) in offspring exposed in utero.* Increased number of squares entered ($p < 0.01$) and frequency of rearing ($p < 0.05$).	LOAEL = 0.4 ppm, gestational	Senichenkova (1991)
Rats	15	0, 0.005, or 0.0005 mg/L (approximately 4 or 0.4 ppm), GDs 1−19	*Changes in motor activity at one and two months in offspring exposed in utero.* Decreased spontaneous mobility at 1 month in both sexes, increased activity at 2 months in both sexes.	LOAEL = 0.4 ppm, gestational	Sheveleva (1971)
Learning and memory					
Adult male and female LEW.1K rats	15/sex	0, 0.1, 0.5, or 5.4 ppm 2 hours for 10 consecutive days	*Impairment in acquisition of a new task*: Male and female rats at all formaldehyde exposures had significantly more errors in completing a water labyrinth ($p < 0.01$). Male and female rats had longer times to completion of the maze at 0.5 and 5.4 ppm ($p < 0.05$, $p < 0.01$).	LOAEL = 0.1 ppm 2 hours/ 10 days	Malek et al. (2003c)
Adult male and female Wistar rats	5−8/ sex	0, 2.6, 4.6 ppm 10 minutes/ day for 90 consecutive days	*Deficit in the retention of a learned task*: Male and female rats committed significantly more errors ($p < 0.05$) and took more time to complete the land maze in across the course of the experiment ($p < 0.04$).	LOAEL = 2.6 ppm 10 minutes/ 90 days	Pitten et al. (2002)
Ferrets		0.25 ppm	*Impairment in acquisition of a new task*: Exposed ferrets only achieved a 75% success rate in training to discriminate odors in a Y-maze versus 90% success rate in controls. Note: The results are confounded with other effects on the olfactory epithelium. The same researchers also reported a decrease in olfactory sensitivity and a reduction in percentage of olfactory cells in similarly treated animals.	None established	Apfelbach and Reibenspies (1991) (abstract only)
Male juvenile and adult rats	5/group	0.25, 0.5 ppm	*Decreases in olfactory thresholds, in juvenile animals but not in adults* ($p < 0.002$).	LOAEL = 250 ppm in juveniles	Weiler and Apfelbach (1992) (abstract only)

Table 4-58. Summary of neurological and neurobehavioral studies of inhaled formaldehyde in experimental animals (continued)

Species	No./ group	Treatment[a]	Observations	LOAEL/ NOAEL	Reference
Neurosensitization endpoints					
Female Sprague-Dawley rats	8−9	0 or 11 ppm 1 hour/day, 7 days	*Increase in cocaine-induced activity*: Increased quadrants crossed after cocaine injection 1 and 2 days after exposure ($p = 0.05$ and $p < 0.04$, respectively). No change in corticosterone levels 28 days postexposure. No change in nociceptive or passive avoidance test or plus-maze (21, 20, and 13 days postexposure, respectively) (21 days).	LOAEL = 11 ppm/ 7 days (unbounded)	Sorg et al. (1996)
Female and male Sprague-Dawley rats	Various up to 24/group	11 ppm, 1 hour/day, 7 days 1 ppm, 1 hour/day, 7 days 1 ppm, 1 hour/day, 5 days/week, 4 weeks	*Increase in cocaine-induced activity*: Increase in rearing after cocaine injection 1 day after exposure but not 4−6 weeks after exposure; 11 ppm for 7 days or 1 ppm for 4 weeks. No change in rats exposed at 1 ppm for 1 week.	LOAEL = 1 ppm 4 weeks NOAEL = 1 ppm 7 days	Sorg and Hochstatter (1999)
Female and male Sprague-Dawley rats	Various up to 24/group	1 ppm, 2 hours/day, 5 days/week, 4 weeks	*Increased conditioned fear response* in formaldehyde-treated, foot-shock-conditioned rats, twofold ($p < 0.05$).	LOAEL = 1 ppm 4 weeks	Sorg and Hochstatter (1999)
Male Sprague-Dawley rats	4−9 or 16	0, 0.7, or 2.4 ppm for 20 or 60 minutes 0, 0.7, or 2.4 ppm 1 hour/day, 5 days/week for 2 or 4 weeks	No change in corticosterone in acute (20- and 60-minute) exposures. *Increase in basal corticosterone*: 0.7 ppm for 2 or 4 weeks. *Hyperresponsive corticosterone response to environment*: 2.4 ppm for 2 or 4 weeks.	LOAEL = 0.7 ppm/ 2 weeks NOAEL = 0.7 ppm/ 20 minutes	Sorg et al. (2001)
Female and male Sprague-Dawley rats	4−9 or 16	0 or 2 ppm 1 hour/day, 5 days/week for 4 weeks	*Increased conditioned fear response* to an olfactory cue in formaldehyde-treated, foot-shock-conditioned male rats. Measured as increased time freezing when presented with a novel environment ($p < 0.05$). *No effect in female rats*.	LOAEL = 2 ppm/ 4 weeks NOAEL = 2 ppm/ 4 weeks	Sorg et al. (2004)

Table 4-58. Summary of neurological and neurobehavioral studies of inhaled formaldehyde in experimental animals (continued)

Species	No./ group	Treatment[a]	Observations	LOAEL/ NOAEL	Reference
Neurochemistry and neuropathology					
8-week-old female C3H/ HeN mice	5	0, 0.08, 0.4, or 1 ppm 16 hours/day, 5 days/week for 1 day or 12 weeks	No change in size of main olfactory bulb by several measures. No change in numbers of PG cells. No change in tyrosine hydroxylase immunopositive PG cells after 1 day. *Increase in tyrosine hydroxylase-immunopositive PG cells* after 12 weeks to 196, 167, and 196% of controls at 0.08, 0.40, and 1 ppm, respectively.	LOAEL = 0.08 ppm/ 12 weeks	Hayashi et al. (2004)
Adult male Sprague-Dawley rats	8	0, 5, 10, 20 ppm 3 hours/day, 1 or 2 days	No change in norepinephrine or 5-hydroxytryptamine in hypothalamus. Increase in DOPAC in hypothalamus after one exposure. Increase in dopamine and 5-HIAA in hypothalamus after two exposures.	LOAEL = 5 ppm/ 3 hours	Boja et al. (1985)
Neurogenesis					
Neonatal Wistar rats	5	0, 6, or 12 ppm, 6 hours/day, 5 days/week for 30 days	*Changes in volume of granular cell layer of the dentate gyrus in the hippocampus at postnatal days 30 and 90* ($p < 0.001$)	LOAEL = 6 ppm/ 30 days	Aslan et al. (2006)
Neonatal Wistar rats	5	0, 6, or 12 ppm, 6 hours/day, 5 days/week for 30 days	*Decreases in brain hemisphere volume at PND 30* ($p < 0.01$) *Changes in volume and cell numbers in the CA region of the hippocampus on PND 30* ($p < 0.01$)	LOAEL = 6 ppm/ 30 days	Sarsilmaz et al. (2007)
Neonatal Wistar rats	7	0, 6, or 12 ppm, 6 hours/day, 5 days/week for 30 days	*Changes in oxidant and antioxidant systems in cerebellum on PNDs 30 and 90* ($p = 0.017-0.001$). Increases in MDA, NO, and GSH-Px and decreases in SOD at both time points.	LOAEL = 6 ppm/ 30 days	Songur et al. (2008)

[a]Treatment is given as the formaldehyde concentration in air (ppm) with the length of exposure each day and the duration of treatment in days, as available.
[b]NA = not available.

Limited data regarding possible neurochemical changes in the brains of formaldehyde-exposed, immunized mice (Ahmed et al., 2007; Fujimaki et al., 2004b; Hayashi et al., 2004; Kakeyama et al., 2004) and rats (Boja et al., 1985) provided conflicting information, and the implications of these data regarding possible formaldehyde neurotoxicity are difficult to determine.

In developmental exposure paradigms, changes in brain structure (Sarsilmaz et al., 2007; Aslan et al., 2006), brain chemistry (Songur et al., 2008), and motor activity (Senichenkova, 1991; Sheveleva, 1971) were seen following neonatal or in utero exposure to formaldehyde. In addition, Weiler and Apfelbach (1992) found juvenile animals to be more sensitive to formaldehyde-induced changes in olfactory thresholds when compared with adult animals. These studies raise concern about possible long-lasting neurological effects of early exposure to formaldehyde. It is important to note, however, that exposure levels in these studies were higher (250−6,000 ppb) than those producing the behavioral effects in adults described above.

Overall, available data provide substantial evidence of behavioral effects, including motor activity changes and changes in learning and retention, following repeated exposure to relatively low levels of formaldehyde. These effects were seen in multiple laboratories, in studies conducted by different authors, and using different behavioral paradigms. These conclusions are also supported by more limited data, indicating possible developmental effects on the nervous system, including changes in brain structure and in the behavior of offspring; the developmental findings are less robust since they were seen only in individual laboratories and occurred following exposure to higher concentrations of formaldehyde. Studies evaluating developmental neurotoxicity at lower doses, comparable to those used in the adult studies, were not available. None of the available data provide sufficient information to allow a determination of the mechanism for these behavioral changes, although it is unlikely that they are attributable to the irritant properties of formaldehyde. The data regarding behavioral sensitization provide some support for a stress-related mechanism for those findings, but the applicability of this mechanism to the behavioral changes seen in the other studies, including the learning deficits, has not been evaluated.

4.2.6.2. *Oral Exposure*

Available data regarding neurotoxic effects of formaldehyde exposure following oral exposure are very limited. Several chronic or subchronic oral toxicity studies evaluated changes in brain weight or histopathology in rats or dogs following repeated oral exposures to formaldehyde at doses as high as 300 mg/kg-day, administered in drinking water (Til et al., 1989, 1988; Tobe et al., 1989; Johannsen et al., 1986). Although data were not presented in the

publications, all stated that no changes in brain weight or pathology were seen in the standard evaluations performed in these studies.

Two studies evaluated changes in behavior following exposure to formaldehyde in drinking water (Venkatakrishna-Bhatt et al., 1997; Venkatakrishna-Bhatt and Panchal, 1992). Venkatakrishna-Bhatt and Panchal (1992) evaluated changes in performance on a conditioned avoidance task in adult male albino rats (five/group). Animals were exposed to formaldehyde in drinking water (10 mg/mL) or by I.P. injection (10 mg/kg) for 60 days. Although it was stated that water consumption was recorded, the data were not presented, and thus actual exposure levels cannot be documented. Prior to the initiation of exposure, rats were trained on the conditioned avoidance task (climbing a wooden pole in response to a warning buzzer, thus avoiding electric shock from a floor grid). Rats were trained to a predetermined performance criterion (not described); animals not achieving the criterion were removed from study. Training and testing conditions (e.g., retest interval and duration of sessions) were not well described. Data were presented as percent response in behavioral performance (apparently separately for the escape or avoidance aspects of the task) or percentage decrease in response. No control data were presented, and pretreatment performance was not described. Figures presented performance at 10-day intervals, starting with day 0, with each data point stated to represent the mean for five experimental sets; again, the interval between experimental sets and the number of trials per set was not specified. Although the authors concluded that a deficit in performance was demonstrated, the data as presented were difficult to interpret and the conclusion could not be verified based on the data as presented.

Venkatakrishna Bhatt and Panchal (1992) examined changes in performance on a conditioned avoidance response, presumably using a procedure similar to the one described above. Albino rats (sex not specified, five/group) were exposed to formaldehyde in drinking water at 0.2 or 0.5 mg/mL for 90 days. As described above, rats were trained in performing the task prior to the start of exposure. Venkatakrishna-Bhatt Bhatr and Panchal (1992) stated that there was a dose-related deterioration of performance, but no data were presented to support these conclusions.

In summary, available data are insufficient to conduct a reliable assessment of neurotoxic effects of formaldehyde following oral exposure. Limited data suggest a lack of overt neuropathological changes at doses up to 300 mg/kg-day (Til et al., 1989, 1988; Tobe et al., 1989; Johannsen et al., 1986), but detailed information regarding the types of neuropathological evaluations performed in those studies is not available, and thus no firm conclusions can be drawn regarding the potential for neuropathological effects. The two available studies evaluating

behavioral changes are not considered to provide useful information, and thus effects on nervous system function could not be evaluated.

4.2.6.3. *Summary*

Overall, there is strong evidence that formaldehyde exposure via inhalation may cause adverse effects on nervous system function in experimental animals at relatively low levels of exposure (LOAELs as low as 100 ppb). Although human data regarding neurotoxicity following formaldehyde inhalation are limited, available data provide support that the types of effects seen in humans are similar to those found in animal studies. Evidence from available human controlled inhalation exposure studies indicates that humans may be affected at doses similar to those used in animal studies; however, the human data are extremely limited.

There are insufficient data to evaluate the potential for neurotoxicity following oral exposure to formaldehyde. Limited evaluations of brain weight or histopathology in available chronic or subchronic oral studies found no evidence of formaldehyde-induced changes (Til et al., 1989, 1988; Tobe et al., 1989; Johannsen et al., 1986). However, reliable studies examining nervous system function or focused studies of neuropathology following oral exposure to formaldehyde are not available.

4.2.6.4. *Other Considerations*

Major data gaps were found regarding the evaluation of changes in nervous system structure or function following formaldehyde exposure by both the inhalation or oral routes.

With respect to inhalation exposure, none of the available human studies resulted in data sufficient to conduct a reliable dose-response assessment for changes in nervous system function. Most of the available animal inhalation studies used short exposure durations (acute or short-term), precluding a reliable evaluation of neurotoxicity following chronic exposure. Available data for neurodevelopmental exposures are also quite limited, consisting of evaluation of neuropathology in only one brain region and functional evaluations focused only on changes in motor activity.

Major data gaps also exist regarding neurotoxicity following oral exposure, with no relevant human data and extremely limited animal data. Available oral exposure studies were insufficient to permit a reliable evaluation of the potential for neurotoxicity following oral exposure to formaldehyde.

4.2.7. Reproductive and Developmental Toxicity

The potential for developmental and reproductive effects after formaldehyde exposure by the inhalation route has generally been considered low, since formaldehyde, as a reactive gas, is not expected to penetrate past the POE (NEG, 2003; IPCS, 2002; Collins et al., 2001). Nevertheless, a number of animal studies have demonstrated effects of formaldehyde on pre- and postnatal development and on the reproductive system. For example, developmental toxicity was observed in two studies that evaluated a standard battery of developmental endpoints resulting from inhalation exposure on GDs 6−10 (Martin, 1990; Saillenfait et al., 1989). Similarly, oral exposures resulted in developmental effects when administered during comparable gestational windows (Marks et al., 1980; Hurni and Ohder, 1973). There have also been reports that identified developmental effects at lower-level formaldehyde exposures that were administered throughout gestation (Senichenkova and Chebotar, 1996; Senichenkova, 1991; Kitaev et al., 1984; Sheveleva, 1971; Gofmekler and Bonashevskaya, 1969; Gofmekler, 1968; Pushkina et al., 1968). Postnatal functional consequences of developmental exposures have also been identified (Sarsilmaz et al., 2007; Aslan et al., 2006; Weiler and Apfelbach, 1992; Senichenkova, 1991; Sheveleva, 1971). Additionally, a number of studies suggest that formaldehyde adversely affects the male reproductive system after both inhalation and oral exposures (Xing et al., 2007; Zhou et al., 2006; Özen et al., 2005, 2002; Sarsilmaz et al., 1999; Chowdhury et al., 1992; Cassidy et al., 1983; Guseva, 1972). This section reviews the available published studies assessing reproductive and developmental endpoints of formaldehyde.

4.2.7.1. *Inhalation Studies Addressing Developmental and Reproductive Toxicity*

Saillenfait et al. (1989) reported a comprehensive and well-documented developmental study in Sprague-Dawley rats. Pregnant rats were exposed beginning on GD 6 in order to cover critical stages of development (e.g., implantation and major organogenesis). Female Sprague-Dawley rats (25/group) were exposed to 0, 5, 10, 20, or 40 ppm (0, 6.15, 12.3, 24.6, or 49.2 mg/m^3) formaldehyde 6 hours/day on GDs 6−20. The onset of pregnancy was determined by the presence of sperm in a vaginal smear. Dams were exposed to formaldehyde in a dynamic flow chamber, and formaldehyde concentrations were determined to be 0, 5.17 ± 0.51, 9.92 ± 0.88, 20.04 ± 0.88, and 38.96 ± 3.70 ppm. Dams were weighed on GDs 0, 6, and 21 and sacrificed on day 21. Upon examination, uterine weights, fetal weights, sex ratio, number of implantation and resorption sites, and live and dead fetuses were recorded. Fetuses were examined for external malformations and cleft palate. One-half of viable fetuses were sectioned to assess soft-tissue alterations. The other half were fixed, stained with alizarin red S, and examined for skeletal alterations.

Body weight gain of dams and body weight of male and female fetuses were reduced by exposure to 40 ppm formaldehyde to 49, 78, and 81% of control values, respectively ($p < 0.01$) (Saillenfait et al., 1989). Reduced weight gain in dams remained significantly decreased when uterine weight was accounted for ($p < 0.01$). Mean fetal weight of male pups was reduced at maternal exposures of 20 and 40 ppm formaldehyde (5.53 and 4.42 g versus 5.61 g in controls). Decreased fetal body weight in females was only seen at 40 ppm (4.27 g versus 5.24 g in controls). All other pregnancy endpoints were unchanged by formaldehyde exposure (e.g., uterine weight, implantation and resorption sites, live fetuses, dead fetuses, and sex ratios). No major malformations were noted in fetuses. Some minor soft tissue and skeletal anomalies, such as dilated ureter, missing sternebrae, extra fourteenth rib, and rudimentary thirteenth rib (statistics not given), were reported. However, these effects occurred at similar frequencies in control and treatment groups. The incidence of delayed ossification of the thoracic vertebrae was 8.7% in fetuses from the 40 ppm exposure group versus 1.8% in controls. However, this difference was not statistically significant. Overall, from these results formaldehyde was neither lethal to embryos nor teratogenic, only exhibiting fetotoxic effects at exposures of 20 ppm and above. These are levels where there was a significant decrease in fetal body weight.

Martin (1990) conducted a similar study exposing pregnant rats during similar stages of development. Mated female Sprague-Dawley rats (25/group) were exposed to 2, 5, or 10 ppm (2.46, 6.15, or 12.3 mg/m^3) formaldehyde 6 hours/day on GDs 6−15. The study included two control groups: dams placed in the exposure chambers once a day but exposed only to clean air and dams fed and housed similarly to the experimental groups but never put into the inhalation chambers. The method of formaldehyde vapor generation and details of the exposure chamber were not described. Mean formaldehyde exposure concentrations were reported as 1.88, 4.88, and 9.45 ppm (variability not given, analytical method not discussed). Food consumption and body weight were recorded. On GD 20, rats were sacrificed, and the following pregnancy parameters were recorded: live fetuses, dead fetuses and resorptions, number of corpora lutea, fetal weights, sex ratios, and preimplantation and postimplantation losses. Fetuses were examined for major malformations, minor external and visceral anomalies, and minor skeletal anomalies (details not given). Weight gain and food consumption in dams were said to be reduced at 10 ppm ($p < 0.05$). Formaldehyde exposure of the dams at 5 and 10 ppm led to an increased incidence of reduced ossification of the pubic and ischial bones in fetuses on GD 20 ($p < 0.05$). Reduced ossification correlated with lower fetal weights, and the author considered both of these findings a result of larger litter size and, therefore, not related to formaldehyde exposure. However, no tables presenting the data or statistical analysis were provided. All other pregnancy parameters and fetal anomalies were described as unaffected by formaldehyde

exposure. However, without data presented for the assessed endpoints, background rates of malformations, trends in the data, and variability, it is difficult to evaluate the Martin (1990) comparisons. However, the author's observations of reduced fetal weight and increased incidence of reduced ossification are consistent with the results of Saillenfait et al. (1989).

Kilburn and Moro (1985) studied similar endpoints but included formaldehyde exposure during earlier gestational windows. The study report, only available in abstract form and not found as a subsequent published article, does not provide many methodological details. Female rats (number and strain not reported) were exposed to 0 or 30 ppm (0 or 37.2 mg/m^3) formaldehyde 8 hours/day during GDs 3−17, 3−12, 8−12, or 9−11. A second experiment included pair-fed controls for dams exposed to 30 ppm formaldehyde during GDs 3−17, 3−12, and 8−12. The authors reported reductions in fetal and maternal weight gain that were greater than decreases in pair-fed controls. Fetal anomalies were noted after 15 days of gestational exposure (e.g., altered organ size and undescended testes). Although the report indicates some maternal toxicity and fetotoxic effects (for example, stunted growth), lack of study details and clear reporting make this report of negligible utility in human health risk assessment.

There are several early studies that examined developmental effects of formaldehyde exposure administered throughout gestation (Gofmekler and Bonashevskaya, 1969; Gofmekler, 1968; Pushkina et al., 1968). It is unclear if these reports represent the same or overlapping experimental groups. They were performed in the same laboratory and are reported with a similar level of detail. The source of formaldehyde, method of vapor generation, exposure conditions (dynamic versus static), confirmation of exposure concentrations, study design, and data presentation details were not provided. Absence of such critical information detracts from the quality of these studies as a coherent record of experimental information, and, thus, these findings can only be utilized qualitatively in the formaldehyde risk assessment.

In the Gofmekler (1968) study, female rats (36, strain not specified) were continuously exposed at 0, low, or high formaldehyde concentrations beginning 10−15 days prior to mating (target concentrations of 0, 0.01, or 0.81 ppm formaldehyde [0, 0.01, or 1 mg/m^3]). The author reported a 14−15% increase in pregnancy duration and a decrease in litter size (data not shown). However, males and females were mated 6−10 days, and no information was provided on how mating and conception were confirmed. No external malformations were attributed to formaldehyde exposure. Concentration-dependent increases in pup body weight and decreases in lung and liver weight were attributed to formaldehyde exposure. Pup weights were increased from 5.6 g in controls to 6.0 and 6.3 g in groups 1 and 2, respectively ($p < 0.01$ and $p < 0.001$). Formaldehyde exposure increased pup adrenal weight in both groups and pup thymus and kidney weight in group 2 only (see Table 4-59).

Table 4-59. Effects of formaldehyde on body and organ weights in rat pups from dams exposed via inhalation from mating through gestation

Exposure (ppm)[a]	Body weight (g)	Relative organ weights (mg/10 g body weight)					
		Thymus	Heart	Lung	Liver	Adrenals	Kidney
0	5.6	26	61.4	287.1	587.7	3.2	51.4
0.01	6.0[b]	25.1	61.5	230.2[c]	557.7[d]	4.2[c]	53.4
0.81	6.3[c]	31.7[c]	64.5	223.2[c]	550.8[b]	3.8[d]	55.7[b]

[a]Dams were exposed to formaldehyde continuously from 10−15 days prior to mating. Exposure concentrations were not validated.
[b]Different from controls, $p < 0.01$.
[c]Different from controls, $p < 0.001$.
[d]Different from controls, $p < 0.05$.

Source: Gofmekler (1968).

In a study by Gofmekler and Bonashevskaya (1969), the researchers evaluated organ histopathology in pups from similarly treated dams. Pregnant female albino rats were continuously exposed at two formaldehyde concentrations (groups 1 and 2, as described above). Adult males were similarly exposed during mating. Offspring were examined for malformations, and the organs were fixed and sectioned for histopathologic examination, including hematoxylin and eosin staining, Brachet stain for RNA, and Feulgen stain for DNA. Liver and kidney sections were also stained with Schiff's reagent (which reacts with aldehydes), with Sudan III for lipids, and Pearl's stain for iron. Placenta, uterus, and ovaries from the dams and testes of the males were sectioned, stained, and evaluated. The authors stated that formaldehyde induced no external anomalies (reported elsewhere, but no reference given). The authors also noted involution of lymphoid tissue and changes in liver, mild hypertrophy of Kupffer cells, and numerous extramedullary myelopoietic centers in pups from dams in group 2. Pups from both treatment groups showed reduced glycogen content in the myocardium and the presence of iron in Kupffer cells. There was a localized increase in positive reaction to Schiff's reagent in the basement membrane and intertubular connective tissue of the kidneys. The authors suggested that this was an indication of functional alterations in the renal tubular apparatus. All other tissues examined and histochemical staining indicated no differences due to formaldehyde exposure.

Researchers in the same laboratory (Pushkina et al., 1968) studied the effects of formaldehyde exposure on vitamin C (ascorbic acid, an antioxidant) and nucleic acid levels in dams and fetuses as general measures of toxicity. Female white rats ($n = 160$) were continuously exposed at two formaldehyde concentrations (groups 1 and 2, as described above) from 20 days

prior to mating (6−10 days) and then throughout gestation. Dams were sacrificed and ascorbic acid and nucleic acid levels determined in harvested organs (methods referenced but not described). No visible malformations in pups were noted. Formaldehyde exposure increased fetal body weight and organ weight in both groups (data not given). There was an average of 11.3 fetuses per litter for control dams versus 9.8 and 8.6 for groups 1 and 2, respectively. The authors reported that formaldehyde exposure decreased DNA levels and increased RNA levels in organs (further details not provided). Formaldehyde exposure resulted in lower vitamin C levels in the whole fetus (76 and 75% of controls) and in maternal liver specimens (82 and 88% of controls) for exposure groups 1 and 2, respectively ($p < 0.05$). In contrast, vitamin C was higher in fetal liver (127% of controls) in group 1 ($p < 0.05$). The significance of these differences is unknown. The authors considered the results as general measures of biochemical changes and therefore toxic.

The reports of Gofmekler and Bonashevskaya (1969), Gofmekler (1968), and Pushkina et al. (1968) lack key methodological details. As discussed above, exposure conditions and actual formaldehyde concentrations cannot be validated. Although methods were not thoroughly detailed, results were reported in data tables with statistics and detailed descriptions of observed pathological changes. However, without validation of exposure concentrations, these findings can only be considered qualitatively.

In another study, Sheveleva (1971) exposed female mongrel (i.e., not a homogeneous genetic strain) white rats to 0, 0.0005, or 0.005 mg/L (0, 0.5, or 5 mg/m^3) (0, 0.4, or 4 ppm) formaldehyde on GDs 1−19 (where GD 1 was defined as the day that spermatozoa were detected in vaginal smears) for 4 hours/day. In each group, 15 dams were terminated on GD 20 for evaluation of ovarian corpora lutea, uterine implantation sites, pre- and postimplantation loss, number of live fetuses, fetal length and weight, and external examination for malformations. Additionally, in each group, six dams were allowed to deliver their litters. Developmental landmarks were monitored (i.e., ear and eye opening, incisor eruption, emergence of hair coat), and the pups were further evaluated at 1 and 2 months of age for body weight, threshold of neuromuscular excitability, total oxygen consumption in 1 hour per 100 g of weight, and spontaneous mobility over 10 minutes. Maternal toxicity (recorded on GD 17) included significantly ($p < 0.05$) decreased leukocyte counts in both treated groups and a number of additional findings at 0.005 mg/L (i.e., significant reductions in the threshold of neuromuscular excitability, rectal temperature, and blood hemoglobin level) as well as an increase in spontaneous mobility over 15 minutes. Fetal examinations on GD 20 identified a 50−70% increase in mean preimplantation loss in both formaldehyde-exposed groups. When pups were 1 month of age, a reduction in spontaneous mobility was noted in both treated groups; in pups at 2

months of age, an increase in mobility was observed in the 0.005 mg/L group. Also, when pups were 2 months of age, there were alterations in hemoglobin levels and leukocyte counts in both treated groups. Detailed descriptions of some study methodologies (particularly in regard to neurological and behavioral assessments) were not provided in the published paper.

Based on a review of the work by Gofmekler (1968) and various epidemiologic studies available at the time, Kitaev et al. (1984) hypothesized that formaldehyde may exert toxic effects in the early days of gestation. To study embryotoxic effects of formaldehyde inhalation exposures, mature female Wistar rats (five to nine per group) were exposed to 0.41 or 1.22 ppm (0.5 or 1.5 mg/m^3) formaldehyde 4 hours/day, 5 days/week for 4 months (Kitaev et al., 1984). Rats were exposed in dynamic flow chambers and formaldehyde levels measured gravimetrically (but not reported). Females were mated on day 120 of exposure and mating confirmed by the presence of sperm in a vaginal smear. Embryos were harvested on the second or third day of pregnancy (GD 2 or 3) and examined by both light and phase contrast microscopy for changes in morphology (i.e., evidence of embryonic degeneration). Additionally, maternal weight gain and organ weights (ovaries, uterus, and adrenal glands) and blood samples (HCT, Hb, and TP) were monitored as indicators of general toxicity. These parameters were unchanged by formaldehyde exposure. Formaldehyde exposure at 1.22 ppm for 4 months resulted in an increased number of degenerating embryos on GD 3 (14.9 versus 4.4% in controls) and a smaller increase of 10.2% (versus 5.1% in controls; statistical significance not assessed) on GD 2. Indications of degeneration included reduced size and changes in appearance (granulation of the ooplasm, wrinkling and degradation of nuclear material). However, it is unclear if litter effects were accounted for in the statistical analyses, and it is unknown how the affected embryos were distributed between litters. For dams exposed to 0.41 ppm formaldehyde, the number of degenerated embryos was not increased on day 2 (3.8 versus 5.1% in controls) but was increased on day 3 (9.1 versus 4.4% in controls; again, unknown if statistically significant) after maternal exposure to 1.22 ppm formaldehyde. This observation may be coincidental since it was seen in dams sacrificed on GD 2 but not in those sacrificed on GD 3. Kitaev et al. (1984) considered these findings to indicate that repeated exposure to formaldehyde over a 4-month period can disturb reproductive function, resulting in adverse effects early in embryonic development.

To further explore the effects of inhalation exposures to formaldehyde on reproductive function, Kitaev et al. (1984) conducted a second series of experiments on 200 similarly treated female rats. After 4 months of repeated formaldehyde exposure at 0.41 or 1.22 ppm as described above, organ weights (ovaries and uterus) and blood levels of gonadotropic hormones and progesterone were determined. However, the day and time of hormone measurement were not given in the report, and normal diurnal variations in these hormones could affect the reported

findings if time of day was not accounted for. The length of the estrous cycle was unchanged during exposure. Formaldehyde exposure modulated gonadotropin levels and relative ovarian weight, suggesting low-level effects on the female rat reproductive system prior to mating (Kitaev et al., 1984). Ovarian weight and blood levels of luteinizing hormone (LH) were both significantly increased after exposures at 0.41 ppm formaldehyde but remained at control levels in rats exposed at 1.22 ppm. Blood levels of follicle-stimulating hormone (FSH) were increased approximately 66% from control after 1.22 ppm formaldehyde exposure ($p < 0.05$). Progesterone levels were unchanged by formaldehyde treatment. Kitaev et al. (1984) suggested a role of the hypothalamus-pituitary system based on increased ovary weight, a greater number of degenerated embryos, and increased LH in rats exposed at 0.41 ppm. They postulated that these effects were not seen at 1.22 ppm due to a toxic effect exhibited as embryonic degeneration, thus the absence of a dose-response did not alter the interpretation of the validity of the adverse response. The study NOAEL was not determined, and the study LOAEL was 0.4 ppm (0.5 mg/m^3), based upon increased early embryo loss and on maternal outcomes (increased ovarian weight and increased blood LH levels) following 4 months of formaldehyde treatment. For the finding of increase blood FSH levels, the endpoint NOAEL was 0.4 ppm (0.5 mg/m^3) and the LOAEL was 1.2 ppm (1.5 mg/m^3).

Senichenkova and Chebotar (1996) and Senichenkova (1991) examined reproductive and developmental effects of daily formaldehyde exposure on GDs 1−19 of pregnancy, including the potential effect of formaldehyde exposure on development early in gestation. Additionally, since anemia adversely affects fetal development, Senichenkova and Chebotar (1996) also examined formaldehyde effects in iron-deficient dams to determine whether coexposure further compromises fetal development. In both studies, female white rats were exposed to 0 or 0.41 ppm formaldehyde (0 or 0.5 mg/m^3), 4 hours/day on GDs 1−19. Formaldehyde concentrations in the dynamic exposure chambers were measured gravimetrically to confirm the exposure concentration but were not reported (methods not provided). It is unclear if gravimetric measurements would be sensitive or accurate enough to validate these low-exposure concentrations without a better understanding of the methodology. This uncertainty in exposure conditions should be considered in evaluating the reported results.

Mongrel female white rats were exposed at a target concentration of 0 or 0.41 ppm (0 or 0.5 mg/m^3) formaldehyde 4 hours/day on GDs 1−19 (Senichenkova, 1991). On GD 20, a subset of the dams was sacrificed and examined for number of corpora lutea, implantation and resorption sites, live/dead fetuses, and fetal weights. Fetuses were examined for gross pathology of the internal organs and skeleton (details not given). Blood pH, partial pressure of CO_2, and partial pressure of oxygen were measured in both dams and embryos. The remaining dams were

brought to term to study postnatal effects of formaldehyde exposure. Rat pups were observed on PNDs 1−25 for viability, physical development, and maturation rate of motor reflexes. Behavior of juvenile offspring (PND 40) was studied in an open field test, and maze learning was tested at sexual maturity. In a follow-up report, Senichenkova and Chebotar (1996) present blood chemistry data, pregnancy outcome, and developmental data for similarly treated dams and their pups in a chemical model of iron deficiency. Intraperitoneal injections of the iron-chelating agent bipyridyl were given on GDs 12−15 at the threshold embryotoxic dose (1 mL, 25% solution). On day 20, the dams were sacrificed and dams and fetuses examined as described above. In addition to blood pH, partial pressure of carbon dioxide and partial pressure of oxygen, acid metabolic products (not detailed), and true bicarbonates were reported for maternal and fetal blood. A review of the data from these reports indicates there may be an overlap of the study groups. Neither paper presents the entire data set; thus, for transparency and brevity, the following text discusses the combined findings from both studies as if they were a single study

Formaldehyde exposure did not affect such indicators of pregnancy outcome as number of corpora lutea, implantation and resorption sites, and live and dead fetuses, all of which were unchanged (Senichenkova and Chebotar, 1996; Senichenkova, 1991). Although fetal weight was slightly increased by formaldehyde exposure, 2.35 versus 2.24 g in controls ($p < 0.001$), neither fetal length nor bone length were changed (femur and humerus) (Senichenkova and Chebotar, 1996; Senichenkova, 1991). Often, increased fetal weight is the result of early physical development, and other signs of development, such as ossification, would be expected to be enhanced as well. The average number of bone centers per limb was increased by formaldehyde exposure from 2.45 and 2.66 to 2.78 and 2.91 in controls for metacarpal and metatarsal bone centers, respectively ($p < 0.05$) (Senichenkova, 1991); these findings were consistent with increased growth and weight. In contrast, Senichenkova (1991) reported a decrease in the number of embryos with ossification centers in the hyoid bone (100% in controls versus 91% for formaldehyde exposure, $p < 0.05$), consistent with the results of Saillenfait et al. (1989) and Martin (1990). However, litter size, a factor influencing fetal weight, was not provided, and it is unclear if Senichenkova (1991) took litter size into account in the analysis.

Senichenkova and Chebotar (1996) reported increased blood acidosis and decreased blood alkaline reserves (bicarbonates and total CO_2) in formaldehyde-treated dams and their embryos ($p < 0.05$). However, this finding should be considered in light of the fact that chronic blood acidosis may increase bone remodeling and decrease bone density in adults. It is unknown if the reported blood acidosis could reduce ossification rates in developing embryos. A better understanding of exposure conditions and the acid metabolic products measured is needed to determine the biological relevance of the reported changes in blood acid balance.

Iron deficiency, induced by injections of bipyridyl (an iron-chelating agent), was found to be fetotoxic. Iron-deficient dams with no formaldehyde exposure had higher rates of postimplantation death than controls (12.6 ± 5.5 versus 4.8 ± 1.3%). Formaldehyde exposure in conjunction with iron deficiency increased postimplantation death to 23.1 ± 5.9%. Fetal weight and litter size were not reported for the bipyridyl treatment groups, but bipyridyl treatment in conjunction with formaldehyde resulted in a decreased number of metatarsal bone centers (2.21 ± 0.12 versus 2.72 ± 0.08 in controls; $p < 0.001$). This decrease was also significant when compared with formaldehyde or bipyridyl alone ($p < 0.02$). However, all pregnancy outcome parameters were not reported for the bipyridyl treatment.

Fetal anomalies were reported after formaldehyde exposure and were increased by iron deficiency. The incidence of litters with internal organ anomalies was increased from 1.4% in controls to 14.2% in formaldehyde-treated dams (Senichenkova, 1991). Undescended testes were the predominant anomaly described: 20.8% in litters from formaldehyde-treated dams versus 1.2% in controls ($p < 0.05$) (Senichenkova, 1991). Similar findings were reported by Senichenkova and Chebotar (1996). Bipyridyl treatment in conjunction with formaldehyde exposure increased the overall incidence of fetal anomalies (13.8 ± 2.1% in controls versus 6.6 ± 1.8% with iron deficiency alone) (Senichenkova and Chebotar, 1996). However, there are discrepancies between the two papers in the reporting of the anomalies, and it is unclear whether the experimental groups overlap between papers, where some parameters are identical (which would lead to double counting of the same animal, including identical SDs) and others are different. Additionally, the reporting is unclear with respect to the basis of the incidence rates reported (for example, overall incidence versus incidence within litter or incidence of litters with anomalies). Unclear reporting, together with some of the uncertainties regarding exposure conditions, suggests that the data may be of limited quality to support risk assessment.

In the second phase of the studies, pups were delivered and postnatal development was assessed (Senichenkova, 1991). Eruption of the upper and lower incisors was delayed in pups from formaldehyde-treated dams, occurring on PND 14 versus PND 12 in controls ($p < 0.01$). All other measures of physical postnatal development were unchanged by formaldehyde. To evaluate postnatal functional outcomes following in utero exposure to formaldehyde, an open field test was conducted in juvenile rats on 3 consecutive days (PNDs 40−42). Juvenile rats from formaldehyde-treated dams exhibited increased mobility (crossed squares), rearings, and defecations/urinations compared with control rats on the second and third open field tests ($p < 0.05$). There were no differences seen in the maze-learning test assessed in mature offspring of formaldehyde-treated dams (Senichenkova, 1991).

Additional assessments of formaldehyde exposure on neurological development are described above in the section on neurological and behavioral toxicity in animal studies (see Section 4.2.6). In brief, studies conducted by Sarsilmaz et al. (2007) and Aslan et al. (2006) exposed 10 neonatal male Wistar rats/group to 0, 6, or 12 ppm (0, 7.36, or 14.7 mg/m^3) formaldehyde 6 hours/day, 5 days/week for 30 days. At that time, five rats/group were killed and subjected to neuropathological examination; the remaining rats were maintained until PND 90, at which time they were killed and evaluated. Aslan et al. (2006) examined the number and volume of granular cells in the hippocampal dentate gyrus, while Sarsilmaz et al. (2007) examined the size and number of the pyramidal cells in the cornu ammonis of the hippocampus. In both studies, lower numbers of cells were observed in both treated groups at PND 90 as compared with PND 30. Although the effects of treatment on the volume and number of granular and pyramidal cells were somewhat inconsistent, a significant decrease in the number of neurons in the pyramidal cell layer of the hippocampal cornu ammonis was observed at both PNDs 30 and 90 (Sarsilmaz et al., 2007).

One other study reported effects on nervous system function following exposure to formaldehyde during postnatal development. An abstract by Weiler and Apfelbach (1992) described a study in which juvenile rats (strain not specified) were exposed to 0.25 ppm (0.31 mg/m^3) formaldehyde from PNDs 30−160 or adult rats were exposed to 0.5 ppm (0.62 mg/m^3) formaldehyde for 90 days. Decreased olfactory sensitivity (i.e., increased olfactory thresholds) was observed and was greater when the exposure was initiated in the young rats, as compared with the adults.

Evaluation of offspring following prenatal, perinatal, and/or juvenile inhalation exposures to formaldehyde have also been reported by Kum et al. (2007), Sandikci et al. (2007), and Songur et al. (2005). Kum et al. (2007) exposed female Sprague-Dawley rats (six dams/group) and their offspring to 0 or 6 ppm (0 or 7.4 mg/m^3) formaldehyde for 8 hours/day in separate groups with exposures starting on GD 1, on postparturition day 1, or in offspring at 4 weeks of age and continuing for 6 weeks. In another group, exposures were initiated in adult rats. Mean body and liver weights were significantly decreased in the offspring exposed in utero and in early postnatal life, and mean liver weights were also significantly decreased in rats with juvenile exposures. However, neither body weight nor liver weight was affected in the group with exposure initiating at an adult age, suggesting a life-stage-related susceptibility to formaldehyde-induced hepatic toxicity. Evaluation of biomarkers of oxidative stress revealed significantly increased catalase (CAT) and MDA in the livers of offspring that were exposed prenatally, significantly decreased GSH levels in the livers of offspring that were exposed in early postnatal life, and significantly decreased SOD levels in the livers of offspring that were exposed starting

at 4 weeks of age. No biomarkers were altered in the livers of rats exposed to formaldehyde only as adults.

A similar study design was used by Sandikci et al. (2007) to examine the effects of 0 or 6 ppm (0 or 7.4 mg/m^3) formaldehyde on bronchus associated lymphoid tissue (BALT) following pre- and perinatal, juvenile, or adult exposures of 6 weeks duration in Sprague-Dawley rats (six/group). The presence of the lysosomal enzyme alpha-naphthylacetate esterase (ANAE) served as a marker of T-lymphocytes in peripheral blood and tissue sections. Significant increases in ANAE-positive T-lymphocytes were found in BALT in all but the in utero exposed groups as compared with control; this outcome is consistent with the postnatal development of BALT in the rat. In peripheral blood, ANAE-positive lymphocyte ratios were significantly ($p <$ 0.001) increased as compared with controls at all life stages tested.

Songur et al. (2005) examined the effect (and reversibility) of formaldehyde exposures during the early postnatal period on zinc, copper, and iron levels and activity of SOD in the lung tissue of Wistar rats. Litters (12−14/group) were exposed to 0, 6, or 12 ppm (0, 7.4, or 14.9 mg/m^3) formaldehyde 6 hours/day, 5 days/week for 30 days. Trace element and biochemical analyses were conducted on PND 30 or 90. Decreased SOD activity, decreased levels of copper and iron levels, and increased zinc levels were observed in the lungs of treated groups following 30 days of treatment and at 90 days (i.e., 60 days post-treatment). Survival was not affected in neonatal rats. Clinical observations during treatment included evidence of respiratory irritation and toxicity. Body weight and food and water consumption were also nonsignificantly decreased as compared with controls.

There are several reports in the literature regarding formaldehyde effects after inhalation exposure on the male reproductive system in animals (Galalipour et al., 2007; Zhou et al., 2006; Özen et al., 2005, 2002; Sarsilmaz et al., 1999; Woutersen et al., 1987; Maronpot et al., 1986; Guseva, 1972). The earliest report examined the effect of simultaneous exposures to formaldehyde from air and water (Guseva, 1972). Male rats (n = 12, strain not specified) were coexposed to formaldehyde in air and drinking water 4 hours/day, 5 days/week for 6 months. There were three exposure levels in the experiment of different air and drinking water concentrations: (1) 0.41 ppm (0.5 mg/m^3) formaldehyde in air and 0.1 mg/L in water; (2) 0.20 ppm (0.25 mg/m^3) formaldehyde in air and 0.01 mg/L in water; or (3) 0.10 ppm (0.12 mg/m^3) formaldehyde in air and 0.005 mg/L in water. Reproductive function was assessed by mating two females per male. The time for the onset of pregnancy and the number of pregnancies per treatment group were recorded. A subset of dams was sacrificed on GD 20 of pregnancy, and the number and weight of fetuses was determined. Postnatal development of the remaining dams was tracked (e.g., times of eye opening and development of hair coat). Nucleic acid levels were

determined in the testes of formaldehyde-exposed rats. Gonadotropic response was assessed by injecting an emulsion of pituitaries from exposed male rats into unexposed infantile females and measuring the weight ratios of the uterus and ovaries. Formaldehyde exposure reduced nucleic acid levels in testes to 88 and 92% of controls in groups 1 and 2, respectively. No other formaldehyde-induced differences were found.

Woutersen et al. (1987) and Maronpot et al. (1986) examined tissue sections from testes and ovaries of exposed animals as part of studies primarily addressing respiratory tract toxicity (see Section 4.2.1.2.2.4 for complete study details). Maronpot et al. (1986) exposed female and male B6C3F1 mice to 0, 2, 4, 10, 20, and 40 ppm (0, 2.45, 4.91, 12.3, 24.5, and 49.1 mg/m^3) formaldehyde 6 hours/day, 5 days/week for 13 weeks. Decreased weight gain due to formaldehyde exposure was seen in both male and female mice. Additionally, there was 80% mortality at the highest exposure (40 ppm). The authors reported endometrial hypoplasia and lack of ovarian luteal tissue in females exposed to 40 ppm, but no compound-related changes were observed in testes sectioned and viewed by light microscopy.

In a study by Appleman et al. (1988), male Wistar rats (40/group) with undamaged or damaged (via bilateral intranasal electrocoagulation) nasal mucosa were exposed for 13 or 52 weeks to 0, 0.1, 1, or 10 ppm (0, 0.124, 1.24, or 12.4 mg/m^3) formaldehyde 6 hours/day, 5 days/week. At study termination, mean body weight was decreased, but relative testes weight was increased in the 10 ppm group (interpreted as a nonadverse outcome that was associated with the decreased body weight). No treatment-related histopathologic findings were reported for male reproductive organs (although it is not clear to what extent they were evaluated since the primary focus of the study was on the nasal epithelium).

Following up on earlier reports of decreased Leydig cell quality in rats administered I.P. injections of formaldehyde (Chowdhury et al. [1992], described in Section 5.2.1.8.3), Sarsilmaz et al. (1999) studied the effects of formaldehyde inhalation on Leydig cells. Adult male Wistar rats (30) were exposed to 0, 10, or 20 ppm (0, 12.3, or 24.6 mg/m^3) formaldehyde 8 hours/day, 5 days/week for 4 weeks. Animals were observed daily and weighed weekly. Rats were sacrificed on day 29 and autopsied, and testes were weighed, fixed, and sectioned for histologic examination. Signs of irritation from formaldehyde exposure were noted (frequent eye blinking, excessive licking, increased frequency of nose cleaning, interrupted breathing, and sneezing). Body weight gain was reduced by formaldehyde exposure from 17.7% gain in control rats to 4.66 and 2.63% in rats exposed at 10 and 20 ppm, respectively ($p < 0.001$). As shown in Table 4-60, relative testes weights were unaffected (reported as proportions but more likely to be percentages), although trends and numerical differences were similar to those reported by Özen et al. (2002). Sarsilmaz et al. (1999) found that both Leydig cell quantity and the percentage of

cells with normal nuclei were reduced by formaldehyde treatment. Although the dose-dependent reduction in Leydig cell quantity was statistically significant at both exposure levels, the study authors considered the data to be within the normal range.

Table 4-60. Formaldehyde effects on Leydig cell quantity and nuclear damage in adult male Wistar rats

Inhalation exposure[a]	Relative testes weight[b,c,d]	Leydig cell quantity[c,e,f]	Appearance of nucleus[e,g]			
			Normal	Pyknotic	Karyorectic	Karyolytic
Control	0.93 (0.03)	47.27 (7.8)	98	2	0	0
10 ppm	0.92 (0.06)	45.04 (7.8)[h]	92	2	4	2
20 ppm	0.89 (0.06)	44.36 (7.5)[i]	76	9	10	5

[a]Rats were exposed 8 hours/day, 5 days/week for 4 weeks.
[b]Stated to represent the ratio of the last day's testicle weight to the body weight but more likely to be the percent of body weight.
[c]Cells within 100 defined areas.
[d]n = 10.
[e]For each exposure group, 100 defined locations were assessed.
[f]n = 100.
[g]Values presented as percentage of cells.
[h]Different from control ($p < 0.05$), as reported by the authors.
[i]Different from control ($p < 0.01$), as reported by the authors.

Source: Sarsilmaz et al. (1999).

It was hypothesized that decreased Leydig cell quality may have been the result of oxidative stress induced by formaldehyde exposure. Özen et al. (2002), in the same laboratory, investigated changes in testicular iron, copper, and zinc levels as measures of oxidative stress and damage. Adult male albino Wistar rats (seven/group) were exposed at 0, 10, or 20 ppm (0, 12.2, or 24.4 mg/m^3) formaldehyde 8 hours/day, 5 days/week for either 4 or 13 weeks. Rats were observed daily and weighed once a week. Rats were sacrificed at the end of the exposure period and autopsied, and the testes were removed and weighed. Zinc, copper, and iron levels were determined in testes tissue by using atomic absorption spectrophotometry. Both weight gain and relative testes weight were decreased in a concentration-dependent and duration-dependent manner (see Table 4-61). Both zinc and copper levels in rat testes were reduced in a concentration- and duration-dependent manner by formaldehyde exposure. For example, zinc was reduced from 277 to 107 mg/kg after a 4-week × 20 ppm exposure and from 260 to 95 mg/kg after a 12-week × 20 ppm exposure ($p < 0.001$) (see Table 4-61). Iron levels in testes were increased from 30 to 39 mg/kg after a 4-week 20 ppm exposure ($p < 0.01$) and from 33 to

58 mg/kg after 12 weeks at 20 ppm ($p < 0.05$). The authors suggested that alterations in trace element levels in the testes were consistent with oxidative damage and may have contributed to changes in Leydig cell function. These researchers also reported alterations in trace metals in lung tissue from Wistar rats exposed to formaldehyde 8 hours/day, 5 days/week for 4 or 13 weeks. Iron levels were increased at 5 ppm for 13 weeks and 10 ppm for either 4 or 13 weeks. Zinc levels decreased for all formaldehyde exposures. In both cases, the authors attributed elevated iron levels to oxidative stress. In addition to citing the role of zinc as a cofactor of cytoplasmic Cu-Zn-SOD, the authors suggested that zinc may have been consumed by increased FALDH activity. Although oxidative stress and increased FALDH activity may be relevant to the POE and therefore impact the lung, it is less clear how these changes would occur in the testes. Taken together, the reports of Özen et al. (2002) and Sarsilmaz et al. (1999) suggested a LOAEL of 10 ppm 8 hours/day for 4 weeks for changes in Leydig cell quantity and quality, decreased testes weight, and changes in trace metal content (zinc, copper, and iron).

Table 4-61. Formaldehyde effects on adult male albino Wistar rats

Inhalation exposure[a]	Weight gain (%)	Testes weight (%)	Zinc (mg/kg)	Copper (mg/kg)	Iron (mg/kg)
4 Weeks					
Control	19.1 (2.7)	0.94 (0.03)	277 (16)	6.4 (0.42)	30 (2.7)
10 ppm	5.8 (2.4)[b]	0.92 (0.02)[c]	132 (8.9)[b]	4.2 (0.33)[b]	35 (2.8)[d]
20 ppm	2.4 (0.6)[b]	0.91 (0.01)[c]	107 (6.9)[b]	3.3 (0.27)[b]	39 (3.1)[d]
13 Weeks					
Control	55.9 (2.3)	0.91 (0.01)	269 (15)	6.0 (0.34)	33 (2.6)
10 ppm	34.7 (3.5)[b]	0.84 (0.03)[b]	112 (8.1)[b]	3.6 (0.30)[b]	52 (3.5)[b]
20 ppm	20.8 (1.4)[b]	0.82 (0.03)[b]	95 (6.4)[b]	1.9 (0.17)[b]	58 (3.0)[b]

[a]Formaldehyde exposure was 8 hours/day, 5 days/week for either 4 or 13 weeks. Values are means ± SDs of seven animals..
[b]Different from control, $p < 0.001$, as calculated by the authors.
[c]Different from control, $p < 0.05$, as calculated by the authors.
[d]Different from control, $p < 0.01$, as calculated by the authors.

Source: Özen et al. (2002).

In another study that assessed testicular toxicity (Özen et al., 2005), male Wistar rats (18/group) were exposed by inhalation to 0, 5, or 10 ppm (0, 6.2, or 12.4 mg/m^3) formaldehyde 8 hours/day, 5 days/week for 91 days. In-life observations in exposed rats included clinical signs of respiratory irritation and decreased mean food and water consumption. At study termination,

serum testosterone levels and mean seminiferous tubule diameters were significantly decreased from control in a dose-responsive manner (see Table 4-62). Immunohistochemical staining of testis tissues showed increased localization of heat shock protein (Hsp) 70 in the cytoplasm of spermatogonia, spermatocytes, and spermatids of treated animals compared with controls (not shown here).

Table 4-62. Formaldehyde effects on testosterone levels and seminiferous tubule diameters in Wistar rats following 91 days of exposure

Inhalation exposure[a]	Testosterone levels (ng/dL)	Seminiferous tubule diameters (µm)
	n = 6	*n* = 100
Control	406.54 ± 16.82	259.22 ± 16.18
10 ppm	244.01 ± 23.86[b]	236.17 ± 13.09[c]
20 ppm	141.30 ± 08.56[b]	233.24 ± 10.13[c]

[a]Formaldehyde exposure was 8 hours/day, 5 days/week for 91 weeks. Values are means ± SEMs.
[b]Different from control, $p < 0.0001$, by one-way ANOVA, as calculated by the authors.
[c]Different from control, $p < 0.001$, by one-way ANOVA, as calculated by the authors.

Source: Özen et al. (2005).

Zhou et al. (2006) investigated the effect of formaldehyde on the testes and the protective effect of vitamin E against oxidative damage by formaldehyde in adult male rats. In this study, adult male Sprague-Dawley rats (10/group) were treated for 2 weeks in the following groups: (1) control rats were administered physiological saline by oral gavage, (2) rats were administered physiological saline by gavage and exposed to 10 mg/m^3 (8.05 ppm) formaldehyde by inhalation for 12 hours/day, and (3) rats were administered daily gavage doses of 30 mg/kg vitamin E and exposed to 10 mg/m^3 (8.05 ppm) formaldehyde by inhalation for 12 hours/day. Formaldehyde treatment resulted in significantly decreased ($p < 0.05$) mean testis weight. Histopathologic findings in treated rats included atrophy of seminiferous tubules, decreased spermatogenic cells, and seminiferous cells that were "disintegrated" and shed into the lumina, which was azoospermic. Interstitial tissue was edematous with vascular dilatation and hyperemia. In the formaldehyde-treated group, epididymal sperm count and percentage of motile sperm were significantly decreased, and the percentage of abnormal sperm was increased ($p < 0.05$), as compared with control. Evaluation of biochemical markers in testes tissue showed the activities of testicular SOD, GPX, and GSH were decreased; MDA levels were significantly increased as

compared with control. All observed effects of formaldehyde treatment (i.e., decreased testes weight, biochemical alterations, histopathologic effects, and sperm count, motility, and morphology findings) were attenuated by administration of 30 mg/kg-day vitamin E.

In a study by Golalipour et al. (2007), testicular changes of increasing severity with increasing duration were reported. A total of 28 Wistar rats, aged 6-7 weeks old, were divided into four groups including three formaldehyde treatment groups (4 hours of exposure/day, 4 days/week for 18 weeks; 2 hours of exposure/day, 4 days/week for 18 weeks; 4 hours of exposure/day, 2 days/week for 18 weeks) and one untreated control. The three formaldehyde-treated groups were exposed via inhalation to formaldehyde. The mean concentration of formaldehyde vapor, based on three measurements during the study (stated as the beginning, during, and end of the study period), was reported as 1.5 ppm. At the end of the study period, the rats were sacrificed by ether anesthesia and subsequent cervical dislocation. The left testis was dissected from each rat and a specimen was taken from each testis. Tissues were fixed, embedded, sectioned (at 4 μm), and stained with hematoxylin and eosin. Using a light microscope, a histopathological examination was performed on the testes tissues, including morphometric evaluation of the diameter and height of 20 seminiferous tubules/testis. Golalipour et al. (2007) reported an formaldehyde exposure frequency (or duration)-dependent increase in testicular germ cells and seminiferous tubule defects. In the most frequent duration treatment group, a severe decrease in germ cells in >85% of the seminiferous tubules and arrested spermatogenesis were observed. In the mid-level frequency of duration treatment group, a decrease in the number of germ cells and an increased thickness of the basement membrane of 75% of the tubules was observed. In the lowest level duration treatment group, a disruption in the Sertoli and germinal cell arrangement, and increased spacing between germ cells was observed. Further, the seminiferous tubule diameter (STD) and seminiferous epithelial height (SEH) was most decreased among the treatment groups (exhibiting the greatest decrease in the group with the greatest hours and days of exposure) compared to the control (see Table 4-63). The results of this study are consistent with the findings of other studies of male reproductive system outcomes with inhalation formaldehyde exposure (e.g., Özen et al., 2005 and Zhou et al., 2006).

Xing et al. (2007) also studied the effects of formaldehyde on sperm development and reproductive capacity in adult male mice. In this study, male mice (12/group, strain not specified) were exposed to 0, 21, 42, or 84 mg/m^3 (0, 16.9, 33.8, or 67.6 ppm) formaldehyde via inhalation for 13 weeks at 2 hours/day, 6 days/week. The males were mated to untreated females in a dominant lethal protocol, and sperm morphology was assessed at study termination. The percent abnormal sperm was increased significantly ($p < 0.05$) in all treated groups, as was the

rate of resorptions ($p < 0.01$) (see Table 4-64). The mean number of live fetuses/litter was decreased in all treated groups, with statistical significance achieved at 84 mg/m^3. Although this study did not assess the number of corpora lutea per dam, thereby precluding the calculation of preimplantation loss, it is nevertheless indicative of formaldehyde-induced sperm morphology changes and dominant lethal effects in male mice.

Table 4-63. Effects of formaldehyde exposure on seminiferous tubule diameter and epithelial height in Wistar rats following 18 weeks of exposure

Inhalation exposure[a]	Seminiferous tubule diameters (µm)	Seminiferous tubule height (µm)
	***n* = 7**	***n* = 7**
Control	252.12 ± 4.82	82.77 ± 2.00
1.5 ppm, 4 h/d, 4 d/w	204.55 ± 3.29[b]	65.26 ± 1.43[b]
1.5 ppm, 2 h/d, 4 h/w	232.45 ± 2.42[b]	69.46 ± 1.78[b]
1.5 ppm, 2 h/d, 2 d/w	238.94 ± 4.37[b]	72.80 ± 2.03[b]

[a] Values are means ± SEMs.
[b] Different from control, $p < 0.05$, as calculated by the authors.

Source: Golalipour et al. (2007).

Table 4-64. Incidence of sperm abnormalities and dominant lethal effects in formaldehyde-treated mice

Dose (mg/m^3)	Sperm abnormalities		Reproductive capacity	
	Total abnormal sperm heads	Aberration rate (%)	Mean live fetuses/litter	Resorption rate (%)
0	391	3.53 ± 0.98	11.00 ± 1.01	2.273
21	568	5.48 ± 1.45	10.67 ± 1.16	9.380[b]
42	849	6.15 ± 1.36	9.63 ± 2.83	10.390[b]
84	974	9.24 ± 2.13[a]	9.04 ± 2.98[a]	12.440[b]

[a]Significantly different from controls ($p < 0.05$), as calculated by the authors.
[b]Significantly different from controls ($p < 0.01$), as calculated by the authors.

Source: Xing et al. (2007).

4.2.7.2. *Oral Exposure Studies Addressing Developmental and Reproductive Toxicity*

No contemporary testing guideline studies, such as a prenatal developmental toxicity study or two-generation reproductive toxicity study, have been performed by the oral route for formaldehyde. However, a number of studies have evaluated developmental toxicity and reproductive parameters in rats, mice, and dogs.

Hurni and Ohder (1973) tested the developmental toxicity of formaldehyde administered as a 40% w/v solution containing 11−14% w/w methanol in 9−10 pregnant beagle dogs that received the compound in their diet on GDs 4−56. Commercial grade formaldehyde (as a 40% solution) was sprayed on the pellets prior to feeding. Each animal was allotted a diet of 300 g of chow (reduced to 200 g 1 week prior to term) that was promptly consumed (within 5−10 minutes) before the formaldehyde volatilized appreciably. The concentrations of formaldehyde in the chow were 0, 125, or 375 ppm, equivalent to doses of 0, 3.1, or 9.3 mg/kg-day, respectively. Dams were allowed to deliver normally and weight gain, gestation length, number of litters, litter size, number of live pups, number of pups surviving through weaning, and pup weights weekly for the first 8 weeks were monitored as indices of the potential reproductive/developmental toxicity of formaldehyde. There were no formaldehyde-related effects in any of the parameters other than progressive pup weights, which were lower by group in litters of dams exposed to formaldehyde (see Table 4-65). A developmental impact of formaldehyde was evident in this strain of dog under the conditions of the experiment. At birth, mean pup body weights were 4 and 8.4% less than control for the low- and high-dose groups, respectively; at 8 weeks of age, the pup weight decrements were 8.3% for the low dose and 12.5% for the high dose, as compared with control, and established a LOAEL of 125 ppm. The contribution of methanol, which is a developmental toxin (Deglitz et al., 2004; Rogers et al., 2004) to these outcomes is not known. No internal or skeletal malformations were observed in any of the 264 live-born and 20 still-born pups.

Marks et al. (1980) conducted a developmental toxicity study of formaldehyde in CD-1 mice in which 29−35 pregnant animals were gavaged on GDs 6−15 with aqueous formaldehyde (containing 10−15% methanol) at 74, 148, and 185 mg/kg-day. Seventy-six controls were gavaged with water alone. All dams were sacrificed on GD 18, and the numbers of implantation sites in each uterine horn were counted. The high dose of formaldehyde was toxic to the dams, as indicated by the deaths of 22 of 34 mice before GD 18. Thus, the dose of 148 mg/kg-day was a NOAEL for maternal toxicity in this study. However, it is unclear to what extent an estimated concurrent dose of up to 75 mg/kg-day methanol may have contributed to this toxic response. To assess the developmental toxicity of formaldehyde, live fetuses were weighed individually, sexed, and examined for external, visceral, and skeletal malformations. Fetuses of surviving

Table 4-65. Body weights of pups born to beagles exposed to formaldehyde during gestation

Time (weeks)	Formaldehyde concentration in chow (ppm)		
	0	125	375
	Average body weight (g)		
0	321	308	294
1	547	526	467
2	818	755	706
3	1,078	987	944
4	1,264	1,247	1,166
5	1,601	1,512	1,429
6	2,020	1,816	1,741
7	2,449	2,263	2,145
8	2,957	2,712	2,587

Source: Hurni and Ohder (1973).

high-dose dams and of those of other groups did not show an increased incidence of malformations. Therefore, Marks et al. (1980) concluded that formaldehyde did not induce fetal abnormalities and that the 185 mg/kg-day dose level was a NOAEL for the developmental toxicity of formaldehyde, nor were fetotoxic effects of methanol apparent under the study experimental conditions.

Seidenberg and Becker (1987) and Seidenberg et al. (1986) included formaldehyde (purity not indicated) in a survey of the behavior of potential toxicants in a developmental toxicity screening assay (Chernoff and Kavlock, 1982). The protocol featured the administration of a borderline toxic dose to 26−30 pregnant ICR/SIM mice on GDs 8−12. Dams were allowed to deliver, and the neonates were examined, counted, and weighed on PNDs 1 and 3. The selected formaldehyde dose of 540 mg/kg-day was fatal for 11/30 dams, but the average weight gain among surviving dams was little changed compared with controls (3.9 ± 2.3 versus 4.0 ± 1.0 g). Similarly, there were no changes in perinatal responses in the neonates of exposed dams compared with controls. For example, the average values for number of neonates/litter, percent survival, and fetal weights on PNDs 1 and 3 were closely similar to those of controls.

Evidence of toxicity to the male reproductive system has been observed following oral administration of formaldehyde in a 40% w/v solution containing 11−14% w/w methanol. Cassidy et al. (1983) administered single oral doses of 100 or 200 mg/kg to five male Wistar

rats/group. Testes from these animals and 20 controls were excised and examined for spermatogenic abnormalities 11 days after dosing. A significant (19%) increase in testicular sperm head counts was observed in rats exposed to 200 mg/kg-day formaldehyde as compared with controls (see Table 4-66). The percentage of abnormal sperm heads was also significantly increased (5%) in the 200 mg/kg-day dose group compared with controls. These data suggest that formaldehyde can induce morphologic abnormalities in the germ cells of male experimental animals at dose levels that did not significantly affect testis weights. The contribution of methanol to these outcomes is unknown.

Table 4-66. Testicular weights, sperm head counts, and percentage incidence of abnormal sperm after oral administration of formaldehyde to male Wistar rats

Dose (mg/kg)	Mean testes weight (g)	Mean sperm heads × 10^6/g testis	Abnormal sperm heads (%)
0	3.30	175	4.76
100	3.27	166	5.22
200	3.16	209[a]	9.77[a]

[a]Significantly different from controls ($p < 0.001$), as calculated by the authors.

Source: Cassidy et al. (1983).

Postmortem evaluation of the reproductive organs was conducted in a number of oral studies that ranged between 4 weeks and 2 years in duration (Til et al., 1989, 1988; Tobe et al., 1989; Johanssen et al., 1986). Johannsen et al. (1986) administered 0, 50, 100, or 150 mg/kg-day formaldehyde in the drinking water to Sprague-Dawley rats (15/sex/group) for 91 days and 0, 50, 75, or 100 mg/kg-day formaldehyde in basal diet to beagle dogs (4/sex/group) for 91 days; the study reported no treatment-related effects on absolute or relative gonad weights or histopathology for either species. In a 4-week drinking water study conducted by Til et al. (1988), formaldehyde was administered to Wistar rats (10/sex/treated group) at nominal levels of 0, 25, and 125 mg/kg-day; gonad organ weights and histopathology were not affected by treatment. Tobe et al. (1989) conducted a chronic (24-month) study in Wistar rats (20/sex/group), with drinking water concentrations of 0, 0.02, 0.1, or 0.5%. According to the study report, gonad weights were measured and histopathology was conducted, but no treatment-related findings were noted. In a chronic (105-week) study (Til et al., 1989) in Wistar rats (70/sex/group), formaldehyde was administered in the drinking water at mean actual levels of 0, 1.2, 15, or 82 mg/kg-day to males and 0, 1.8, 21, or 109 mg/kg-day to females; serial sacrifices

were conducted at 53, 79, and 105 weeks of study. At study termination (105 weeks), mean testes weights were 30% increased ($p < 0.01$) in high-dose males as compared with controls, and histopathology evaluation revealed Leydig cell tumors in treated males (incidences of 0/50, 3/50, 3/50, and 2/50 for the control through high-dose groups, respectively; historical control tumor incidence data were not provided). The study authors did not judge these findings to be treatment related. By design, none of the subacute to chronic studies included measures of reproductive function (e.g., estrous cyclicity, sperm measures, or reproductive performance). With the exception of Til et al. (1989), detailed mean organ weight and histopathology incidence data were not provided in the published reports, and Til et al. (1988) only included tumor (not nontumor) data.

4.2.7.3. *Intraperitoneal Studies Addressing Developmental and Reproductive Toxicity*

Other studies in which formaldehyde was administered by I.P. injection have confirmed the potential effects on the male reproductive system.

Chowdhury et al. (1992) administered I.P. injections of 0, 5, 10, or 15 mg/kg-day formaldehyde to Charles foster adult male rats (10/group) for 30 days. On study day 31, blood was collected for serum testosterone measurements and the rats were sacrificed. The testes were removed, weighed, fixed in Bouin's solution, and processed for histopathology. The study authors reported adverse findings in all treated groups, including significant ($p < 0.01$) mean body weight gains, serum testosterone levels, and testes weights. Histopathologic evaluation revealed normal spermatogenic processes and Leydig cells in control animals. However, in treated rats, gradual cellular degeneration in seminiferous tubules and in Leydig cells was observed. Marked nuclear damage was noted in the 10 and 15 mg/kg-day groups, with significantly ($p < 0.001$) decreased Leydig cell populations and nuclear diameters observed in all treated groups. Additionally, a decrease in 3β-Δ^5-hydroxy steroid dehydrogenase was noted in the Leydig cell region of treated rat testes.

In a 30-day study performed by Majumder and Kumar (1995), 10 mg/kg-day formaldehyde was administered I.P. to eight male Wistar rats. All animals were sacrificed at term, and the testes, prostate, seminal vesicles, and epididymides were excised and weighed. With the use of methodologies that were not described in the report other than by reference to the *Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction* (WHO, 1987), sperm counts, motility, and viability were compared with those of 10 controls (injected I.P. with water alone). As shown in Table 4-67, striking reductions in sperm count and motility were noted in formaldehyde-treated rats compared with controls. Sperm

viability was also significantly reduced by formaldehyde treatment, though to a lesser overall extent than sperm count and motility.

Table 4-67. Effect of formaldehyde on spermatogenic parameters in male Wistar rats exposed intraperitoneally

Parameters	Control (*n* = 10)	Treated (*n* = 8)
Sperm count (10^6/mL)	46.30 ± 5.01	20.40 ± 2.01[a]
Sperm viability (%)	87.10 ± 0.83	72.60 ± 2.32[a]
Sperm motility (%)	75.00 ± 10.90	22.00 ± 6.40[a]

[a]Significantly different from controls ($p < 0.0001$), as calculated by the authors.

Source: Majumder and Kumar (1995).

Majumder and Kumar (1995) also carried out an in vitro experiment in which sperm from normal rats were incubated with different concentrations of aqueous formaldehyde at concentrations ranging from 125 pg/mL to 2.5 µg/mL. Viability of control sperm remained close to 80% for a period of 1 hour, whereas the presence of formaldehyde dose-dependently reduced viability. Thus, only 50% spermatozoa were viable for 30 minutes in the presence of 5 ng/mL formaldehyde compared with 6 minutes in the presence of 500 ng/mL. Sperm motility also was sensitive to the presence of formaldehyde. Less than 10% of sperm was motile for 10 minutes in the presence of 125 pg/mL formaldehyde. The authors of the study considered their data to be good evidence that functional parameters of spermatozoa, such as viability and motility, can be adversely affected by exposure to formaldehyde. Moreover, they suggested that the cumulative effects of I.P. administration of formaldehyde on the male rat reproductive system raise an alert that formaldehyde might impair the reproductive health of males who are occupationally exposed to the compound.

Odeigah (1997) conducted two short-term in vivo assays to examine sperm head abnormalities and dominant lethal mutations. In the sperm assessment, five daily I.P. injections of 0, 0.125, 0.25, or 0.5 mg/kg formaldehyde were administered to male albino rats (six/group; strain not specified). The rats were killed 3 weeks after the last injection, and epididymal sperm counts and abnormalities were assessed. A dose-related decrease in sperm count was observed, and significantly increased incidences of sperm head abnormalities were found at all treatment levels (see Table 4-68).

Table 4-68. Incidence of sperm head abnormalities in formaldehyde-treated rats

Dose (mg/kg)	Total abnormal sperm heads	Frequency (%) ± SEM
0	90	1.50 ± 0.11
0.125	184	3.09 ± 0.16 [a]
0.25	436	7.27 ± 0.30 [b]
0.5	514	8.57 ± 0.33 [b]

[a]Significantly different from controls ($p < 0.05$), as calculated by the authors.
[b]Significantly different from controls ($p < 0.001$), as calculated by the authors.

Source: Odeigah (1997).

In the dominant lethal assay (Odeigah, 1997), five daily I.P. injections of 0, 0.125, 0.25, or 0.5 mg/kg formaldehyde were administered to male rats (5 control rats, 12/treated group). Subsequently, each male was mated with two untreated virgin females per week for 3 consecutive weeks. The females were killed 13 days after the midpoint of the mating period and evaluated for live and dead uterine implants. In general, the number of live embryos was decreased with treatment, and the number of dead implants was increased (see Table 4-69). Additionally, there was a reduction in fertile matings in females mated 1−7 days after the males had been treated. This study did not assess the number of corpora lutea and therefore precluded the determination of preimplantation loss. Nevertheless, it is indicative of dominant lethal effects on the male germ cells.

4.2.7.4. *Dermal Exposure Studies Addressing Developmental and Reproductive Toxicity*

In a study designed to assess the embryotoxic effects of dermal exposure to formaldehyde, Overman (1986) applied 0.5 mL of a 37% formaldehyde solution directly to the dorsal skin of female Syrian golden hamsters (four−six/group) on GDs 8, 9, 10, or 11 for 2 hours. To prevent grooming during the treatment period, the animals were anesthetized with Nembutal. At the end of the 2-hour treatment period, the application site was washed thoroughly to remove any remaining formaldehyde. The dams were terminated on GD 15 (i.e., one day prior to expected delivery, since the typical gestation period for the Syrian golden hamster is 16−18 days). The fetuses were removed and fixed in either Bouin's solution or 95% ethanol for visceral or skeletal evaluation, respectively. The uteri were examined for implantation sites. Fixed fetuses were weighed, measured (crown-rump), and examined for external abnormalities; fetuses that had been placed in Bouin's fixative were evaluated for visceral anomalies by using a

Table 4-69. Dominant lethal mutations after exposure of male rats to formaldehyde

Dose (mg/kg)	Time of mating (days)	Fertile matings[a] (%)	Implants per female[a] (mean ± SE)	Live embryos per female (mean ± SE)	Dead implants per female (mean ± SE)	Dominant lethal mutation index[b]
0	0−21	96.67 (29)	7.86 ± 0.2 (29)	7.43 ± 0.3	0.43 ± 0.8	0
0.125	1−7	75.0 (18)	7.18 ± 0.3 (18)	5.95 ± 0.2	1.23 ± 0.5	19.92
	8−14	79.17 (19)	7.38 ± 0.5 (19)	6.30 ± 0.5	1.08 ± 0.3	15.21
	15−21	91.67 (22)	7.68 ± 0.2 (22)	6.89 ± 0.3	0.79 ± 0.5	7.27
0.25	1−7	33.33 (8)	5.75 ± 0.3 (8)	2.05 ± 0.3	3.70 ± 0.4	72.41
	8−14	50.0 (12)	6.60 ± 0.2 (12)	3.91 ± 0.2	2.69 ± 0.2	47.38
	15−21	87.5 (21)	7.25 ± 0.4 (21)	6.63 ± 0.3	0.62 ± 0.5	10.77
0.5	1−7	25.0 (6)	5.05 ± 0.03 (6)	1.10 ± 0.5	3.95 ± 0.22	85.20
	8−14	29.17 (7)	5.27 ± 0.01 (7)	1.50 ± 0.6	3.77 ± 0.28	79.81
	15−21	83.33 (20)	7.08 ± 0.04 (20)	5.79 ± 0.4	1.29 ± 0.17	22.07

[a]Number of females with implants presented in parentheses.
[b]Dominant lethal mutation index:

$$\text{Index} = 1 - \frac{\text{(Live implants experiment group per female)}}{\text{(Live implants of control group per female)}} \times 100$$

Source: Odeigah (1997).

free-hand sectioning technique, and those that were placed in ethanol were macerated, stained, and cleared for skeletal examination. In this study, the dams exhibited signs of dermal irritation and irritability, but the author reported no treatment-related effects on maternal body weight gain. The percent of resorption sites was increased (although not significantly) in treated litters as compared with control (0, 4.2, 8.1, 4.6, and 3.2% resorbed implantation sites for control and GDs 8, 9, 10, and 11 treatment groups, respectively). No treatment-related effects on fetal weight, length, or visceral or skeletal development were observed.

4.2.7.5. *Summary of Reproductive and Developmental Effects*

Formaldehyde exposures up to 40 ppm 6 hours/day from GDs 6−15 or 6−20 did not result in external or internal malformations (Martin, 1990; Saillenfait et al., 1989). Martin (1990) reported delayed skeletal ossification and dose-dependent decreases in fetal body weight at 5 ppm. Formaldehyde exposure at 40 ppm to pregnant female Sprague-Dawley rats reduced fetal body weights in male and female progeny and in male pups of dams exposed to 20 ppm

formaldehyde (Saillenfait et al., 1989). Based on these studies (see Table 4-70), the LOAEL for developmental effects in rats is 5 ppm, with a NOAEL of 2 ppm for decreased fetal weight and delayed skeletal ossification, based on inhalation exposures during GDs 6−20.

Developmental studies during earlier gestational windows of inhalation exposure to formaldehyde have reported additional adverse health effects, including delayed ossification, changes in relative organ weight, undescended testes, biochemical changes (e.g., ascorbic acid and nucleic acids), and blood acidosis (Senichenkova and Chebotar, 1996; Senichenkova, 1991; Kilburn and Moro, 1985; Gofmekler and Bonashevskaya, 1969; Gofmekler, 1968; Pushkina et al., 1968). Kitaev et al. (1984) hypothesized that formaldehyde may affect reproductive function by stimulating the hypothalamo-pituitary-gonadal (HPG) axis based on their observations of increased ovary weight, increased number of ovulating cells, and changes in blood levels of gonadotropins (LH and FSH). Evidence of preimplantation loss, which may be related to HPG disruption, was observed in this study and by Sheveleva (1971). Additional studies are needed to better understand developmental effects of formaldehyde exposure during early gestational windows.

The prenatal developmental toxicity of oral and dermal exposures to formaldehyde has not been thoroughly studied. Reductions in postnatal growth in beagle pups was observed by Hurni and Ohder (1973) following in utero exposure to 125 ppm maternal dietary formaldehyde during GDs 4−56 in beagle dogs. However, gavage dosing during gestation of mice to overtly maternally toxic doses (Seidenberg and Becker, 1987; Marks et al., 1980) (see Table 4-71) and dermal application during gestation to hamsters at a dose that caused dermal irritation and irritability (Overman, 1986) did not result in any observed fetal toxicity (see Table 4-72).

Few studies identified effects on maternal toxicity or female reproductive capacity. As summarized in Table 4-70, exposure of rat dams at 10−40 ppm formaldehyde during pregnancy has been shown to result in significantly decreased weight gain (Martin, 1990; Saillenfait et al., 1989; Kilburn and Moro, 1985). Maronpot et al. (1986) reported endometrial hypoplasia with a lack of ovarian luteal tissue in female rats exposed at 40 ppm but not at 20 ppm. Changes in LH and FSH levels were reported in dams exposed to 0.41 ppm formaldehyde by Kitaev et al. (1984), establishing an unbounded LOAEL for maternal toxicity.

Studies designed to assess male reproductive system endpoints in rats following repeated inhalation exposures to formaldehyde have shown concentration-dependent decreases in Leydig cell number and quality, effects on seminiferous tubules, decreases in testes weight, alterations in sperm measures, decreased testosterone levels, alterations in trace metals in the testes, and/or dominant lethal effects (Zhou et al., 2006; Özen et al., 2005, 2002; Zhou et al., 2006Sarsilmaz et al., 1999) (see Table 4-73). Based on available studies, the LOAEL for changes in the male

Table 4-70. Summary of reported developmental effects in formaldehyde inhalation exposure studies

Species, strain, sex	*n*/Group	Dose; time of treatment[a]	Reported study findings[b]		LOAEL/NOAEL[c]		Reference
			Maternal	Offspring	Maternal	Offspring	
Rat, strain NR, female	12	0, 0.01, or 0.81 ppm (reported as 0.012, and 1 mg/m^3)[d]; continuous dosing 10−15 days prior to mating and during gestation	At 0.01 and 0.81 ppm: ↑ pregnancy duration (dose-dependent data not shown)[e]	Fetuses: At 0.01 and 0.81 ppm: ↓ fetuses/dam (dose dep., data not shown)[e] ↑ body wt (dose dep., stat. sig.) ↓ lung and liver wt (dose dep., stat. sig.) ↑ adrenal wt (dose dep., stat. sig.) At 0.81 ppm: ↑ thymus and kidney wt (stat. sig.)	L: 0.01 ppm *N*: ND	L: 0.01 ppm *N*: ND	Gofmekler (1968)
Rat, "albino" strain NR, female	12	0, 0.01, or 0.81 ppm (reported as 0.012, and 1 mg/m^3)[d]; continuous dosing 10−15 days prior to mating and during gestation	NE	Age of assessment NR. At 0.81 ppm: histologic effects in liver (e.g., ↑ extramedullary hematopoietic centers), kidney (e.g., ↑ polymorphism of renal epithelial cell nuclei) and thymus	NE[e]	*N*: 0.01 ppm L: 0.08 ppm	Gofmekler and Bonashevskaya (1969)[f,h]
Rat, strain NR, male	4	Inhalation and drinking water coexposure: 0; 0.10 ppm plus 0.005 mg/L water; 0.20 ppm plus 0.01 mg/L water; or 0.41 ppm plus 0.1 mg/L water; all treatments 4 hours/day, 5 days/week for 6 months	No effects	No effects	ND[g]	ND	Guseva (1972)

Table 4-70. Summary of reported developmental effects in formaldehyde inhalation exposure studies (continued)

Species, strain, sex	*n*/Group	Dose; time of treatment[a]	Reported study findings[b]		LOAEL/NOAEL[c]		Reference
			Maternal	Offspring	Maternal	Offspring	
Rat, strain NR, female	NR	Expt 1: 0 or 30 ppm[d] Expt 2: 0, pair-fed control (15, 10, or 5 days), or 30 ppm; 8 hours/day for 15 days (GDs 3−17), 10 days (GDs 3−12), 5 days (GDs 8−12), or 3 days (GDs 9−11)	At 30 ppm 50% mortality (10 and 15 day exp) ↓ wt gain (duration dep.; 3, 5, 10, and 15 day exp.) ↓ wt of liver, kidney, spleen and thymus ↑ wt of lung and adrenal[e]	Fetuses: At 30 ppm ↓ fetal wt and growth (duration dep., 10 and 15 day exp.) ↑ dev. defects (undescended testes, large hearts, small thymuses, small lungs)[e]	*N*: ND L: 30 ppm[e]	*N*: ND L: 30 ppm[e]	Kilburn and Moro (1985)[f] Ab
Rat, Wistar, female	Embryo dev expt: 5−9/group (42 adult animals); maternal effects: NR (200 adult females total) [i]	0, 0.4, or 1.2 ppm (converted from reported 0, 0.5 or 1.5 mg/m^3); 4 hours/day, 5 days/week for 4 months; exposed females mated to unexposed males on 120th day exp.	At 0.4 ppm: ↑ wt of ovaries (stat. sig[e]) ↑ LH level (stat. sig.[e]) At 1.2 ppm: ↑ FSH level in blood (stat. sig.; nonsig. at 0.4 ppm[e])	At 0.4 ppm: ↑ no. of embryos and 2 blastomere stage embryos (stat. sig. in 2nd day preg.) At 1.2 ppm: ↑ no. degenerating embryos (stat. sig. in 3rd day preg.)	L: 0.4 ppm *N*: ND	L: 0.4 ppm *N*: ND	Kitaev et al. (1984)[g]
Rat, Sprague-Dawley, female and offspring of both sexes	25	0 (air control group), 0 (room control group), 2, 5, or 10 ppm; 6 hours/day GDs 6−15. Exposed females mated to unexposed males	At 10 ppm: ↓ food consumption (stat. sig.) ↓ wt gain (stat. sig.)	At 5 and 10 ppm: Fetuses: ↑ incidence of reduced ossification of pubic and ischial bones (stat. sig. compared with air control group) ↓ fetal wts (nonsig.) ↑ litter size (nonsig.)	L: 10 ppm *N*: 5 ppm	L: 5 ppm *N*: 2 ppm	Martin (1990)[e,f]

Table 4-70. Summary of reported developmental effects in formaldehyde inhalation exposure studies (continued)

Species, strain, sex	*n*/Group	Dose; time of treatment[a]	Reported study findings[b]		LOAEL/NOAEL[c]		Reference
			Maternal	Offspring	Maternal	Offspring	
Rat, "white" strain NR, female and offspring of both sexes	5−12 (NR for formalde-hyde only)	0, 0.01, or 0.81 ppm (reported as 0.012 and 1 mg/m^3)[d]; continuous 10−15 days prior to mating through gestation	At 0.01 and 0.81: ↓ vit. C level in liver (stat. sig.) ↓ vit. C level in placenta (nonsig.)	Fetuses: At 0.01 and 0.81 ppm: ↓ fetuses/female[e] ↑ body wt and organ wt (data not shown[e]) ↓ vit. C level in whole fetus (stat. sig.) At 0.01 ppm: ↓ vit. C level in fetal liver (stat. sig.)	L: 0.01 ppm *N*: ND	L: 0.01 *N*: ND	Pushkina et al. (1968)[f]
Rat, Sprague-Dawley, female and offspring of both sexes	25	0 (air control), 5, 10, 20, 40 ppm; 6 hours/day, GDs 6−20. Exposed females mated to unexposed males.	GD 21 dams: At 5 ppm: ↑ absolute body wt gain (5 ppm only) At 40 ppm: ↓ body wt gain GDs 6−21 (stat. sig.) ↓ absolute body wt gain (stat. sig., dose-dependent trend 20 and 40 ppm)	GD 21 fetuses: At 20 and 40 ppm: ↓ fetal body wt, male (stat. sig.) At 40 ppm: Delayed ossification of thoracic vertebrae (stat. sig., trend 20 ppm) ↑ unossified sternebrae (nonsig. at 40 ppm) ↓ fetal body wt, female (stat. sig.)	L: 40 ppm *N*: 20 ppm	L: 20 ppm *N*: 10 ppm	Saillenfait et al. (1989)

Table 4-70. Summary of reported developmental effects in formaldehyde inhalation exposure studies (continued)

Species, strain, sex	*n*/Group	Dose; time of treatment[a]	Reported study findings[b]		LOAEL/NOAEL[c]		Reference
			Maternal	Offspring	Maternal	Offspring	
Rat, mongrel white, female and offspring of both sexes	NR[i]	0 or 0.41 ppm (reported as 0.5 mg/m^3) formaldehyde (also a 3rd group of gasoline exposure, not described in this table); 4 hours/day GDs 1−19	Dams GD 20: ↓ corpora lutea (nonsig.), embryos dead before implantation (not stat. sig.), and implanted embryos (nonsig.) ↑ blood pCO_2 (stat. sig.)	Fetuses (GD 20): Stat. sig. findings include ↑ fetal wt ↑ litters w/internal organ anomalies ↓ fetuses w/ossification centers in hyoid bone ↑ metacarpal bone centers ↑ metatarsal bone centers ↑ developmental defects ↑ blood pCO_2 and pO_2 Pups: ↓ pup wt Dev. delays (data not shown)	L: 0.41 ppm *N*: ND	L: 0.41 ppm *N*: ND	Senichenkova (1991)
Mouse, mongrel, female and offspring of both sexes	NR (254 dams) [i]	0 + ethyl alcohol; 0.41 ppm formaldehyde; 0.41 ppm formaldehyde + bipyridyl; 4 hours/day GDs 1−19. Induced maternal iron deficiency anemia by I.P. bipyridyl injections on GDs 12−15; controls injected w/25% ethyl alcohol.	Dams GD 20: formaldehyde alone: ↑ blood pCO_2 (stat. sig.) formaldehyde + bipyridyl: ↓ blood acid metabolic products (stat. sig.) ↓ blood true bicarbonates and CO_2 conc. (stat. sig.)	Fetuses (GD 20): Formaldehyde alone: ↑ cryptorchidism Formaldehyde + bipyridyl: ↑ birth defects (stat. sig.) ↓ dev. delay (stat. sig.) ↓ blood acid-base measures of embryos (stat. sig.)	L: 0.41 ppm *N*: ND	L: 0.41 ppm *N*: ND	Senichenkova and Chebotar (1996)

Table 4-70. Summary of reported developmental effects in formaldehyde inhalation exposure studies (continued)

Species, strain, sex	*n*/Group	Dose; time of treatment[a]	Reported study findings[b]		LOAEL/NOAEL[c]		Reference
			Maternal	Offspring	Maternal	Offspring	
Rat, mongrel, white, female and offspring of both sexes	15/group terminated GD 20, 6/group littered	0, 0.0005, or 0.005 mg/L (0, 0.4, or 4 ppm), GDs 1−19, 4 hours/day	At 0.4 ppm: ↓ leukocyte counts At 4 ppm: ↓ leukocyte counts; reduced threshold of neuromuscular excitability, ↓ rectal temperature, ↓ blood hemoglobin; ↑ spontaneous mobility	At 0.4 ppm: ↑ preimplantation loss; at 1 mo. of age, ↓ spontaneous mobility; at 2 mo. of age, ↓ hemoglobin levels and leukocyte counts At 4 ppm: ↑ preimplantation loss; at 1 and 2 mo. of age, ↓ spontaneous mobility; at 2 mo. of age, ↓ hemoglobin levels and leukocyte counts	L: 0.4 ppm *N*: ND	L: 0.4 ppm *N*: ND	Sheveleva (1971)
Rat, Sprague-Dawley, female and offspring of both sexes	6 dams	0 or 6 ppm 8 hours/day, 6 weeks, starting at GD 1, PND 1, 4 weeks of age, or adult age	NE	In offspring exposed in utero and during early postnatal life: ↓ mean BW and liver weight; ↑ markers of oxidative stress In offspring exposed as juveniles: ↓mean liver weight; ↑ markers of oxidative stress In offspring exposed only as adults: no effect	NE	L: 6 ppm *N*: ND	Kum et al. (2007)
Rat, Sprague-Dawley, female and offspring of both sexes	6 dams	0 or 6 ppm 8 hours/day; 6 weeks, starting at GD 1, PND 1, 4 weeks of age, or adult age	NE	In offspring exposed in early postnatal life, as juveniles, or as adults, ↑ ANAE-positive T-lymphocytes in BALT In all exposure initiation groups, ↑ ANAE-positive lymphocyte ratios	NE	L: 6 ppm *N*: ND	Sandikci et al. (2007)

Table 4-70. Summary of reported developmental effects in formaldehyde inhalation exposure studies (continued)

Species, strain, sex	*n*/Group	Dose; time of treatment[a]	Reported study findings[b]		LOAEL/NOAEL[c]		Reference
			Maternal	Offspring	Maternal	Offspring	
Rat, Wistar, female and offspring of both sexes	12−14 dams	0, 6, or 12 ppm, 6 hours/day; 5 days/week, 30 days	NE	At 6 and 12 ppm, at postnatal days 30 and 90: respiratory irritation and toxicity; decr. BW, FC, WC; ↓ SOD activity, ↓ levels of copper and iron levels in lungs, ↑ zinc levels in lungs	NE	L: 6 ppm *N*: ND	Songur et al. (2005)

ND: not determined; NE: not evaluated; NR: not reported; Ab: abstract only; wt: weight; stat. sig.: statistically significant; p: pressure; l: length;
To convert concentrations in air (at 25°C) from mg/m^3 to ppm: 1 ppm = 1.23 mg/m^3; 1 mg/m^3 = 0.813 ppm.

[a]Treatment is given as the formaldehyde concentration in air (ppm) with the length of exposure each day and the duration of treatment in days, as available.
[b]Studies with negative findings are included.
[c]L: LOAEL; *N*: NOAEL.
[d]Exposure concentrations not validated; details of formaldehyde vapor generation not reported; exposure during gestation not well characterized in study report.
[e]No statistics provided.
[f]Lack of study details.
[g]See Table 4-73 for reproductive effects.
[h]Gofmekler and Bonashevskaya (1969) seem to report on different findings from the same study (i.e., same animals) as Gofmekler (1968).
[i]Number/group not clear from study report.

Table 4-71. Summary of reported developmental effects in formaldehyde oral exposure studies

Species, strain, sex	*n*/ Group	Dose; time of treatment	Reported developmental effects [a]		LOAEL/NOAEL[b]		Reference
			Maternal	Offspring	Maternal	Offspring	
Dog, beagle, female and pups	9−10	0, 125, or 375 ppm[c] (corresponding to 0, ~3.1, or ~ 9.3 mg/kg-d[d]), dietary, GDs 4−56	No effects	At 125 (3.1 mg/kg-day), and 375 ppm (9.3 mg/kg-day): ↓ birth wt and wt gain through postnatal week 8	L: ND *N*: 9.3 mg/kg-day (375 ppm)	L: 3.1 mg/kg-day (125 ppm) *N*: ND	Hurni and Ohder (1973)
Mouse, CD-1, female	29−35 total	0, 74, 148, or 185 mg/kg-day, GDs 6−15 (aqueous formaldehyde solution contained 10−15% methanol)	At 185 mg/kg-day: Mortality	No effects at GD 18	L: 185 mg/kg-day *N*: 148 mg/kg-day	L: ND *N*: 185 mg/kg-day	Marks et al. (1980)
Mouse, ICR/SIM, female	26−30 total	0 or 540 mg/kg-day, GDs 8−12	At 540 mg/kg-day: Mortality	No effects in pups on PND 1 and 3	L: 540 mg/kg-day *N*: ND	L: ND *N*: 540 mg/kg-day	Seidenberg and Becker (1987)

ND: not determined.
[a]Studies with negative findings are included.
[b]L: LOAEL; *N*: NOAEL.
[c]Reported dose units.
[d]mg/kg-day doses are based on a conversion presented in Hurni and Ohder (1973).

Table 4-72. Summary of reported developmental effects in formaldehyde dermal exposure studies

Species, strain, sex	*n*/ Group	Dose; time of treatment[a]	Reported developmental effects		LOAEL/NOAEL[a]		Reference
			Maternal	Offspring	Maternal	Offspring	
Hamster, Syrian golden , female	4−6	0 or 37%; 0.5 mL applied to dorsal skin (hair clipped) for 2 hours then washed; GDs 8, 9, 10, or 11	Signs of dermal irritation and irritability	At all GDs of treatment, ↑ percent resorptions (not sig.)	L: 37% *N*: ND	L: 37% *N*: ND	Overman (1986)

[a]L: LOAEL; *N*: NOAEL.

ND = not determined.

Table 4-73. Summary of reported reproductive effects in formaldehyde inhalation studies

Species, strain, sex	*n*/ Group	Dose; time of treatment[a]	Reported reproductive effects[b]	LOAEL/ NOAEL[c]	Reference
Rat, strain NR, male	4	Inhalation plus drinking water coexposure: 0; 0.10 ppm plus 0.005 mg/L water; 0.20 ppm plus 0.01 mg/L water; or 0.41 ppm plus 0.1 mg/L water; all treatments; 4 hours/day, 5 days/week for 6 months. Exposed males mated to unexposed females	At 0.20 ppm + 0.01 mg/L water and 0.41 ppm + 0.1 mg/L water: ↓ nucleic acid in testes (dose dep.; data not shown; stat. sig.)	L: 0.20 ppm + 0.01 mg/L water *N*: ND	Guseva (1972)[d,e]
Rat, Wistar, female	NR (200 female)	0, 0.4, or 1.2 ppm ; 4 hours/day, 5 days/week for 4 months. Exposed females mated to unexposed males on 120th day of exposure.	At 0.4 ppm: ↑ wt of ovaries (stat. sig.[e]) ↑ LH level in blood (stat. sig. at 0.4 ppm[f]) At 1.2 ppm: ↑ FSH level in blood (stat. sig., dose dep. trend[f])	L: 0.4 ppm [h] *N*: ND [h]	Kitaev et al. (1984)[e]
Mouse, B6C3F1, male and female	10	0, 2, 4, 10, 20, or 40 ppm; 6 hours/day, 5 days/week for 13 weeks	Males and females: At 20 ppm:↓ wt gain At 40 ppm: ↑ mortality (13 weeks exp.); ↓ wt loss Females: At 40 ppm: ↑ Uterine endometrial and ovarian hypoplasia	L: 20 ppm *N*: ND	Maronpot et al. (1986)
Rat, albino Wistar, male	7	6 groups: 0, 10, or 20 ppm; 8 hours/day, 5 days/week for 4 weeks (subacute) or 13 weeks (subchronic)	At 10 and 20 ppm (both durations): ↓ wt gain (stat. sig., dose dep.) ↓ relative testes wt (stat. sig., dose and conc. dep.) ↓ zinc and copper in testes (stat. sig., dose and conc. dep.) ↑ iron in testes (stat. sig., dose and conc. dep.) No effect on testes wt.	L: 10 ppm *N*: ND	Özen et al. (2002)[f]
Rat, Wistar, male	18	0, 5, or 10 ppm; 8 hours/day, 5 days/week, 91 days	At 5 and 10 ppm: clinical signs of respiratory irritation, ↓ BW, FC, WC; ↓serum testosterone; ↓ mean seminiferous tubule diameters; ↑ localization of heat shock protein 70 in cytoplasm of spermatogonia, spermatocytes, and spermatids	L: 5 ppm *N*: ND	Özen et al. (2005)

Table 4-73. Summary of reported reproductive effects in formaldehyde inhalation studies (continued)

Species, strain, sex	*n*/ Group	Dose; time of treatment[a]	Reported reproductive effects[b]	LOAEL/ NOAEL[c]	Reference
Rat, albino Wistar, male	10	0, 10, or 20 ppm 8 hours/day, 5 days/week for 4 weeks	Dose NR: Irritation: standing hair, interrupted breathing, ↑ eye blinking, licking, nose cleaning, and sneezing. At 10 and 20 ppm: ↓ Body wt gain (dose dep.; stat. sig.) ↓ Leydig cell quantity (stat. sig.) ↑ Nuclear damage of Leydig cells (dose dep.; stat. sig.)	L: 10 ppm *N*: ND	Sarsilmaz et al. (1999)
Mouse, strain not specified, male	12	0, 21, 42, or 84 mg/m^3 (0, 16.9, 33.8, or 67.6 ppm); 2 hours/day, 6 days/week; 13 weeks. Exposed males mated to unexposed females.	In all treated groups: ↑ percentage abnormal sperm, ↑ resorption rate, and ↓ live fetuses	L: 16.9 ppm *N*: ND	Xing et al. (2007)
Rat, Wistar, male	7	0 or 1.5 ppm, 18 weeks formaldehyde exposures: (1) 4 hours/day, 4 days/week (2) 2 hours/day; 4 days/week (3) 2 hours/day, 4 days/week	In all treated groups: sig. ↓ seminiferous tubular diameter and epithelial height. Other effects in exposure groups: (1) sig. ↓ germ cells; arrested spermatogenesis (2) ↓ cells, increased thickness in basal membrane (3) ↑ spaces between germ cells; disrupted association between Sertoli and germinal cells	L: 1.5 ppm *N*: ND	Golalipour et al. (2007)
Rat, Sprague-Dawley, male	10	(1) 0 (gavage saline); (2) 10 mg/m^3 (8.05 ppm), 12 hours/day, 2 weeks; or (3) 10 mg/m^3 (8.05 ppm), 12 hours/day, 2 weeks, plus 30 mg/kg-day vitamin E orally	At 10 mg/m^3: sig. ↓ testis weight, atrophy of seminiferous tubules, ↓ spermatogenic cells, disintegrated and sloughed seminiferous cells; edematous interstitial tissue with vascular dilatation and hyperemia; ↓ epididymal sperm count and percentage motile sperm, ↑ percentage abnormal sperm; ↓ SOD, GSH-Px, GSH and ↑ MDA in testes; vitamin E attenuated all effects	L: 8.05 ppm *N*: ND	Zhou et al. (2006)
Rat, Wistar, male	40	0, 0.1, 1, or 10 ppm; 6 hours/day, 5 days/week, 13 or 52 weeks	No effects: testis weight; histopathologic findings[g]	L: ND *N*: 10 ppm	Appleman et al. (1986)

[a]Treatment is given as the formaldehyde concentration in air (ppm) with the length of exposure each day and the duration of treatment in days.
[b]Studies with negative findings are included.
[c]L: LOAEL; *N*: NOAEL.
[d]Guseva (1972) was a drinking water and inhalation study.

Table 4-73. Summary of reported reproductive effects in formaldehyde inhalation studies (continued)

[e]Developmental effects shown in Table 4-70.
[f]Statistics not provided in study report.
[g]Focus of study was not the reproductive system; only reproductive system findings are addressed in the table; NOAEL and LOAEL in table are based only on reproductive system findings.
[h] For increased FSH, the LOAEL was 1.2 ppm (1.5 mg/m^3) and the NOAEL was 0.4 ppm (0.5 mg/m^3).

ND: not determined; NR: not reported.
To convert concentrations in air (at 25°C) from mg/m^3 to ppm: 1 ppm = 1.23 mg/m^3; 1 mg/m^3 = 0.813 ppm.

reproductive system in rats following 5 days/week of inhalation exposures is 5 ppm for 3 months of daily exposures and 10 ppm for 4 weeks of daily exposures; these dose levels are unbounded. Abnormal sperm were also noted in mice at an inhalation dose of 16.9 ppm 2 hours/day, 6 days/week for 13 weeks (Xing et al., 2007), but, in contrast, Maronpot et al. (1986) reported no histologic abnormalities in male mice after formaldehyde exposures at 40 ppm 6 hours/day, 5 days/week for 13 weeks. Varied results among studies may be due to species differences or differences in methods. Although several oral subchronic and chronic studies with formaldehyde did not identify effects on the testes (Tobe et al., 1989; Til et al., 1988; Johanssen et al., 1986), Cassidy et al. (1983) observed spermatogenic abnormalities after a single oral dose of 200 mg/kg to rats, and a chronic drinking water study in rats (Til et al., 1989) reported low incidences of Leydig cell tumors in all treated groups, compared with none in control (see Table 4-74). Additionally, studies utilizing I.P. injection of formaldehyde in rats have demonstrated testes and sperm anomalies (Majumder and Kumar, 1995; Chowdhury et al., 1992) and dominant lethal effects (Odeigah, 1997) (see Table 4-75).

4.3. GENOTOXICITY

Formaldehyde has been extensively studied for its mutagenic and genotoxic activity in a variety of assay systems. The first reported mutagenic activity of formaldehyde was when Rapoport (1946) described the induction of sex-linked recessive lethals in *Drosophila* larvae fed on a medium containing formalin. A variety of genotoxic and mutagenic effects have been subsequently demonstrated in both in vitro and in vivo test systems, including the formation of DNA-protein crosslinks (DPXs or DPCs), point mutations, DNA strand breaks, increased micronuclei (MN), and chromosomal aberrations (CAs) (Auerbach et al 1977; Ma and Harris, 1988; Conaway et al 1996; IARC 1995; 2006).

In this section, reactions of formaldehyde with cellular macromolecules, such as DNA and proteins, and formaldehyde-induced clastogenicity are described. In addition, the evidence for formaldehyde-induced mutations is considered in the context of the current EPA cancer guidelines (U.S. EPA, 2005a). Particular emphasis is given to the genotoxic effects of formaldehyde in humans, described in Section 4.3.4.2.

4.3.1. Formaldehyde-DNA Reactions

Formaldehyde is a reactive chemical and interacts with DNA in several ways, forming DPXs or DPCs, DNA adducts, and DNA-DNA cross-links (DDXs) (Fennell, 1994; Casanova et al., 1989; Heck and Casanova, 1987; Casanova-Schmitz et al., 1984a, b; Casanova-Schmitz and Heck, 1983; Ohba et al., 1979; Dönecke, 1978; Brutlag et al., 1969). Formaldehyde also may

Table 4-74. Summary of reported reproductive effects in formaldehyde oral studies

Species, strain, sex	*n*/ Group	Dose; time of treatment	Reported reproductive effects[a]	LOAEL/ NOAEL[b]	Reference
Rat, Wistar, male	5 (20 control)	0, 100, or 200 mg/kg, single gavage dose	At 200 mg/kg: ↑ (19%) testicular sperm head counts (stat. sig.) ↑ (5%) abnormal sperm head (stat. sig.)	L: 200 mg/kg *N*: 100 mg/kg	Cassidy et al. (1983)
Rat, Sprague-Dawley, both sexes	15	0, 50, 100, or 150 mg/kg-day, drinking water; 91 days	No effects: absolute or relative gonad weights; histopathologic findings of reproductive organs[c]	L: ND *N*: 150 mg/kg-day	Johanssen et al. (1986)
Dog, beagle, both sexes	4	0, 50, 75, or 100 mg/kg-day, dietary; 91 days	No effects: absolute or relative gonad weights; histopathologic findings of reproductive organs[c]	L: ND *N*: 100 mg/kg-day	
Rat, Wistar, both sexes	10	0, 25, or 120 mg/kg-day, drinking water, 4 weeks	No effects: gonad weights; histopathologic findings of reproductive organs[c]	L: ND *N*: 120 mg/kg-day	Til et al. (1988)
Rat, Wistar, both sexes	70	0, 1.2, 15, or 82 mg/kg-day (males), 0, 1.8, 21, or 109 mg/kg-day (females),[d] drinking water; 105 weeks	In all treated groups: Leydig cell tumors observed at 105 weeks of study[c] At 82 mg/kg-day: ↑ mean testes weights	L: 1.2 mg/kg-day *N*: ND	Til et al. (1989)
Rat, Wistar, both sexes	20	0, 0.02, 0.1, or 0.5% in drinking water; 24 months	No effects: gonad weights; histopathologic findings of reproductive organs[c]	L: ND *N*: 0.5%	Tobe et al. (1989)

[a]Studies with negative findings are included.
[b]L: LOAEL; *N*: NOAEL.
[c]Focus of study was not the reproductive system; only reproductive system findings are addressed in the table; NOAEL and LOAEL in table are based only on reproductive system findings.
[d]Actual concentrations.

ND = not determined.

Table 4-75. Summary of reported reproductive effects in formaldehyde intraperitoneal studies

Species, strain, sex	*n*/ Group	Dose; time of treatment[a]	Reported reproductive effects[a]	LOAEL/ NOAEL[b]	Reference
Rat, Charles foster, male	10	0 or 5 mg/kg-day; 30 days	↓ body weight gain ↓ Leydig cell population and cell nuclear diameter ↓ serum T levels ↓ testes weights cellular degeneration of seminiferous tubules	L: 5 mg/kg-day *N*: ND	Chowdhury et al. (1992)
Rat, Wistar, male	8	0 or 10 mg/kg-day; 30 days	↓ sperm count, motility and sperm viability	L: 10 mg/kg-day *N*: ND	Majumder and Kumar (1995)
Rat, "albino" strain NR, male	6	0, 0.125, 0.250, or 0.60 mg/kg-day; 5 days	At all treatment levels: ↓ sperm count and ↑ sperm head abnormalities (3 weeks after the last injection)	L: 0.125 mg/kg-day *N*: ND	Odeigah (1997)
Rat, "albino" strain NR, male	12	0, 0.125, 0.250, or 0.60 mg/kg-day; 5 days. Exposed males mated to unexposed females.	At all treatment levels (at GD 13): Delayed time to mating ↓ mean no. implants and live embryos ↑ dead implants and dominant lethal index following mating to untreated females	L: 0.125 mg/kg-day *N*: ND	

[a]Studies with negative findings are included.
[b]L: LOAEL; *N*: NOAEL.

ND = not determined; NR = not reported; T = testosterone.

facilitate the formation of adducts between other chemicals or drugs (endogenous or xenobiotic) and DNA (Fennell, 1994; Koppel et al., 1991; Casanova et al., 1989; Heck and Casanova, 1987; Lam et al., 1985; Casanova-Schmitz et al., 1984a; Casanova-Schmitz and Heck, 1983; Ohba et al., 1979; Dönecke, 1978; Brutlag et al., 1969).

The high reactivity of formaldehyde results in little specificity in reaction sites, indicating that a range of adducts and cross-links might be expected. However, the spectrum of formaldehyde-DNA reaction products is difficult to quantify in vivo as many of these products are labile and difficult to measure directly (Fennell, 1994; Casanova et al., 1989). Additionally, formaldehyde is metabolically incorporated into nucleic acids, and therefore DNA and RNA assays incorporating radiolabeled formaldehyde need careful interpretation to distinguish between covalently bound and metabolically incorporated formaldehyde (Casanova et al., 1989; Heck and Casanova, 1987; Casanova-Schmitz et al., 1984a, b; Casanova-Schmitz and Heck, 1983). Hence, reports of formaldehyde-DNA reactivity in cell-free system results may not provide a useful measure of exposure (Fennell, 1994). Besides, the question of biological relevance must also be considered. On the other hand, methods used to extract and measure formaldehyde-DNA reaction products after in vivo exposures should be evaluated to ensure that formaldehyde reaction products are neither created nor removed during sample preparation (Fennell, 1994; Casanova et al., 1989).

4.3.1.1. *DNA-Protein Cross-Links (DPXs)*

Formaldehyde forms DPX by reacting with the amino or imino groups of proteins (e.g., lysine and histidine side chains) or of nucleic acids (e.g., cytosine) resulting in a Schiff base formation which then reacts with another amino group (McGhee and von Hippel 1975a, b). Evidence from numerous experimental models, ranging from cell-free systems to single cells and in vivo animal and human exposures, suggests that formaldehyde reacts readily with DNA forming DPXs (Reviewed in Conaway et al 1996; IARC 2006, 1995). As shown in Table 4-76, cross-links between histones and DNA have been demonstrated in isolated chromatin samples on exposure to formaldehyde from earlier studies (Ohba et al., 1979; Dönecke, 1978; Brutlag et al., 1969). Several in vitro studies demonstrated induction of DPX by formaldehyde exposure in bacteria (Wilkins and McLeod 1976), yeast (Magana-Schwencke and Ekert, 1978) and mammalian cells including animal cells (Chinese hamster ovary [CHO] cells, Chinese hamster V79 lung epithelial cells, mouse leukemia L1210 cells, mouse hepatocytes, rat Yoshida lymphosarcoma cells, rat C18 tracheal epithelial cells, hepatocytes, nasal, tracheal, buccal epithelial cells, kidney cells and aortic endothelial cells) and human cells (lung and bronchial epithelial cells, fibroblasts, white blood cells, peripheral blood lymphocytes, Epstein-Barr Virus (EBV)-Burkitt's lymphoma cells, Jurkat E6-1 cells, HeLa cells, lymphoblastoid cells, gastric

Table 4-76. Formaldehyde-DNA reactions (DPX formation)

Species/Strain	Cell/Strain	Result	References
DNA Interaction			
DPX formation in vitro			
In vitro	Nucleohistone	+	Brutlag et al., 1969
		+	Döenecke, 1978
		+	Ohba et al., 1979
Bacteria	*Escherichia coli*	+	Wilkins and McLeod, 1976
Yeast	*Saccharomyces cerevisiae*	+	Magana-Schwencke and Ekert, 1978
Hamster/Chinese	Ovary cells	+	Marinari et al., 1984
		+	Zhitkovich and Costa, 1992
		+	Olin et al., 1996
		+	Garcia et al., 2009a
Hamster/Chinese	V79 lung epithelial cells	+	Swenberg et al., 1983
		+	Merk and Speit 1998, 1999
		+	Speit et al., 2007a
Mouse	Leukemia L1210 cells	+	Ross and Shipley, 1980
		+	Ross et al., 1981
	Hepatocytes	+	Casanova and Heck, 1997
		+	Casanova et al., 1997
Rat/unspecified	Yoshida lymphosarcoma cells	+	O'Connor and Fox, 1987
	C18 tracheal epithelial cell line	+	Cosma and Marchok, 1988
Rat/F344	Hepatocytes	+	Casanova and Heck, 1997
	Nasal mucosa	+	Casanova-Schmitz and Heck, 1983
	Nasal epithelium	+	Bermudez and Delehanty, 1986
	Primary tracheal epithelial cells	+	Cosma et al., 1988
Rat/Wistar	Aortic endothelial cells	+	Lin et al., 2005
Human	Lung/bronchial epithelial cells	+	Fornace et al., 1982
		+	Saladino et al., 1985
		+	Grafstrom et al., 1986, 1990
	Bronchial epithelial cells	+	Grafstrom et al., 1983
	Lung/bronchial epithelial cells, fibroblasts, skin fibroblasts	+	Grafstrom et al., 1984
	Foreskin fibroblasts	+	Snyder and Van Houten, 1986
	Bronchial/Skin fibroblasts	+	Olin et al., 1996

Table 4-76. Formaldehyde-DNA reactions (DPX formation) (continued)

Species/Strain	Cell/Strain	Result	References
Human (continued)	White blood cells	+	Shaham et al., 1996
	EBV-Burkitt's lymphoma cells	+[a]	Costa et al., 1997
	Gastric mucosa cells	+	Blasiak et al 2000
	Peripheral blood lymphocytes	+	Quievryn and Zhitkovich 2000
		+	Andersson et al., 2003
		+	Liu et al 2006
	Fibroblasts	+	Speit et al 2000
	Primary skin fibroblasts and keratinocytes	+	Emri et al 2004
	Buccal epithelial cells	+	Li et al 2004
	Jurkat E6-1 cells	+	Saito et al 2005
	HeLa cells	+	Liu et al 2006
	Whole blood cultures	+	Schmid and Speit 2007
	Lung/bronchial epithelial cells	+	Speit et al 2008
	Lymphoblastoid/TK6	+	Craft et al., 1987
	Lung carcinoma (A549) cells	+	Speit et al., 2010
DPX formation in vivo			
Rat	Nasal mucosa	+	Casanova-Schmitz and Heck 1983[b]
		+	Casanova-Schmitz et al 1984b
		+	Lam et al 1985
		+	Heck et al 1986
		+	Heck Hd and Casanova 1987
		+	Casanova et al 1989, 1994
	Tracheal implants	+	Cosma et al., 1988
	Peripheral blood cells	-	Speit et al 2009
	Bronchoalveolar lavage cells	-	Neuss et al 2010
Rhesus monkeys	Nasal, larynx, trachea, and carina	+	Casanova et al 1991
Human	White blood cells	+	Shaham et al., 1996
	Peripheral blood lymphocytes	+	Shaham et al., 1997, 2003

[a] indicates that DNA-protein cross-links formed at cytotoxic concentrations.
[b] used homogenates of nasal mucosa.
'+' indicates a positive test result.
'-' indicates a negative test result.

mucosal cells and whole blood cultures) and in vivo studies involving experimental animals and occupationally exposed human populations as summarized in Table 4-76. Some relevant studies are described here.

Ross and Shipley (1980) showed that formaldehyde induces single strand breaks (SSBs) and DPXs in mouse leukemia cells; SSBs are formed at concentrations >200 μM and a reduction of radiation-induced breaks (indirect measure of DPXs) at 50 μM. The authors used a [^{14}C]-thymidine-incorporated mouse L1210 cell line to monitor formaldehyde-induced DNA strand breaks and DPXs. They exposed cells to varying concentrations of formaldehyde for 2.5 hours. An alkaline-elution technique in the presence or absence of proteinase K was used to measure strand breaks. In order to detect DPXs, some cells were exposed to 300 R of X-rays immediately after formaldehyde treatment. Formaldehyde-induced DPXs were repaired 24 hours after the compound was removed from the culture (Ross and Shipley, 1980).

Casanova-Schmitz and Heck (1983) have shown that homogenates of rat nasal mucosa incubated with formaldehyde in vitro followed by extraction with a strong aqueous-immiscible organic solvent demonstrated increased DPX formation in DNA obtained after enzymatic proteolysis from the aqueous-organic interface (IF), termed as "interfacial DNA" (IF-DNA).

Bermudez and Delehanty (1986) observed the formation of DPXs, scheduled (S) and unscheduled DNA synthesis (UDS), and synthesis of RNA when cultured F344 rat nasal epithelial cells from the naso- and maxillary turbinates were incubated with formaldehyde. Unscheduled and scheduled DNA synthesis was stimulated (0.05−0.1 mM) and then inhibited (0.1−1 mM), depending on the formaldehyde concentration. Experiments by Cosma et al. (1988) and Cosma and Marchok (1988) showed the induction of DPXs and DNA SSBs in cultured C18 rat tracheal epithelial cells exposed to 200 μM formaldehyde for 90 minutes (Cosma et al., 1988; Cosma and Marchok, 1988).

Several human cells (epithelial cells, fibroblasts, buccal cells) or cell lines (lymphoblastoid cells) exposed to formaldehyde have been shown to form DPX (Craft et al 1987; Costa et al 1997; Emri et al 2004; Li et al 2004).

Craft et al. (1987) detected DPXs by alkaline elution in TK6 human lymphoblastoid cells immediately after a 2-hour exposure (zero time) to 0, 15, 50, 75, 100, 150, 300, and 600 μM formaldehyde with a significant nonlinear increase in DPXs above 50 μM concentration, which correlated with the onset of cytotoxicity, but DPXs were completely removed in cultures held for 24 hours before processing. In the zero-time sample, significant increases in DPXs were first observed at 50 μM and increased linearly up to 150 μM. In cells held for 24 hours, there was no detectable increase in DPXs.

However, Costa et al. (1997) detected DPXs with paraformaldehyde (which dissociates to release formaldehyde) at doses that were cytotoxic (>0.003%) but could not discriminate between the DPX-inducing and cytotoxic effects of this chemical in EBV-human Burkitt's lymphoma cells (Costa et al., 1997). Grafström et al. (1983) reported that the number of DPXs induced by 100 μM formaldehyde in vitro in human epithelial cells and fibroblasts of bronchial origin was similar and that the frequency of these cross-links was proportional to the concentration of formaldehyde. Besides the bronchial epithelial cells and fibroblasts, the authors also noted that formaldehyde exposure resulted in DPXs and DNA SSBs in skin fibroblasts and DNA excision repair-deficient skin fibroblasts. However, formaldehyde was only moderately cytotoxic to normal bronchial epithelial cells and fibroblasts at concentrations that induced substantial DNA damage. Repair of the formaldehyde-induced DNA SSBs and DPXs appeared to be inhibited by the continued presence of formaldehyde in the culture medium (Grafström et al., 1984).

Emri et al. (2004) detected a significant increase in DPX formation in primary human skin fibroblasts and keratinocytes at 8 hours of exposure in vitro to formaldehyde at 25 μM with an approximately linear increase up to 100 μM. These cells were exposed to 0, 12.5, 25, 50, and 100 μM formaldehyde for 8 hours and then exposed to 250 μM methyl methane sulfonate (MMS) for 2.5 hours. The induction of DPX formation was measured by the ability of formaldehyde to reduce DNA migration in the comet assay induced by MMS in this study (Emri et al., 2004).

Li et al. (2004) measured DNA damage in primary human buccal cells by using the comet assay. The appearance of SSBs, suggesting compound-induced fragmentation of DNA, occurred at formaldehyde concentrations of 5 and 7.5 μM. At higher concentrations, the response slope decreased, indicating formation of DPXs or DDXs (Li et al., 2004). The same laboratory reported similar findings in primary human peripheral blood lymphocytes and HeLa cells (Liu et al., 2006). Peak response for SSBs was seen at 10 μM in both cells, with higher concentrations resulting in cross-link formation. SSBs in HeLa cells induced by 10 μM formaldehyde were repaired by 60 minutes after cells were washed to remove formaldehyde.

Schmid and Speit (2007) tested formaldehyde for its ability to induce DPXs in blood cultures. They used an indirect method to monitor DPX formation in which the extent of DNA migration in the comet assay in response to γ radiation was compared in formaldehyde-treated cultures versus controls. A concentration of 25 μM was required for DPX formation, and repair of these lesions was rapid, with DPXs induced by concentrations of formaldehyde up to 100 μM and completely removed after 8 hours. Overall, several in vitro studies in cultured mammalian

cells demonstrated the formation of DPX and SSB formation confirming the genotoxicity of formaldehyde.

Several in vivo studies involving rodents, monkeys and humans have demonstrated DPX formation following formaldehyde exposure (see Table 4-76). As mentioned earlier, in vitro studies by Casanova-Schmitz and Heck (1983) have shown that reaction of formaldehyde with cellular macromolecules in tissue homogenates causes a decrease in the extractability of DNA into aqueous phase during solvent extraction and the formaldehyde-bound DNA-protein complex migrates into the interface. In the same study Casanova-Schmitz and Heck (1983) have also shown that DNA isolated from the nasal, but not olfactory, mucosa of rats exposed to formaldehyde (2, 6, 15, and 30 ppm 6 hours/day for 2 days) via inhalation showed significant increase in DPXs in the interfacial DNA ≥6 ppm, which was shown to be linear in the exposure range of 2−30 ppm (2.45−36.8 mg/m^3). However, DNA in the aqueous phase did not show DPX formation. Thus, the cross-linked DNA that could be extracted from the interface after proteolysis was considered to be supporting evidence of chemically induced DPX formation. The inability of this study to detect DPXs at lower levels of formaldehyde exposure is likely be due to the protective mechanism of GSH, which catalyzes the conversion of formaldehyde to formate. Later, Lam et al., (1985) have shown that coexposure of rats with 2 ppm acrolein and 6 ppm formaldehyde for 6 hours resulted in higher DPX in the nasal mucosa of rats compared to the rats given formaldehyde alone, suggesting that GSH depletion by acrolein enhanced the DNA binding of formaldehyde. In this study inhalation exposure of rats to acetaldehyde alone at 0, 0.1, 0.5, 1 and 2.5 ppm for 6 hours induced a concentration-dependent depletion of GSH in rat nasal mucosa with no detectable DPX levels at 2 ppm dose. However, acetaldehyde forms DPX (as interfacial DNA) in rat nasal mucosa at much higher concentrations (100-1000 ppm) compared to formaldehyde.

So, in a later study, Casanova and Heck (1987) reported that GSH depletion caused an increase in DPX formation in the IF-DNA in the nasal mucosa of F344 rats when a dual-isotope (^{3}H/^{14}C) method was used. The dual isotope method helps in making the distinction between metabolic incorporation and covalent binding of formaldehyde. Oxidation by removal of one hydrogen atom is required for metabolic incorporation of formaldehyde into cellular macromolecules, but not in the formation of DNA adducts or DNA-protein crosslinks. Thus, the ratio of ^{3}H/^{14}C of DNA containing DPX will be higher than the macromolecules where formaldehyde is metabolically incorporated. However, the authors further demonstrated that, when the double isotope method was used, the ^{3}HCHO is oxidized significantly more slowly than H^{14}CHO under these conditions, resulting in an overestimate of the concentration of cross-links due to an isotopic effect on the oxidation of ^{3}HCHO catalyzed by formaldehyde

dehydrogenase (FDH). Besides, this method leaves residual formaldehyde that is likely to form DNA adducts by reacting with deoxyribonucleosides in the DNA hydrolysates (Heck and Casanova, 1987).

To overcome this, Casanova et al. (1989) used an improved method, which is based not on the analysis of residual formaldehyde bound to deoxyribonucleosides in DNA hydrolysates but on the determination of the total ^{14}C-formaldehyde bound to DNA. This study showed that formaldehyde was exclusively bound to interfacial DNA, indicating the formation of DPXs. Hydrolysis of DPXs in different samples quantitatively released formaldehyde. Besides, DPX formation was detectable at all concentrations of exposure to formaldehyde (0.3−10 ppm for 6 hours). Overall, these studies clearly show that formaldehyde induces DPXs in nasal epithelial cells of rodents. However, there are no published rodent studies that assess DPXs beyond the nasal passages of the upper respiratory tract.

Formaldehyde-induced DPXs were also found in the nasal mucosa and extranasal tissues of rhesus monkeys exposed to 0, 0.71, 2, or 6 ppm (0, 0.86, 2.45, or 7.36 mg/m^3) formaldehyde 6 hours/day for 3 days (Casanova et al., 1991). These data were used as a basis for cross-species prediction of formaldehyde-induced DPXs in humans. The presence of DPXs in rhesus monkeys confirms formaldehyde‘s DNA reactivity as a general effect. Additionally, DPXs were detected in the larynx/trachea/carina (pooled sample) and in intrapulmonary airways of monkeys exposed to 2 or 6 ppm formaldehyde. These data demonstrate direct effects of formaldehyde on DNA in tissues that correspond to observed tumor sites in humans (nasal and nasopharynx).

4.3.1.2. *DNA Adducts*

In addition to the formation of DPX, there is evidence that formaldehyde forms hydroxymethyl (hm) DNA adducts in vitro in a variety of cell-free systems (Zhong and Que Hee, 2004a; Cheng et al., 2003; Kennedy et al., 1996; Fennell, 1994; Beland et al., 1984) and nasal epithelial cells (Zhong and Que Hee 2004b). In cell-free systems, formaldehyde directly reacts with DNA forming hmDNA adducts (Cheng et al., 2003; Kennedy et al., 1996; Fennell, 1994; McGhee and von Hippel, 1977a, b, 1975a, b). The results on formaldehyde-induced DNA adduct formation are summarized in Table 4-77.

Beland et al. (1984) first reported hmDNA adducts in Chinese hamster ovary (CHO) cells incubated with 1 mM of radiolabeled formaldehyde. After a 2-hour incubation, small amounts of N^6-hydroxymethyldeoxyadenosine (N^6-hmdA) adducts were detected with concomitant metabolic incorporation of formaldehyde. Besides N^6-hmdA, various other forms of hmDNA adducts, including N^4-hydroxymethyldeoxycytidine (N^4-hmdC), and N^2 hydroxymethyldeoxyguanosine (N^2-hmdG), were detected by high performance liquid

Table 4-77. Formaldehyde-DNA reactions (DNA adduct formation)

Species/Strain	Cell/Strain	Result	References
DNA Interaction			
DNA Adduct Formation in vitro			
Cell-free system	Deoxyribonucleosides	+	Cheng et al 2008
	Guanosine	+	Kennedy et al 1996
	Guanosine	+	Cheng et al 2003
Placental DNA	In vitro	+	Zhong and Que Hee 2004a
Calf thymus DNA	In vitro	+	Beland et al 1984
Calf thymus DNA	In vitro	+	Von Hippel and Wang 1971
Cell-free system	In vitro	+	McGhee and von Hippel 1975a
		+	McGhee and von Hippel 1975b
		+	McGhee and von Hippel 1977a
		+	McGhee and von Hippel 1977b
		+	Fennell 1994
		+	Cheng et al 2003
Rats	Nuclei	+	Fennell 1994
	Nasal epithelial cells	+	Casanova et al 1989
	Nuclei	+	Heck Hd and Casanova 1987
Hamster	CHO cells	+	Beland et al 1984
Human	Nasal epithelial cells	+	Zhong and Que Hee 2004b
DNA adduct formation in vivo			
Drosophila	Larvae	+	Alderson, 1985
Rats	Indirect evidence	+	Wang et al 2007
Rat/F344	Nasal epithelium	+	Lu et al 2010
Rat/F344	White blood cells, bone marrow cells, lung, liver, spleen, thymus	-	Lu et al 2010
Human	White blood cells	+	Wang et al 2009

'+' indicates a positive test result.
'-' indicates a negative test result.

chromatography (HPLC) following in vitro reaction between formaldehyde and calf thymus DNA or individual deoxynucleotides.

Since the formaldehyde adducts are labile, Fennel (1994) developed a method by derivatizing them with sodium bisulfite to their sulfomethyl form, whereby he detected

N^6-sulfomethyldeoxyadenosine (SOMedA) and N^2-sulfomethyldeoxyguanosine (SOMedG) by using HPLC. However, the levels of SOMedA in DNA isolated following incubation of radiolabeled formaldehyde with isolated rat hepatic nuclei were similar to those in control nuclei. And in human TK6 lymphoblastoid cells treated with formaldehyde, detection of SOMedA adducts was precluded by additional radioactive spots. These observations suggest that N^6-sulfomethyldeoxyadenosine adducts are formed at very low levels in formaldehyde-incubated rat hepatic nuclei and that measurement of hydroxymethyldeoxyadenosine would not provide a useful measure of formaldehyde exposure. Fennel (1994) also reported that ^{32}P-postlabeling studies (Gupta et al. 1982) allowed for much greater analytical sensitivity but did not confirm the level of SOMedA adduct detected by HPLC. However, either estimate of adduct formation is much less than the estimate of DPX formation (120 pmol/mg DNA) in similarly treated rat nuclei (Heck and Casanova, 1987).

Casanova et al. (1989) demonstrated that detection of hmDNA adduct formation was sensitive to the methodology used, particularly the buffer used for sample preparation. Specifically, Tris buffer can prevent hmDNA adduct formation due to the abundance of formaldehyde-reactive primary amine sites in the buffer. In contrast, the tertiary amine sites that predominate in Bis-Tris buffer do not react with formaldehyde.

Zhong and Que Hee (2004a) observed hmDNA adducts (N^6-hmdA, N^2-hmdG, and N^4-hmdC) in placental DNA exposed to 100 ppm formaldehyde in vitro for 20 hours at 37°C followed by hydrolysis of formaldehyde-reacted DNA using bis-Tris buffer. However, deoxythymidine did not form hydroxymethyl derivatives in this study (Zhong and Que Hee, 2004a). On the other hand, the same investigators were able to detect N^6-hmdA and N^2-hmdG adducts in human nasal epithelial cells cultured in the presence of 0, 10, 25, 50, 100, 250, 400, or 500 µg/mL formaldehyde and using Tris buffer during hydrolysis of adducted DNA. The toxicity threshold for <90% viability appeared to be between 100 and 250 µg/mL initial formaldehyde culture concentration, and even at 500 µg/mL concentration it was not toxic, with a viability was 70% in this study (Zhong and Que Hee, 2004b).

There are only a few reports of formaldehyde-induced hmDNA adducts in vivo. Alderson (1985) hypothesized that a N6-hydroxymethyl adenosine, an RNA adduct formed in the reaction of formaldehyde and adenosine is likely to play a mutagenic role in *Drosophila*, based on the observation that flies fed on a medium containing formalin was mutagenic only when adenosine or adenylic acid, but not adenine was present in the medium. An indirect evidence from the study of Wang et al., (2007) has shown that N^6-hydroxymethyl-deoxyadenosine (N^6-hmdA) adducts were formed in hepatic and pulmonary DNA from rats exposed to *N*-nitrosodimethylamine (NDMA) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-

butanone (NNK), suggesting that these two nitrosamines could be a source of formaldehyde-DNA adducts. The same group recently has shown that formaldehyde-DNA adducts are detectable in the leukocytes of smokers and nonsmokers. More recently, Lu et al (2010) have shown that in rats exposed to formaldehyde by inhalation deoxyguanosine monoadducts and DDX were detected in nasal epithelium, while endogenous formaldehyde monoadducts and DDX were detectable in white blood cells and several internal organs including liver, lung, spleen, thymus and bone marrow.

4.3.1.3. *DNA-DNA Cross-Links (DDXs)*

Formaldehyde, besides forming DPXs and DNA adducts, has also been shown to form DDX in vitro. Li et al. (2004) showed that formaldehyde induces DNA strand breaks at low-exposure concentration and DDXs and DPXs at higher concentrations in buccal cells. The authors also showed that formaldehyde induces DDXs in human peripheral blood lymphocytes exposed in vitro when the concentration was more than 25 μM. Recently, Lu et al (2010) reported DDXs in the nasal epithelium of F344 rats that were exposed by nose-only to 10 ppm formaldehyde for 1 or 5 days (6 hrs/day). The relevance of these modifications in formaldehyde-induced genotoxicity is not known at the moment.

Overall, formaldehyde forms predominantly DPXs that are detected in cell-free systems and single cells in vitro and in animal and human tissues in vivo. In rodents, DPXs are formed in nasal epithelia but not in extranasal passages, which are completely removed within a day after formation. The DPXs are detected in nasal and extranasal tissues of monkeys, suggestive of direct effects of formaldehyde in tissues that correspond to observed tumor sites (nasal and nasopharynx) in humans. Besides, this is used as a basis for cross-species comparison with humans. Formaldehyde-DNA adducts are labile, constituting a minor fraction of the DNA-reaction products. DPXs but not DNA adducts appear to play an important role in the genotoxicity of formaldehyde.

4.3.1.4. *Single Strand Breaks*

Formaldehyde has been shown to induce DNA single strand breaks in a number of mammalian cell systems in vitro as well as in vivo as shown in Table 4-78. In the in vitro studies, most studies were positive, a few were negative and one study was equivocal for strand breaks induced by formaldehyde. Among the in vivo studies, Im et al (2006) observed a dose-dependent increase in DNA damage, analyzed by the Oliver tail movements in the comet assay in both blood lymphocytes as well as livers of rats exposed to 5 and 10 ppm formaldehyde. Sul et al (2007) also observed a dose-dependent increase in SSBs of Sprague-Dawley rats exposed to

Table 4-78. Formaldehyde-DNA interactions (single strand breaks)

Species/Strain	Cell/Strain	Result	References
DNA single strand breaks (in vitro)			
Hamster/Chinese	V79 lung epithelial cells	-	Speit et al 2007a
Mouse	Leukemia L1210 cells	(+)	Ross and Shipley, 1980
		-	Ross et al 1981
Rat	Hepatocytes	+	Demkowicz-Dobrzanski and Castonguay 1992
Rat	Yoshida lymphosarcoma cells	+	O'Connor and Fox 1987
Rat/F344 trachea	Tracheal epithelial cell/Primary culture	+	Cosma et al., 1988
Human	Bronchial cell/Skin fibroblast	+	Grafstrom et al., 1984
	Peripheral blood lymphocytes	+	Liu et al 2006
	HeLa cells	+	Liu et al 2006
	Skin fibroblast	+	Snyder and Van Houten, 1986
	Lung/bronchial epithelial cells	+	Saladino et al 1985
		+	Grafstrom et al 1984
		+	Grafstrom 1990
		+	Fornace et al 1982
Human	Lung/bronchial epithelial cells	+	Vock et al 1999
	Skin keratinocytes/fibroblasts	-	Emri et al 2004
DNA single strand breaks (in vivo)			
Mouse	Liver (maternal)	+	Wang and Liu 2006
	Liver (fetal)	+	Wang and Liu 2006
Rats/Sprague-Dawley	Lung epithelial cells	+	Sul et al 2007
Rats/Sprague-Dawley	Peripheral blood lymphocytes and liver	+	Im et al 2006

'no' indicates test was not done in vivo; '+' indicates a positive test result; 'yes' indicates test was done in vivo; - indicates a negative test result; (+) indicates a weak positive test result.

0, 5, and 10 ppm formaldehyde by inhalation for 2 weeks (6 hrs/day, 5 days/wk). Additionally, there is some evidence that DNA single strand breaks (SSBs) may be induced directly by formaldehyde reactivity (Grafström et al., 1984). Thus, formaldehyde has been shown to induce dose-dependent DNA damage in vivo in rodents. Besides, formaldehyde has also been shown to induce SSBs in cultured human cells such as lung/bronchial epithelial cells (Vock et al 1999; Saladino et al 1985; Grafstrom et al 1984; Grafstrom 1990; Fornace et al 1982), skin fibroblasts

(Grafstrom et al., 1984; Snyder and van Houten, 1986; Emri et al., 2004) and HeLa cells (Liu et al., 2006).

4.3.1.5. *Other Genetic Effects of Formaldehyde in Mammalian Cells*

Formaldehyde induces several other genetic and related effects in mammalian cells which are evaluated by in vitro assays such as unscheduled DNA synthesis (UDS), DNA repair inhibition and cell transformation as summarized in Table 4-79.

Table 4-79. Other genetic effects of formaldehyde in mammalian cells

Species/Strain	Cell/Strain	Result	References
Unscheduled DNA synthesis (UDS)			
Rat/F344	Nasal epithelial cells	+	Bermudez and Allen 1984
	Nasal epithelial cells	+	Bermudez and Delehanty 1986
	Hepatocytes	+	Williams et al 1989
Hamster/Syrian	Embryo cells	+	Hamaguchi and Tsutsui 2000
Human	HeLa cells	+	Martin et al 1978
	Bronchial epithelial cells	-	Doolittle et al 1985
DNA repair inhibition			
Human	Bronchial epithelial cells/skin fibroblasts	+	Grafstrom et al 1984
	Normal fibroblasts (MRC5CV), XPA cell line, and FA cell line	+	Speit et al 2000
	Skin fibroblasts/keratinocytes	+	Emri et al 2004

XPA, xeroderma pigmentosum, complementation group A (deficient in NER pathway).
FA = Fanconi's anemia (cell line has genetic defect leading to hypersensitivity to DNA-DNA cross links; NER = nucleotide excision repair.

UDS, which represents DNA repair activity following excision of DNA damage, has been reported in nasal epithelial cells of F344 rats (Bermudez and Allen 1984; Bermudez and Delahanty 1986), rat hepatocytes (Williams et al 1989) and Syrian hamster embryo cells (Hamaguchi and Tsutsui 2000) exposure to formaldehyde. UDS was observed in HeLa cells (Martin et al 1978), but not in human bronchial epithelial cells (Doolittle et al 1985) upon formaldehyde exposure. These studies suggest that following formaldehyde-induced DNA damage was followed by DNA repair.

Studies involving human bronchial epithelial cells and skin fibroblasts or keratinocytes (Grafstrom et al 1984; Emri et al 2004), DNA repair proficient or −deficient cell lines (e.g.

xeroderma pigmentosum) or cell lines hypersensitive to DNA-DNA crosslinks (e.g., Fanconi's anemia) (Speit et al 2000) have been shown that formaldehyde causes DNA repair inhibition at a concentration range of 0.125 mM to 10 mM. Emri et al (2004) have shown that DNA repair was inhibited in human keratinocytes and fibroblasts after irradiation with UVB and UVC, but not UVA followed by treatment with low concentrations of formaldehyde (10 μM). UVA, UVB, and UVC induced wavelength- and time-specific changes of DNA migration in comet assay. They observed that DNA SSB induced by UVB or UVC irradiation alone were repaired within 3-6 hours of exposure, while cells with UV irradiation followed by formaldehyde exposure still had the strand breaks at the same timepoints suggesting that formaldehyde is likely to contribute to UV-induced carcinogenesis.

4.3.2. In Vitro Clastogenicity

Clastogenic effects, including increased MNs, CAs, and SCEs are also reported in a range of in vitro study systems as shown in Table 4-80.

Several studies demonstrated formaldehyde-induced chromosomal aberrations (CAs) in a variety of mammalian cells, such as CHO cells (Garcia et al 2009; Natarajan et al 1983), Chinese hamster lung fibroblasts (Ishidate et al., 1981), Syrian hamster embryo cells (Hagiwara et al., 2006; Hikiba et al., 2005), mouse lymphoma cells (Speit and Merk 2002) and human peripheral blood lymphocytes (Dresp and Bauchinger, 1988; Schmid et al 1986; Miretskaya and Shvartsman 1982) and fibroblasts (Levy et al., 1983).

Miyachi and Tsutsui (2005) measured the induction of sister chromatid exchanges (SCEs) in Syrian hamster embryo (SHE) cells. Cells were exposed to 0, 3.3, 10, and 33 μM formaldehyde for 24 hours. SCE levels after 3.3 μM formaldehyde were not different from controls, but significant increases were observed at both 10 and 33 μM. Toxicity as measured by reduced cloning efficiency was seen only at 33 μM (Miyachi and Tsutsui, 2005). The same laboratory used SHE cells to measure the induction of CAs (Hikiba et al., 2005). Cells were exposed to 0, 33, 66, and 99 μM formaldehyde for 24 hours prior to staining for analysis and the percentages of aberrant metaphases were 0, 6, 6, and 71, respectively. The aberrations were predominantly chromosome gaps and chromosomal breaks and exchanges. The relative colony-forming efficiency remained high (at least 85%) for the concentrations of formaldehyde used in the experiment (Hikiba et al., 2005).

Table 4-80. In vitro clastogenicity of formaldehyde

Species	Cell/Tissue origin	Without activation	With activation	References
Cytogenetic Assays				
Chromosomal aberrations (CA)				
Hamster/Chinese	Ovary cells	(+)	(+)	Galloway et al., 1985
		-	ND	Dresp and Bauchinger, 1988
		+	ND	Natarajan et al 1983
		+	ND	Garcia et al 2009
	Lung fibroblasts	+	ND	Ishidate Jr et al 1981
Hamster/Syrian	Embryo cells	+	ND	Hikiba et al 2005
		+	ND	Hagiwara et al 2006
Mouse	Lymphoma cells	+	+	Speit and Merk 2002
Human	Peripheral blood lymphocytes	+	+	Schmid et al 1986
		+	ND	Miretskaya and Shvartsman 1982
		+	ND	Dresp and Bauchinger, 1988
	Fibroblasts	+	ND	Levy et al 1983
Micronucleus (MN)				
Hamster/Chinese	V79 lung epithelial cells	+	ND	Speit et al 2007b
		+	ND	Merk and Speit 1998
Human	Fibroblasts	+	ND	Emri et al 2004
	Whole blood cultures	+	ND	Schmid and Speit 2007
	Human MRC5CV (normal) and XP(Repair-deficient) and FA (repair-deficient) cell lines	+[a]	ND	Speit et al 2000
Sister Chromatid Exchange (SCE)				
Hamster/Chinese	Ovary cells	(+)	(+)	Galloway et al., 1985
		+	ND	Natarajan et al 1983
		+	ND	Garcia et al 2009
		+	ND	Obe and Beek 1979
Hamster/Chinese	V79 lung epithelial cells	+	(+)	Basler et al. 1985
		+	ND	Speit et al 2007b
		+	ND	Merk and Speit 1998, 1999
		+	ND	Neuss and Speit 2008

Table 4-80. In vitro clastogenicity of formaldehyde (continued)

Species	Cell/Tissue origin	Without activation	With activation	References
Hamster/Syrian	Embryo cells	+	ND	Miyachi and Tsutsui 2005
Human	A549 lung epithelial cells	+	ND	Neuss and Speit 2008
	A549 + V79 (cocultivated)	+[c]	ND	Neuss and Speit 2008
	A549 + V79 (cocultivated)	-[d]	ND	Neuss and Speit 2008
	Lymphocytes	+[b]	ND	Garry et al., 1981
		+	ND	Krieger and Garry 1983
		+	ND	Schmid et al 1986
		+	ND	Obe and Beek 1979
	Whole blood cultures	+	ND	Schmid and Speit 2007
Premature chromosome Condensation (PCC)				
Hamster/Chinese	Ovary cells	+	ND	Dresp and Bauchinger, 1988

[a] MN frequency increased in repair-deficient cell lines compared to normal cell lines.
[b] indicates SCE with significant loss of cell viability.
[c] A549 cells exposed for 1 h with formaldehyde then cocultivated with V79 cells.
[d] A549 cells exposed for 1 h with formaldehyde, cells washed and then cocultivated with V79 cells.

'+' indicates a positive test result.
'ND' indicates test was not done.
- indicates a negative test result.
(+) indicates a weak positive test result.
XP, xeroderma pigmentosum; FA = Fanconi's anemia.

Schmid and Speit (2007) observed that SCEs were induced in lymphocytes of whole blood cultures at a formaldehyde concentration of 200 µM, an effect apparently associated with cytotoxicity. This was indicated by a concomitant reduction in the proliferative index. These authors also observed the formation of MNs in their cultures. This effect was statistically significant at a formaldehyde concentration of 300 µM and above. However, MN formation was confined to those cultures in which formaldehyde treatment commenced 44 hours after the start of the culture. This prompted the conclusion that the level of DPX formation would need to be high for MN formation and that the cells must be exposed after the first mitosis. In examining MN formation more closely, Schmid and Speit (2007) used the FISH technique, employing a "biotin-labeled pan-centromeric chromosome paint specific for all human centromeres."

Indicative that formaldehyde was inducing a clastogenic (rather than aneugenic) effect, 81% of MNs in binucleated cells were centromere-negative.

In summary, formaldehyde forms MNs, SCEs, and CAs in isolated animal and human cells following in vitro exposure (see Table 4-80).

4.3.3. In Vitro Mutagenicity

Mutations may occur during repair of formaldehyde-induced DNA damage (DPXs, DNA adducts, SSBs, or clastogenic effects) or as a result of replication errors during mitogenesis. The in vitro evidence for formaldehyde-induced mutations is strengthened by examining the correlation between these genotoxic and clastogenic events and induction of mutations. Therefore studies are presented with respect to relevance to one or more of the following lines of evidence for mutagenicity recommended for consideration in the EPA guidance (U.S. EPA, 2005a): (1) that the chemical is DNA reactive and/or has the ability to bind to DNA, (2) that the chemical generates positive results in in vitro mutagenic test systems (specifically gene mutations and CAs), and (3) that the chemical induces indications of genetic damage in in vivo tests (specifically gene mutations and CAs). Numerous studies have demonstrated formaldehyde-induced DNA mutations under a variety of experimental conditions (reviewed in IARC 1995, 2006; Ma and Harris 1988; Auerbach et al. 1977; Conaway et al 1996; NTP 2009).

4.3.3.1. *Mutagenicity in Bacterial Systems*

A number of research reports describe the mutagenicity of formaldehyde in bacterial test systems using reverse and forward mutation assays as well as specific strains detecting deletions, insertions and point mutations. Among the bacterial strains, *Salmonella typhimurium* TA102 and the *Escherichia coli* strains containing an AT base pair at the primary reversion site are often used to detect oxidative compounds, cross-linking agents and hydrazines. In an early National Toxicology Program (NTP) collaborative study with three laboratories, formaldehyde consistently tested positive for mutagenicity in *Salmonella typhimurium* strain TA100 in the presence of a rat or hamster liver S9 activating system (Haworth et al., 1983). Formaldehyde was mutagenic with and without metabolic activation in a number of other studies using reverse mutation assays with *S. typhimurium* strains TA98, TA100, TA102, TA104, TA2638, and TA2638a and *E. coli* strains WP2 (pkM101), WP2 *uvr*A (pkM101), and hrs/r30R (Ryden et al., 2000; Dillon et al., 1998; Watanabe et al., 1996; Le Curieux et al., 1993; O'Donovan and Mee, 1993; Zielenska and Guttenplan, 1988; Schmid et al., 1986; Connor et al., 1983, 1985; Orstavik and Hongslo, 1985; Takahashi et al., 1985; Fiddler et al., 1984; Frei et al., 1984; Donovan et al., 1983), while other studies (Muller et al., 1993; Jung et al., 1992; Wilcox et al., 1990; Marnett et

al., 1985) show both positive and negative results. These results are summarized in Table 4-81 and some of the studies are described in greater detail.

Formaldehyde has been shown to be mutagenic in forward mutation assays using *S. typhimurium* (Couch et al 1982; Donovan et al 1983; Temcharoen and Thilly 1983) as well as in *E. coli* (Bosworth et al 1987). Temcharoen and Thilly (1983) examined the toxicity and mutagenicity of *S. typhimurium* strain TM677, using forward mutation to 8-azaguanine resistance, and have shown that formaldehyde induced both toxicity and mutagenicity at minimum concentrations of 0.17 mM (−S9) and 0.33 mM (+S9). It has also been shown that formaldehyde formed as an intermediate by oxidation at the methyl group of *N*-nitrodimethylamine, a biologically active *N*-nitramine of environmental significance, is mutagenic to *S. typhimurium* TA100 strain at low concentrations and toxic above 2 μmol/plate (Frei et al., 1984).

Bosworth et al (1987) developed a forward mutation assay in *E. coli* D494 *uvr*B strain transformed with a multi-copy mutator plasmid pGW1700, in which mutations are scored by an increase in ampicillin-resistant colonies after exposure of bacterial cells during the logarithmic growth by the test chemicals. This assay is more sensitive to base-pair substitutions, but less sensitive to frameshift mutations compared to Salmonella/microsome-based assays. In this assay, the authors (Bosworth et al 1987) observed positive curvilinear response to formaldehyde exposure. Crosby et al (1988) used four *E. coli* strains GP120, GP120A, 7-2, and 33694 containing the xanthine guanine phosphoribosyl transferase (*gpt*) gene (which detects point mutations, deletions and insertions) tested the mutagenicity of formaldehyde by exposing for 1 hour at 4 and 40 mM concentrations. They observed 41% large insertions, 18% large deletions and 41% point mutations. However, at 40 mM dose there were 92% point mutations, a majority of them (62%) being transition mutations at a single AT base pair in the *gpt* gene. In the same study they observed frameshift mutations in *E. coli* that was transformed with naked pSV2gpt plasmid DNA exposed to 3.3 or 10 mM formaldehyde. Thus, the mutation pattern appear to differ depending on the concentration of formaldehyde exposure to the bacterial strain as well as the nature of DNA.

Formaldehyde has also been shown to induce primary DNA damage in *E. coli* and mutagenic activity in the Ames fluctuation test in *S. typhimurium* TA100, TA102, or TA98 strains (Le Curieux et al., 1993).

Table 4-81. Summary of mutagenicity of formaldehyde in bacterial systems

Species	Strain	Metabolic activation		References
		+S9	-S9	
Mutagenicity Assays				
Reverse Mutation				
S. typhimurium	TA98, 100, 1535, 1537, 1538	-	-	De Flora, 1981
	TA100	ND	(+)	Couch et al., 1982
	TA100	+	-	Haworth et al., 1983
	TA1535, 1537	-	-	Haworth et al., 1983
	TA98	(+)	-	Haworth et al., 1983
	TA98, TA100	+	+	Connor et al., 1983[a]
	UTH8414, UTH8413	-	-	Connor et al., 1983[a]
	TA97, 98, 100	+	+	Donovan et al., 1983
	TA102	+	+	De Flora et al., 1984
	TA100	+	ND	Frei et al., 1984
	TA100	ND	+	Fiddler et al., 1984
	TA100	+	(+)	Connor et al., 1985
	TA98	(+)	-	Connor et al., 1985
	UTH8414, UTH8413	-	-	Connor et al., 1985
	TA100	(+)	-	Ashby et al., 1985[b]
	TA97, 98, 1535, 1537, 1538	-	-	Ashby et al., 1985[b]
	TA98, 100, 102	ND	(+)	Takahashi et al., 1985
E. coli	WP2, WP2 *uvrA*	ND	+	Takahashi et al., 1985
	H/R30R, HS30RuvrA	ND	+	Takahashi et al., 1985
	NG30*recA, 016polA*	ND	-	Takahashi et al., 1985
S. typhimurium	TA97, 98, 100	ND	-	Marnett et al., 1985
	TA102, 104	ND	+	Marnett et al., 1985
	TA98, 100	+	+	Oerstavik and Hongslo, 1985
	TA100	+	+	Schmid et al., 1986
	TA104	+	ND	Zielenska and Guttenplan, 1988
	TA102	ND	-	Wilcox et al., 1990
E. coli	WP2 *uvrA*/(pKM101)	ND	+	Wilcox et al., 1990
	WP2 (pKM101)	ND	-	Wilcox et al., 1990

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Table 4-81. Summary of mutagenicity of formaldehyde in bacterial systems (continued)

Species	Strain	Metabolic activation		References
		+S9	-S9	
S. typhimurium	TA102	+	ND	Jung et al., 1992
	TA102	ND	+	Le Curieux et al., 1993
	TA102	+	ND	Muller et al., 1993
	TA98, 100, 102	ND	+	O'Donovan and Mee, 1993
	TA1535, 1537, 1538	ND	-	O'Donovan and Mee, 1993
E. coli	WP2 (pKM101),	ND	+	O'Donovan and Mee, 1993
	WP2uvrA (pKM101)			O'Donovan and Mee, 1993
	K12 (AB1157)(WT)	ND	+	Graves et al., 1994
	K12 (AB1886)/(uvrA), K12(AB2480)/(recA/uvrA)	ND	-	Graves et al., 1994
S. typhimurium	TA102, 2638	ND	+	Watanabe et al., 1996
E. coli	WP2 (pKM101), WP2uvrA (pKM101)	ND	+	Watanabe et al., 1996
Mutagenicity Assays				
S. typhimurium	TA1535 [c]	-	-	Sarrif et al., 1997
	TA1537 [c]	+	+	Sarrif et al., 1997
	TA98, 100 [c]	+	-	Sarrif et al., 1997
	TA97[c]	ND	+	Sarrif et al., 1997
	TA1535, 1537 [d]	-	-	Sarrif et al., 1997
	TA98 [d]	+	+	Sarrif et al., 1997
	TA100 [d]	-	+	Sarrif et al., 1997
	TA100 [e]	+	+	Sarrif et al., 1997
	TA100, 104 [d]	+	+	Dillon et al., 1998
E. coli (Lac+ reversion)	WP3101P, WP3106P	+		Ohta et al 1999
S. typhimurium	TA102, 2638 [d]	ND	+	Ryden et al., 2000
Forward Mutation				
S. typhimurium	TM677	ND	(+)	Couch et al., 1982
		+	+	Donovan et al., 1983
		+	+	Temcharoen and Thilly, 1983
E. coli	D494*uvrB*	+		Bosworth et al 1987

Table 4-81. Summary of mutagenicity of formaldehyde in bacterial systems (continued)

Species	Strain	Metabolic activation		References
		+S9	-S9	
Deletion Mutation				
E. coli	GP120, GP120A 7-2, 33694	ND	+ [f]	Crosby et al., 1988
Point Mutation				
E. coli	GP120, GP120A 7-2, 33694	ND	+	Crosby et al., 1988
Insertion Mutation				
E. coli	GP120, GP120A 7-2, 33694	ND	+	Crosby et al., 1988

[a] Indicates the use of formalin in mutagenicity assay.
[b] Indicates the use of hexamethylmelamine (HEMLA), a formaldehyde-releasing compound, in mutagenicity assay.
[c] Indicates use of the Standard Plate Method.
[d] Indicates use of the Preincubation Plate Method.
[e] Indicates use of the Suspension Method.
[f] Indicates loss of DNA.
'+' indicates a positive test result.
'ND' indicates test was not done.
'-' indicates a negative test result.
(+) indicates a weak positive test result.

O'Donovan and Mee (1993) observed clear mutagenicity by the preincubation exposure method in *S. typhimurium* TA98, TA100, and TA102 strains and both *E. coli* WP2(pKM101) and WP2uvrA(pKM101) strains, while the standard plate-incorporation assays showed consistent mutagenicity only with TA100 and WP2*uvr*A(pKM101) strains and no evidence of mutagenicity in TA1535, TA1537, or TA1538 strains using either method of exposure in the absence of metabolic activation. The *S. typhimurium* and *E. coli* strains used in this study are histidine and tryptophan auxotrophs, with an AT base pair at the critical mutation site within the *his*G and *trp*E genes, respectively, with an intact excision repair system facilitating the detection of cross-linking agents and both strains carrying the mutator plasmid, pKM101, which enhances error-prone repair. These salmonella strains detect frameshift (TA98 and TA1537) and base-pair substitutions (TA100, TA102, and TA1535), while the *E. coli* strains detect base-pair substitutions (WP2*uvr*A). These findings are consistent with the suggestion that formaldehyde induces excision-repairable lesions in bacteria and indicate that the presence of the R-factor plasmid may be required for the expression of its mutagenicity in excision repair-deficient salmonella (O'Donovan and Mee, 1993).

Dillon et al. (1998) employed salmonella strains TA100, TA102, and TA104 because of the latter two strains being more sensitive to oxidative mutagens. Formaldehyde was clearly mutagenic between 6 and 50 μg/plate in all three strains with and without metabolic activation using Aroclor-induced S9 from male F344 rats or male B6C3F1 mice, except for an equivocal response in TA102 with mouse S9 (Dillon et al., 1998). Using a set of six tester strains (WP3101−WP3106) of *E. coli*, each reversible by a mutation involving a single DNA base pair substitution, Ohta et al. (1999) determined that formaldehyde preferentially induced GC to TA transversion mutations. Ryden et al. (2000) demonstrated a statistically significant increase in the number of revertants in *S. typhimurium* TA102 (2.5-fold) and TA2638a (3-fold) strains by formaldehyde at ≥17 μg/plate compared with solvent controls.

In summary, formaldehyde induces mutations in several bacterial strains containing an AT base pair at the primary reversion site that are used to detect oxidative compounds and cross-linking agents without metabolic activation by exogenous enzyme-activating systems. This evidence is strengthened by examining the correlation between genotoxic and clastogenic events and mutation induction.

4.3.3.2. *Mutagenicity in Other Nonmammalian Cell Systems*

Formaldehyde has been shown to be mutagenic in several nonmammalian systems also. It has been shown to cause gene conversion, strand breaks, crosslinks, homozygosis and related damage in yeasts (*Saccharomyces cervisiae*), forward and reverse mutations in molds (*Neurospora crassa*), micronuclei formation in spiderworts (*Tradescantia pallida*), DNA damage and mutations in several plants, genetic cross-over or recombination, sex-linked recessive lethal mutations, dominant lethal mutations, heritable translocations and gene mutations in insects (*Drosophila melanogaster*) and recessive lethal mutations in nematodes (*Caenorhabditis elegans*), but failed to show micronuclei formation in newt larvae (*Pleurodeles waltl*) (Reviewed in Conaway 1996; IARC 2006).

4.3.3.3. *Mutagenicity in Mammalian Cell Systems*

Several studies demonstrated the mutagenicity of formaldehyde in mammalian cells. In its report, the Federal Panel on Formaldehyde underlined the role of formaldehyde as an inducer of gene mutations and CA in a variety of test systems (Report of the Federal Panel on Formaldehyde, 1982). Results from several studies are summarized in Table 4-82.

Table 4-82. Mutagenicity in mammalian cell systems

Species/Strain	Cell/Strain	*In Vivo test*	Metabolic activation		References
			-S9	+S9	
Mutagenicity Assays					
Dominant Lethal Mutation					
Rat/Albino	Spermatocyte, Live implants	yes	+	ND	Odeigah, 1997
Rat	Dominant lethal	yes	(+)		Kitaeva et al 1990
Mouse	Dominant lethal	yes	-		Epstein and Shafner 1968
		yes	-		Epstein et al 1972
		yes	(+)		Fontignie-Houbrechts 1981
Deletion Mutation					
Hamster/Chinese	V79 cells (*Hprt* locus)	no	-		Merk and Speit 1998
	V79 cells *Hprt* locus)	no	-		Merk and Speit 1999
	V79/HPRT	no	+	ND	Grafstrom et al., 1993
	Ovary HPRT	no	-	+	Graves et al., 1996
Mouse	Lymphoma L5178Y cells (Tk $^{+/-}$ locus)	no	+		Macerer et al 1996
	Lymphoma L5178Y cells	no	+	ND	Speit and Merk 2002
Human	Bronchial cell	no	+		Grafstrom et al., 1983
	Bronchial fibroblasts/epithelial cells (HPRT locus)	no	+		Grafstrom et al 1985
	Bronchial fibroblasts/epithelial cells (HPRT locus)	no	+		Grafstrom 1990
	Lymphoblast/HPRT	no	+[a]	ND	Crosby et al., 1988
	Lymphoblast/tk	no	+		Craft et al 1987
	Peripheral blood lymphocytes	yes	+	ND	Shaham et al 2003
	Lymphoblast (TK6)	no	+		Goldmacher and Thilly 1983
Point Mutation					
Hamster/Chinese	Ovary HPRT	no	+	ND	Graves et al., 1996
Mouse	Lymphoma cell/TK+/-	no	+	+	Blackburn et al., 1991
Mouse	Lymphoma cell/TK+/-	no	+	ND	Wangeheim and Bolcsfoldi, 1988
Human	Lymphoblast/TK6	no	+	ND	Liber et al., 1989

Table 4-82. Mutagenicity in mammalian cell systems (continued)

Species/Strain	Cell/Strain	*In Vivo test*	Metabolic activation		References
			-S9	+S9	
Insertion Mutation					
Hamster/Chinese	Ovary HPRT	no	+	ND	Graves et al., 1996
Heritable Mutation					
Mouse	Heritable mutation	yes	+		Liu et al 2009
DNA Repair enzyme activity					
Human	Peripheral lymphocyte	yes	-		Hayes et al., 1997
Mutagenicity Assays					
Cell Transformation			**-S9**	**+S9**	
Mouse	C3H10T1/2 cells		+[b]		Ragan and Boreiko 1981
	Embryo fibroblast/C3H/10T1/2	no	[+]	ND	Boreiko et al., 1983
	Embryo fibroblast/C3H/10T1/2	no	[+]	ND	Frazelle et al., 1983
Hamster	Kidney cell/BHK-21/cI.13	no	+	+	Plesner and Hansen, 1983
p53 mutation and/or p53 protein expression					
Rats/F344	Nasal squamous cell carcinomas	yes	+[c]		Recio et al 1992
Rats/F344	Nasal tumor cell lines	No	+		Bermudez et al 1994
Rats/F344	Nasal squamous cell carcinomas	Yes	+[d]		Wolf et al 1995
Human	Peripheral blood lymphocytes	yes	+		Shaham et al 2003

[a] indicates loss of DNA.
[b] Positive only in the presence of 12-O-tetradecanoylphorbol 13-acetate (TPA).
[c] p53 mutations.
[d] p53 mutated protein detected by immunohistochemistry.
'no' indicates test was not done in vivo
'+' indicates a positive test result.
'ND' indicates test was not done.
'yes' indicates test was done in vivo.
- indicates a negative test result.
(+) indicates a weak positive test result.
[+] indicates positive test result after TPA or *N*-methyl-*N*-nitro-*N*-nitrosoguanidine promoter treatment.

Snyder and Van Houten (1986) demonstrated that formaldehyde increases the levels of misincorporation of bases into synthetic polynucleotides catalyzed by *E. coli* DNA polymerase I,

indicating that the mutagenicity of formaldehyde may be due to covalent alteration of DNA bases. They have also shown that formaldehyde-induced DNA damage in human fibroblasts was not susceptible to repair by the typical "long patch" excision repair mechanism.

Craft et al. (1987) measured the induction of mutations at the thymidine kinase (*tk*) locus or at the ouabain resistance (*Oua*^{r}) locus in TK6 human lymphoblastoid cells. The *tk* mutations can result from a variety of mutational events, including base pair substitution, small and large deletions, and chromosome exchange events, while mutations to *Oua*^{r} require specific base pair substitutions. Single treatment of formaldehyde (0, 15, 30, 50, 125, and 150 μM) for 2 hours resulted in a nonlinear increase in *tk* mutagenesis with increasing slope >125 μM (see Figure 4-31). To explore a dose-response effect, cells were also exposed as follows: three treatments of 50 μM for 2 hours or five treatments of 30 μM or 10 treatments of 15 μM for 2 hours (treatments were spaced 2−4 days apart) with multiple treatments causing an increase in *tk* mutations, although their combined effect was less than a single treatment of equivalent C × t (150 μM for 2 hours). Lymphoblasts given four treatments of 150 μM formaldehyde for 2 hours failed to induce mutations at the *Oua*^{r} locus. Dose-response increases were seen in all exposure scenarios, with 30 μM being the level of statistical significance. There was little indication of a dose-response effect until the cumulative concentration was greater than 100 μM. Formaldehyde-induced DPXs were no longer evident after 24 hours of exposure; mutants induced in the TK6 lymphoblast cell line showed a similar dose-response curve to the DPXs measured immediately after exposure ended (Craft et al., 1987).

The same group also studied mutations induced at the X-linked hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus by eight repetitive treatments of 150 μM formaldehyde in TK6 human lymphoblast cell line by Southern blot analysis, wherein half (14/30) of induced mutants contained partial or complete deletions with most of the partial deletions showing unique deletion patterns, while only a third (5/15) of spontaneous mutants had partial or complete deletions, indicating that formaldehyde can induce large losses of DNA in human lymphoblast cells (Crosby et al., 1988).

Liber et al. (1989) followed up the findings of Crosby et al. (1988) by performing Southern blot, Northern blot, and DNA sequence analysis on the 16 induced and 10 spontaneous human lymphoblast mutants not showing deletions. Northern blot analysis showed that the point mutations fell into four categories: normal size and amount of RNA, normal size but reduced amounts of RNA, reduced size and amounts of RNA, and no RNA. Sequence analysis of recombinant DNAs from *hprt* mRNA in formaldehyde-induced mutants showed a preferential AT to CG transversion at a specific site, with other changes represented to a lesser degree (Liber et al., 1989).

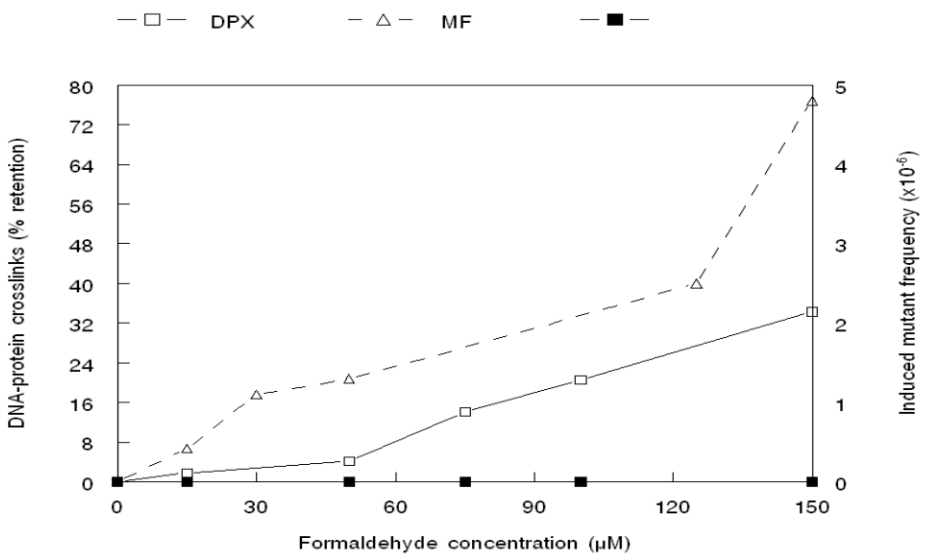

Figure 4-31. DNA-protein cross-links (DPX) and thymidine kinase (*tk*) mutants in TK6 human lymphoblasts exposed to formaldehyde for 2 hours.

Note: □ DPXs immediately after exposure, ■DPXs 24 hours after exposure, △ *tk* mutants. Relative survival was 100% at 0, 15, 30, and 50 μM, 30% at 125 μM, and 20% at 150 μM.

Source: Adapted from Craft et al. (1987).

Even in CHO cells formaldehyde has been shown to induce *hprt* mutations involving mostly single-base pair transversions mostly occurring at AT sequences, including three AT to TA at position 548 of exon 8 and two AT to CG and one GC to TA transversion at other sites (Graves et al., 1996). In another study, formaldehyde-induced forward mutations to trifluorothymidine resistance in mouse lymphoma L5178Y $tk^{\pm}$ cells both in the absence and presence of rat liver S9 (higher concentrations required for effect with S9). Both toxicity and mutagenicity were abolished when FADH was incorporated in the exposure medium (Blackburn et al., 1991).

Formaldehyde-induced DPXs are removed in part through spontaneous hydrolysis and in part due to active repair processes (Quievryn and Zhitkovich, 2000). Inhibition of specific proteosomes in XP-A cells inhibited DPX repair, thereby supporting the role of enzymatic degradation (Quievryn and Zhitkovich, 2000). The half-life of formaldehyde-induced DPXs in

human cell lines was consistent with the findings of Craft et al. (1987), ranging from 11.6 to 13 hours (Quievryn and Zhitkovich, 2000). In the same report, removal of DPXs from human peripheral lymphocytes was much slower, with a half-life of 18.1 hours. This difference was primarily in slower active repair of DPXs, with a $t_{1/2}$ of 66.6 hours for human lymphocytes versus 23.3 hours for human cell lines (Quievryn and Zhitkovich, 2000).

Since DPX repair involves proteolytic removal of proteins from the DNA, Speit et al. (2000) hypothesized that single peptides or small peptide chains cross-linked to the DNA are critical to formaldehyde-induced mutation. However, these authors did not find significant difference in the induction and repair of DPXs in normal and DNA repair-deficient cell lines but observed increased susceptibility of the repair-deficient cell lines to formaldehyde-induced MN induction. In this study, a normal human cell line (MRC5CV1), a xeroderma pigmentosum cell line deficient in nucleotide excision repair (NER), and a Fanconi anemia cell line, which has a genetic defect leading to hypersensitivity towards DDXs, were exposed to 125, 250, and 500 μM formaldehyde for 2 hours. The authors suggest that more than one repair pathway is involved in the repair of cross-links and that the altered NER pathway has more severe consequences to formation of CAs than disturbed cross-link repair (Speit et al., 2000).

The correlation of early DPX formation and mutation is at first counterintuitive since the cross-linking of protein to DNA inhibits DNA replication. Without active DNA replication, formaldehyde-DNA adducts and DPXs would not induce replication error and would be unlikely to result in a change in DNA sequence or mutation. Recent evidence indicates that residual peptides and short polypeptides that remain cross-linked to DNA after DPX removal may in fact be the cause of DPX-associated, formaldehyde-induced mutation (Speit et al., 2000).

A study by Merk and Speit (1998) indicated that formaldehyde-induced DPXs did not result in direct gene mutations in the *hprt* locus of V79 Chinese hamster cells, leading the authors to speculate that formaldehyde was not mutagenic. Since, the *hprt* locus in the V79 Chinese hamster cell line is primarily sensitive to point mutations and other studies show the formation of deletion mutations by formaldehyde at the same locus in human lymphoblasts (Crosby et al., 1988), Merk and Speit (1998) concluded that the *hprt* mutation assay is insensitive to deletion mutations.

Later, using the mouse lymphoma assay, Speit and Merk (2002) demonstrated that exposure to formaldehyde for 2 hours was mutagenic in a concentration-dependent manner in the L5178Y mouse lymphoma cells, which was mainly contributed by a strong increase in small colony mutants, suggestive of CAs (Speit and Merk, 2002). Detailed analysis of both spontaneous and formaldehyde-induced lesions indicates that recombination or deletion of DNA from the *tk* locus was primarily responsible for the loss of heterogeneity, thereby leading to the

observed mutant phenotype. Therefore, it is believed that formaldehyde is mutagenic in the L5178Y cell mouse lymphoma system by a clastogenic mechanism rather than through point mutations. This finding is consistent with that of Craft et al. (1987), who demonstrated formaldehyde mutagenicity at the *tk* locus of TK6 cells, and also with the findings of Grafström et al. (1984), who demonstrated increased SSB formation in formaldehyde-exposed cell lines.

Formaldehyde has also been shown to induce cell transformation in mouse embryo fibroblasts (Ragan and Boreiko 1981; Boreiko et al 1983; Frazelle et al 1983) . At low concentrations of 0.017 mM formaldehyde has shown to cause cell transformation in C3H10T1/2 mouse cells (Ragan and Boreiko 1981) and hamster kidney cells in vitro (Plenser and Hansen 1983).

More recently, Shaham et al. (2003) examined the frequency of DPXs and the incidence of mutant versus wild type p53 tumor suppressor genes in the peripheral blood lymphocytes of a cohort of workers exposed to formaldehyde. The adjusted mean levels of DPXs were greater in the lymphocytes of exposed subjects compared with those of unexposed subjects, and exposure to formaldehyde increased the likelihood of their having a higher level of pantropic p53 (>150 pg/mL). The authors speculated on a possible causal relationship between DPXs and mutations in p53. Recio et al (1992) demonstrated point mutations in the p53 tumor suppressor gene in 45% (5 out of 11) of the primary nasal squamous cell carcinomas (SCCs) obtained from F344rats that were chronically exposed to 15 ppm formaldehyde for 2 years (Recio et al ., 1992).

In summary, the results of in vitro experiments demonstrate the mutagenicity of formaldehyde. Mutagenicity is observed below levels of significant cytolethality in mammalian cell lines. Formaldehyde is clearly a DNA-reactive genotoxicant inducing lesions (DPXs) that show clastogenicity (SSBs, MNs, etc.). The experiments by Speit and Merk (2002) explore mechanistic links between DPXs, clastogenicity, and the observed locus-specific mutations in the mouse lymphoma in vitro testing system.

4.3.4. In Vivo Mammalian Genotoxicity

4.3.4.1. ***Genotoxicity in Laboratory Animals***

As discussed above, formaldehyde is clearly reactive at the POE in animal studies, resulting in increased DPXs in the nasal mucosa. Despite formaldehyde's reactivity and mutagenicity in isolated mammalian cells, clear evidence of mutagenicity does not emerge from animal bioassays (see Table 4-83).

Table 4-83. Genotoxicity in laboratory animals

Species/Strain	Cells/Organ/Tumor	Result	References
Cytogenetic Assays			
Chromosomal aberrations (CA)			
Mice/Q strain	Spermatocyte	-	Fontignie-Houbrechts et al., 1981
	Spermatogonia	-	Fontignie-Houbrechts et al., 1982
Mice/CBA	Polychromatic erythrocytes	-	Natarajan et al., 1983
	Spleen cells	-	Natarajan et al., 1983
Rats/F344	Lymphocytes	-	Kligerman et al 1984
Rats/Sprague-Dawley	Gastric epithelial cells	+	Migliore et al 1989
Rats/Wistar	Bone marrow	+	Kitaeva et al 1990
Rats/Sprague-Dawley	Bone marrow	-	Dallas et al 1992
Rats/Sprague-Dawley	Pulmonary lavage cells	+	Dallas et al 1992
Rats/F344	Peripheral blood cells	-	Speit et al 2009
Micronucleus (MN)			
Mouse/NMRI	Bone marrow	-	Gocke et al 1981
Mice/CBA	Femoral polychromatic erythrocyte and spleen cell	-	Natarajan et al., 1983
Mice/B6C3F1	Bone marrow	+	Ward et al 1983
Rats/Sprague-Dawley	Gastric epithelial cells	+	Migliore et al 1989
Sister Chromatid Exchange (SCE)			
Rats/F344	Lymphocyte	-	Kligerman et al 1984
Rats/F344	Peripheral blood cells	-	Speit et al 2009

'+' indicates a positive test result.
'-' indicates a negative test result.

In a chromosomal analysis study (Fontignie-Houbrechts, 1981), formaldehyde given I.P. at 50 mg/kg to male Q strain mice and analyzed 8−15 days after treatment did not induce any chromosomal lesions in spermatocytes. Also, in another study from the same group (Fontignie-Houbrechts et al., 1982), formaldehyde (30 mg/kg) given along with hydrogen peroxide (90 mg/kg) as a mixture to male Q strain mice failed to produce significant increases in chromosomal lesions in the spermatogonia.

Ward et al. (1983) studied the cytogenetic effects of commercial formalin on the bone marrow of B6C3F1 mice. Since commercial formalin contains 10-15% of methanol, another group was dosed with methanol (1000 mg/kg) and also included a negative control (water) and a

positive control group dosed with 100 mg/kg cyclophosphamide (CP). Mice from all groups were administered 1 mg/kg colcemid (which arrests cells in metaphase), 28 h after dosing with test compounds. Two hours after colcemid treatment, mice were sacrificed; bone marrows from femurs were prepared and analyzed the metaphase chromosomes for structural aberrations. They reported that formalin, methanol, water and CP, respectively produced structural chromosomal aberrations (%) such as aneuploidy (101, 65, 6, 3), breaks (3, 3, 1, 32), exchanges (40, 24, 10,17), aberrant chromosomes with gaps (45, 32, 2.1, 63) and without gaps (43, 27, 1.7, 55) in bone marrow cells of male B6C3F1 mice. The cytogenetic effects seen in bone marrow suggest that formalin or other chemicals given at a single high dose (gavage) were able to reach bone marrow and induce genotoxicity, specifically, aneuploidy and sister chromatid exchanges. It is recognized that the results reported were preliminary; however, they are significant in terms of genotoxic potential of formaldehyde exposure via oral route of exposure.

In a different study Natarajan et al. (1983) failed to detect significant differences in MN induction in bone marrow cells or CAs in spleen cells of male and female CBA mice given I.P. 6.25, 12.5, and 25 mg/kg formaldehyde compared with saline-treated controls. However, the same study showed a positive induction of MNs and CAs in vitro. The authors suggest that the lack of genotoxicity in vivo may be due to the inability of formaldehyde to reach the target cells in sufficient quantity to induce biological effects.

Kligerman et al. (1984) also found no difference in the incidence of SCEs or chromosome breakage in the peripheral lymphocytes of male and female F344 rats exposed to formaldehyde in air at 0.5, 6, or 15 ppm (0.61, 7.36, or 18.4 mg/m^3) 6 hours/day for 5 days. However, in a different study (Migliore et al., 1989), clastogenic effects, such as increased MNs and CAs, were reported in GI epithelial cells of male Sprague-Dawley rats after oral exposures to 200 mg/kg formaldehyde. In this study, micronucleated cells and nuclear anomalies were increased in a time-dependent manner in the stomach, duodenum, ileum, and colon of rats, and the mitotic index was unchanged for these cells compared with controls at 16, 24, and 30 hours. These clastogenic effects were seen without regenerative cell proliferation, supporting formaldehyde-induced mutations as primary effects of formaldehyde rather than secondary to regenerative cell proliferation.

Kitaeva et al. (1990) observed cytopathological and cytogenetic effects of formaldehyde chronic inhalation in 0.5 and 1.5 mg/m^3 doses in the female rat's germ cells and bone marrow cells, where formaldehyde-induced harmful effects were seen in germ cells at <1.5 mg/m^3 doses, while the reliable clastogenic and cytogenetic effects on the marrow cells were induced even at the 0.5 mg/m^3 dose, suggesting differences among effects of small doses of formaldehyde on different cell systems.

Dallas et al. (1992) observed a slight increase (7.6 and 9.2%) in CAs in pulmonary lavage cells from male Sprague-Dawley rats exposed to 15 ppm (18.4 mg/m^3) formaldehyde in air 6 hours/day, 5 days/week for 1 or 8 weeks by inhalation compared with corresponding controls (3.5 and 4.8%), respectively. However, the small study, limited as it was to five animals/group, showed statistically significant increase at the highest dose tested (15 ppm) but not at lower doses (0.5 and 3 ppm). In the same study, no clastogenic effects were seen in bone marrow, which is consistent with formaldehyde acting primarily at the site of first contact.

Speit et al (2009) investigated the genotoxicity of formaldehyde in peripheral blood samples of Fischer-344 rats exposed to 0 to 15 ppm formaldehyde by whole-body inhalation for 4 weeks (6 h/day, 5 days/week). In this study, the authors found no significant increase in the genotoxic assays such as comet assay with or without gamma-irradiation of blood samples (DNA migration as determined by tail movement or intensity), sister chromatid exchange (SCE) assay and micronucleus test (MNT) compared to controls. However, rats given 50 mg/kg methylmethane sulfonate (MMS) by gavage for 4 hrs (positive control for Comet and SCE assays) or 10 mg/kg cyclophosphamide (CP) given twice orally (positive control for MNT) induced significant increase in genotoxicity in this study. The lack of genotoxicity in this study is not surprising since earlier studies by Casanova-Schmitz et al (1984a) have shown that formaldehyde does not cause toxicity to bone marrow possibly due to the inability of this chemical to reach the bone marrow. Although MMS and CP used in this study were positive in the genotoxicity assays, the data from positive controls can not be used for validation since the exposure routes of formaldehyde (inhalation) and the positive controls (oral) were different.

No animal studies have examined clastogenic effects of formaldehyde in nasal or respiratory epithelial cells. Therefore, it is unknown whether similar changes would occur in response to exposure to formaldehyde via inhalation. However, the negative finding in bone marrow cannot be considered definitive evidence on the question of the mutagenic potential of formaldehyde for cells present at the POE. With weak positive results in pulmonary lavage cells and clear clastogenicity in GI epithelial cells below exposures that trigger regenerative cell proliferation, the existing evidence, however incomplete, supports the concept of genotoxic action of formaldehyde at the POE.

4.3.4.2. *Genotoxicity in Humans*

The majority of the studies on the effects of formaldehyde in exposed humans have measured various cytogenetic endpoints, such as MNs, SCEs, or CAs in nasal and oral mucosal cells (considered to be in direct contact with formaldehyde) as well as peripheral lymphocytes. Since genotoxicity at the proximal sites (oral, nasal) can be readily linked to the reactive nature

of formaldehyde, these studies are discussed first, noting where researchers also collected blood lymphocyte samples. A subsequent discussion is focused on results in blood lymphocytes. Finally, the few studies that measured DPXs in exposed humans are discussed. Table 4-89 provides a summary of human cytogenetic studies of formaldehyde.

4.3.4.2.1. ***Nasal, buccal, and oral mucosal cells.***

Epithelial cells of the URT and oral cavity are potential targets of formaldehyde's DNA reactivity and genotoxicity. Several studies indicate that formaldehyde exposure results in measurable increases in SCEs, MN formation, and DPXs in nasal, buccal, and oral mucosal cells; however, these genotoxic effects vary with the type of exposure. Study quality, sample size, availability of exposure measurements, and assay methodology may in part contribute to variability in study findings. The studies fall into three general categories: workers (industrial or professional), students and staff attending anatomy and mortuary science courses, and subjects in a controlled clinical trial.

Ballarin et al. (1992) observed significantly higher frequency of micronucleated cells in a formaldehyde exposed group in a plywood factory compared with controls (0.9 ± 0.47 versus 0.25 ± 0.22, $p < 0.01$). In this study, the frequency of MNs and cytology of respiratory nasal mucosal cells was examined in 15 nonsmokers exposed to levels of formaldehyde that ranged between 0.1 and 0.39 mg/m^3 (~0.32 ppm) for an average of 6.8 years. Exposed subjects were compared with age- and sex-matched controls.

Ye et al. (2005) reported significant increases in MNs per thousand cells in nasal mucosal cells for 18 nonsmoking workers (2.70 ± 1.50) in a formaldehyde manufacturing plant in the Hugei province of China as compared with controls (1.25 ± 0.41). In addition, higher frequencies of SCEs in peripheral lymphocytes of workers were also reported (8.24 ± 0.89 versus 6.38 ± 0.41). In this study, the average age of workers was 29 ± 6.8 years, the average duration at work was 8.5 years (range 1−15 years), and the reported 8-hour TWA was 0.985 mg/m^3 (0.8 ppm). The control group consisted of 23 undergraduate students with an average age of 19 ± 2.3 years. The 8-hour TWA in the student dormitories was 0.011 mg/m^3 (9 ppb). A group of 16 waiters with an average exposure duration of only 12 weeks and an 8-hour TWA of 0.107 mg/m^3 (90 ppb) was also included in the study. The incidence of MNs and SCEs in the waiters was the same as that in controls. Overall, results from this study suggest that the genotoxic potential of high-level formaldehyde exposure may have occupational risks in long-term exposure.

However, in a different study, Speit et al. (2007b) showed that formaldehyde did not induce MNs in exfoliated buccal mucosa cells of humans exposed up to a maximum of 1 ppm

and a cumulative exposure of 13.5 ppm-hours over 2 weeks. In this study, volunteers exposed to formaldehyde in closely controlled conditions (4 hours/day for 10 days) with a complex exposure schedule, amounting to a cumulative total of 13.5 ppm-hours (16.6 mg/m^3-hours), were used. Samples of the buccal mucosa were taken from subjects 1 week before the start of the experiment, at the start of the experiment, at the conclusion of the series of exposures, and at 7, 14, and 21 days after the completion of exposure. Thus, the subjects served as their own controls. Two thousand cells per data point were assessed for the frequency of MNs on slides that were coded by an independent quality assurance organization. As shown in Table 4-84, the frequency of MN formation was statistically unchanged from that in controls. The apparent slight increase in subjects evaluated at the conclusion of exposure was caused by frequencies of MNs in two subjects (5.0 and 4.5 MNs per 1,000 cells). The data as reported show a high variability, where the SD approaches or exceeds the mean for each sample point, suggestive of data with an asymmetrical distribution.

Table 4-84. MN frequencies in buccal mucosa cells of volunteers exposed to formaldehyde

Sampling point	Group	MN/1000 cells (± SD)
Control data		
1 week before exposure	1	0.95 ± 0.67
Immediately before exposure series	2	0.86 ± 0.84
Test data		
Immediately after exposure series	3	1.33 ± 1.45
7 days after exposure	4	0.94 ± 0.73
14 days after exposure	5	0.85 ± 0.86
21 days after exposure	6	0.44 ± 0.38[a]

[a]Statistically significantly different from control values ($p < 0.05$), as calculated by the authors.
Source: Speit et al. (2007b).

The best evidence of formaldehyde-induced clastogenic changes in peripheral lymphocytes is found in studies of anatomy class and mortuary class students. Since genetic damage accumulates with age, the studies in younger adults, where cells are analyzed before and after exposure, may have greater sensitivity and fewer confounding factors.

Suruda et al. (1993) showed a 12-fold increase in the MN frequency of epithelial cells from the buccal area of the mouth in mortuary science students exposed to embalming fluids

containing formaldehyde following an 85-day exposure period (see Table 4-85). Overall, students were exposed to 0.33 ppm (0.4 mg/m^3) formaldehyde as an 8-hour TWA on days when embalming was performed (an average of 6.9 embalmings). Blood, oral, and nasal samples were collected pre- and postexposure. As shown in Table 4-85, nasal epithelial MNs increased by 22% (frequency of micronucleated lymphocytes increased by 28%). By contrast, SCE frequency decreased by 7.5% after formaldehyde exposure.

Table 4-85. MN and SCE formation in mortuary science students exposed to formaldehyde for 85 days

Sampling point	Buccal mucosa (MN/1,000)	Nasal epithelium (MN/1,000)	Blood (MN/1,000)	Blood (SCEs/cell)
Before course	0.046 ± 0.17	0.41 ± 0.52	4.95 ± 1.72	7.72 ± 1.26
After course	0.60 ± 1.27[a]	0.50 ± 0.67	6.36 ± 2.03[a]	7.14 ± 0.89

[a]Statistically significant ($p < 0.05$), as calculated by the authors.
Source: Suruda et al. (1993).

Another group (Titenko-Holland et al., 1996) also reported a significant increase in MN frequency of buccal, but not nasal, epithelial cells from mortuary students exposed to embalming fluid. In this study, 28 out of 35 students were sampled before and after a 90-day embalming class. The mean formaldehyde exposure for the subjects providing data on buccal cell MNs was 14.8 ± 7.2 ppm-hours (18.2 ± 8.8 mg/m^3-hours) for the entire 90-day period and 16.5 ± 5.8 ppm-hours (20.3 ± 7.1 mg/m^3-hours) for students providing data on nasal cell MNs. Cells were recorded as having either whole chromosomes with centromeres (MN^+) or acentric fragments and no centromeres (MN^-). Cells with multiple nuclei were present only in samples taken after exposure to embalming fluid. There was a ninefold increase in the MN frequency in buccal cells ($p < 0.5$) and only a twofold increase ($p > 0.05$) in nasal cells. In addition, there was a twofold increase in the MN^+ frequency in buccal cells (see Table 4-86). The authors suggested that chromosomal breakage appears to be the primary mechanism of MN formation.

Ying et al. (1997), however, observed higher frequencies of MNs in the nasal exfoliative cells (3.85 ± 1.48 versus 1.20 ± 0.676, paired t-test, $p < 0.001$) and oral exfoliative cells (0.857 ± 0.558 versus 0.568 ± 0.317, $p < 0.001$) after formaldehyde exposure, although there was no significant increase in the frequency of lymphocyte MNs ($p > 0.05$) in students exposed to formaldehyde in anatomy classes (three classes per week for 3 hours over an 8-week duration). In this study, blood samples and nasal swabs were collected before and after the study. The TWA concentration of formaldehyde in anatomy laboratories and student dormitories was 0.508

± 0.299 mg/m^3 and 0.012 ± 0.0025 mg/m^3, respectively, suggesting that nasal mucosa cells exposed through respiration are the primary target of formaldehyde-induced genotoxicity.

Table 4-86. Incidence of MN formation in mortuary students exposed to formaldehyde for 90 days

	Buccal cells (*n* = 19)			Nasal epithelial cells (*n* = 13)		
Sampling point	**Total MN**	**MN$^+$**	**MN$^-$**	**Total MN**	**MN$^+$**	**MN$^-$**
Pre-exposure	0.6 ± 0.5	0.4 ± 0.4	0.1 ± 0.2	2.0 ± 1.3	1.2 ± 1.3	0.5 ± 0.5
Postexposure	2.0 ± 2.0[a]	1.1 ± 1.3	0.9 ± 1.1[a]	2.5 ± 1.3	1.0 ± 0.8	1.0 ± 0.6[a]
p value	0.007	0.08	0.005	0.20	0.31	0.03

[a]Statistically significant at the level shown, as calculated by the authors.

Source: Titenko-Holland et al. (1996).

Ying et al. (1997), however, observed higher frequencies of MNs in the nasal exfoliative cells (3.85 ± 1.48 versus 1.20 ± 0.676, paired t-test, $p < 0.001$) and oral exfoliative cells (0.857 ± 0.558 versus 0.568 ± 0.317, $p < 0.001$) after formaldehyde exposure, although there was no significant increase in the frequency of lymphocyte MNs ($p > 0.05$) in students exposed to formaldehyde in anatomy classes (three classes per week for 3 hours over an 8-week duration). In this study, blood samples and nasal swabs were collected before and after the study. The TWA concentration of formaldehyde in anatomy laboratories and student dormitories was 0.508 ± 0.299 mg/m^3 and 0.012 ± 0.0025 mg/m^3, respectively, suggesting that nasal mucosa cells exposed through respiration are the primary target of formaldehyde-induced genotoxicity.

In a different study (Ying et al., 1999), however, the same group showed that exposure to formaldehyde affected the composition of lymphocyte subsets (B cells, total T cells, T helper-inducer cells, T cytotoxic-suppressor cells), but no significant difference was reported between lymphocyte proliferation rate and SCEs at the given levels and durations of formaldehyde exposure. This study involved 23 nonsmoking students exposed to 0.508 ± 0.299 mg/m^3 formaldehyde for a period of 8 weeks (3 hours , 3 times per week).

Burgaz et al. (2002) reported significantly ($p < 0.05$) higher mean MN frequencies in buccal mucosal cells from shoe workers as well as anatomy and laboratory workers (0.62 ± 0.45% and 0.71 ± 0.56%, respectively) compared with unexposed controls (0.33 ± 0.30%). In this study, the measured air concentrations of formaldehyde in the breathing zone of the anatomy and pathology laboratory workers were between 2 and 4 ppm (2.5 and 5 mg/m^3). MN count per

3,000 cells was measured in buccal smears from shoe workers and from anatomy and pathology staff, and eighteen male university staff were used as controls.

In a critical review, Speit and Schmid (2006) examined data from studies that have reported the formation of MNs in nasal or buccal cells of persons either environmentally or occupationally exposed to formaldehyde. The authors identified a number of issues relating to study design, exposure regimen, and confounding factors, including MN levels in nasal and buccal cells well below established background levels, reports limited by the number of cells observed, variation in standard techniques, and nonconcordance between buccal and nasal findings. However, the authors concluded that, despite these limitations, the weight of evidence supports the finding that formaldehyde may be genotoxic in human cells in direct contact with formaldehyde.

4.3.4.2.2. *Peripheral blood lymphocytes.*

Mature lymphocytes are present at the POE as intraepithelial lymphocytes and within germinal centers in the mucosa. Because more lymphocytes may be available in the nasal mucosa than the oral mucosa, mouth versus nose breathing may contribute to variability in findings. Since some of the lymphocytes traffic around the body, it is reasonable to find clastogenic effects in these relatively long-lived cells reflected in peripheral blood lymphocytes. Thus, lymphocytes proliferating in response to antigen would be more vulnerable to DNA reactivity of formaldehyde and to the clastogenic effects in general.

A cytogenetic evaluation by Fleig et al. (1982) of 15 employees exposed for an average of 28 years in a formaldehyde manufacturing plant revealed no statistically significant increase in the frequency of CAs in peripheral blood lymphocytes compared with a matched control group. Although some of the workers are smokers in this study, smokers did not show an increased incidence of CAs. Likewise, in a different study (Thomson et al., 1984), no exposure-related differences were evident in the frequency of CAs and MNs in lymphocytes from six pathology workers and five unexposed controls. This study did not account for differences in smoking.

Bauchinger and Schmid (1985) observed an increased incidence of CAs (dicentric and ring chromosomes) in the peripheral lymphocytes of 20 male paper mill workers and supervisors exposed to formaldehyde (average exposure of 14.5 ± 7.2 years) compared with unexposed workers. When workers and supervisors were analyzed separately, significant increases were only seen for supervisors. The average length of exposure for supervisors (n = 11) and workers (n = 9) was 18.9 years and 7.2 years, respectively. Information regarding formaldehyde concentrations for the two groups was not provided. However, the incidence of SCEs among

workers was actually slightly lower than among the 20 controls. In contrast, Ye et al., (2005) observed that the frequency of SCEs in peripheral lymphocytes of 18 nonsmoking formaldehyde factory workers (mean exposure duration of 8.6 years) was significantly increased over nonsmoking controls (8.24 ± 0.89 versus 6.38 ± 0.41) (described in Section 4.3.4.2.1).

Vargová et al. (1992) observed that the percentage of aberrant cells and number of breaks per cell in the peripheral blood lymphocytes of formaldehyde-exposed workers was 3.08 and 0.045 versus 3.6 and 0.030 in controls in a pressed board factory, respectively, suggesting both groups to be at an increased risk. However, normal unexposed population had only 1−2% aberrant cells. The authors also noted that the mitotic index was significantly decreased in exposed workers compared with controls. This study did not specify about the smoking background of the two groups, however, the authors indicate that both exposed and unexposed workers have similar habits and social status.

Kitaeva et al. (1996) evaluated the genotoxic effects of formaldehyde among 15 industrially exposed workers and 8 academic laboratory instructors and observed an increase in the frequencies of CAs and MNs in the lymphocytes of exposed subjects compared with 6 unexposed controls. This study did not provide information about smoking history.

Shaham et al. (1996, 1997) found significantly higher levels of DPXs and SCEs in peripheral blood lymphocytes of workers occupationally exposed to formaldehyde (physicians and technicians) compared with unexposed control workers. The authors also observed a linear relationship between years of exposure to formaldehyde and levels of DPXs and SCEs. Although both studies have smokers and nonsmokers, smoking did not influence DPX formation in either of the studies (Shaham et al 1996; 1997).

Formaldehyde-induced genotoxicity has also been reported in peripheral blood lymphocytes of anatomy class students and mortuary workers. Vasudeva and Anand (1996) did not observe significant differences in the incidences of CAs between the formaldehyde exposed students and the matched, unexposed controls. In this study, peripheral blood lymphocytes from 30 medical students exposed to formaldehyde in a gross anatomy laboratory for 15 months with average exposures of less than 1 ppm (1.23 mg/m^3) formaldehyde were used. No smoking history is available for this study.

He et al. (1998) used the cytokinesis-blocked MN (CBMN) assay to detect the frequency of micronucleated peripheral lymphocytes in 13 students exposed to formaldehyde during a 12-week (10 hours/week) anatomy class. Sampling of breathing zone air showed a mean concentration of 2.37 ppm (3.17 mg/m^3). Ten students from the same school, without exposure to formaldehyde, were used as controls. All study subjects were nonsmokers. CAs and SCEs were observed in both groups, and there were significant increases ($p < 0.01$) in the frequencies

of micronucleated cells and CAs in the formaldehyde-exposed group compared with the control group.

In a study involving 97 plasticware workers (34 males and 63 females) exposed to 0.5 to 0.9 mg/m^3 formaldehyde, 4.4 to 6.2 mg/m^3 styrene and 0.5 to 0.75 mg/m^3 phenol for 2 months to 25 years, Lazutka et al (1999) observed significantly higher CAs than controls (nonexposed donors matched by age and similar smoking habits as the exposed workers). Although workers with short and long exposures showed significant increases in the frequency of CAs, the cytogenetic damage did not increase with exposure duration.

Sari-Minodier et al. (2001), using the CBMN assay in anatomy/pathology laboratory workers who were occupationally exposed to formaldehyde for a mean exposure period of 9 yrs (range 1-16 yrs), reported higher frequency of micronucleated peripheral blood lymphocytes (18.8% ± 13.1) than in matched controls (8.8% ± 4.4).

Shaham et al. (2002) observed a mean ± S.E. number of 0.27 ± 0.003 SCEs per chromosome in the peripheral lymphocytes of a hospital pathology department workers with a mean exposure period of 15.4 yrs (range 1-39 yrs) compared with 0.19 ± 0.002 SCEs in control subjects from the administrative staff of the same hospital ($p < 0.01$) after adjusting for age, sex, smoking habits, etc. This study involved 90 individuals employed in 14 hospital pathology laboratories and 52 unexposed controls.

Yu et al. (2005) reported dose-dependent increase in MNs and comet assay parameters (olive tail moment and comet tail length) in peripheral lymphocytes in 151 workers from two plywood factories compared with 112 unexposed controls. Both exposed and control subjects are nonsmokers in this study. The TWA exposure level in the working environment was 0.1−7.88 mg/m^3 (0.08−6.42 ppm) formaldehyde compared with a background level of <0.01 mg/m^3 (<0.008 ppm) formaldehyde applicable to controls. In the comet assay, the authors observed olive tail moments averaging 0.93 (0.78−1.1), 3.03 (2.49−3.67), and 3.95 (3.53−4.43) for control, low-, and high-exposure individuals, respectively. For the same subjects, comet tail lengths were 6.78 (6.05−7.6), 11.25 (10.12−12.5), and 12.59 (11.8−13.43), respectively. In the CBMN assay, MNs/100 cells were 0.27 ± 0.13, 0.41 ± 0.25, and 0.65 ± 0.36, respectively, for control, low-, and high-exposure individuals.

In a population of 18 workers exposed to formaldehyde at a plant in China, with a mean employment of 8.5 years (range 1 to 15 years), Ye et al (2005) examined nasal epithelial cells and lymphocytes for cytogenetic effects. This study also included a second group of 16 waiters who worked in a newly fitted ball room for 12 weeks with a low level exposure to formaldehyde from building material, tobacco smoke and furniture and a group of 23 college students as a control group. The background indoor air concentration of 0.009 ppm formaldehyde was

reported in students' dorms. Significantly increased frequencies of MNs in the nasal mucosal cells and SCEs in peripheral blood lymphocytes were reported for the workers, but not the waiters in this study.

Orsière et al. (2006) reported no apparent effect on the DNA damage in peripheral blood lymphocytes as assessed by a chemiluminescence microplate assay in pathology and anatomy laboratory workers (n = 59) before and after a 1-day exposure to formaldehyde. This study had 59 exposed workers and 37 controls with similar smoking history. However, with the CBMN assay, the authors reported statistically significant differences in the frequency of binucleated micronucleated cells (1.69 ± 0.93 versus 1.11 ± 0.6%) in exposed versus control subjects. Discrimination between clastogenic and aneugenic events by using fluorescence *in situ* hybridization (FISH) technique with a pan-centromeric DNA probe resulted in a higher rate of binucleated micronucleated cells (1.91 ± 1.01 versus 1.19 ± 0.56% in controls) and showed that the frequency of centromeric nuclei was higher in the exposed group than in controls, though not significantly. Among the centromeric MNs, the frequency of MNs with only one centromere (C1+MN) was significantly greater in pathologists/anatomists than in controls (1.1 ± 0.62 versus 0.31 ± 0.24%, $p < 0.001$). The authors interpreted their data on monocentromeric nuclei in anatomists/pathologists as an indication that formaldehyde exposure might be associated with aneugenic (rather than clastogenic) events.

Based on pooled analysis of two reports (Iarmarcovai et al., 2006a, b) (see Table 4-87), MN frequency ratios in the peripheral lymphocytes of cancer patients, welders, and anatomists/pathologists were significantly increased compared with the corresponding controls. The data were taken from three biomonitoring studies by using CBMN/FISH. The incidence of MNs was scored and then evaluated further for the presence of centromere-negative MNs (C−MNs), centromere-positive MNs (C+MNs), and, for the latter case, those containing a single centromere (C1+MNs) and those containing two or more centromeres (Cx+MNs). Applying their findings to considerations of the aneugenic mechanism of action of formaldehyde, the authors hypothesized that the use of centromeric signals enables the identification of endpoints representing impaired chromosomal migration (with C1+MN formation) or centrosome amplification (with Cx+MN formation).

Recently, Costa et al. (2008) observed a significant increase in the genotoxicity of formaldehyde-exposed pathological anatomy laboratory workers (n = 30) compared with controls (n = 30) in cytogenetic assays. In this study, the authors evaluated the level of exposure to formaldehyde near the breathing zone of workers, and TWA of exposure was calculated for each subject, giving a mean level of exposure to be 0.44 ± 0.08 ppm (range: 0.04−1.58 ppm). As compared with control subjects, peripheral blood lymphocyte cultures of formaldehyde exposed

Table 4-87. Multivariate repression models linking genomic instability/occupational exposures to selected endpoints from the MN assay

Study populations	Number	MN[a]	C−MN	C+MN	C1+MN	Cx+MN
Cancer patients versus controls	10/10	**1.85 (1.18−2.87)**	**2.05 (1.07−3.94)**	**1.81 (1.02−3.21)**	1.68 (0.80−3.53)	1.28 (0.63−2.59)
Welders versus controls	27/30	**1.37 (1.09−1.72)**	1.39 (0.99−1.95)	**1.37 (1.03−1.83)**	1.10 (0.80−1.53)	1.31 (0.99−1.74)
Pathologists/anatomists versus controls	18/18	1.28 (0.86−1.90)	0.79 (0.46−1.36)	**1.65 (1.05−2.59)**	**3.29 (2.04−5.30)**	0.68 (0.38−1.20)

[a]Bolded values indicate statistical significance ($p < 0.05$).

Source: Iarmarcovai et al. (2006b).

workers showed significant increases in MN frequency (5.47 ± 0.76 versus 3.27 ± 0.69; $p = 0.003$), SCEs (6.13 ± 0.29 versus 4.49 ± 0.16; $p < 0.05$), and comet assay as determined by tail length (TL) (60.00 ± 2.31 versus 41.85 ± 1.97; $p < 0.05$). Smoking did not affect MN, TL and SCE. In addition, Costa et al. (2008) observed a positive correlation between formaldehyde exposure levels and MN frequency ($r = 0.384$; $p = 0.001$) and TL ($r = 0.333$; $p = 0.005$) (see Table 4-88). However, polymorphic genes of xenobiotic metabolizing and DNA repair enzymes did not show any significant effect on the genotoxic endpoints. This is the lowest level of exposure to formaldehyde in the studies observed so far, wherein a clear indication of genotoxic effects of formaldehyde was demonstrated.

Table 4-88. Genotoxicity measures in pathological anatomy laboratory workers and controls

	MN assay	SCEs	Comet assay
	Mean MN ± SEM (range)	**Mean SCE ± SEM (range)**	**Mean TL (μM) ± SEM (range)**
Controls ($n = 30$)	3.27 ± 0.69 (0−17)	4.49 ± 0.16 (3.10−3.06)	41.85 ± 1.97 (28.85−66.52)
Exposed ($n = 30$)	5.47 ± 0.76 (1−17)	6.13 ± 0.29 (3.64−8.80)	60.00 ± 2.31 (33.76−99.09)
p value	0.003	<0.05	<0.05

Source: Costa et al. (2008).

4.3.5. Summary of Genotoxicity

Formaldehyde's genotoxicity has been demonstrated in a variety of in vitro and in vivo test systems including bacteria (*S. typhimurium* and *E. coli*), molds (*N. crassa*), yeasts (*S. cerevesiae*), fruit flies (*Drosophila*), plants, several mammalian primary cells and cell lines, tumor cell lines, human lymphoblasts and lymphocytes, in vivo studies in rodents (different strains of mice, rats and hamsters), nonhuman primates (monkeys) and peripheral blood lymphocytes of occupationally exposed workers (industrial as well as professional) workers. Among these test systems several genotoxicity endpoints, such as DPXs (or DPCs), DNA adducts, DDXs, SSB, cytogenetic endpoints (MNs, SCEs, CAs), mutations (point, dominant lethal, deletion, heritable), *p*53 mutations and mutant *p*53 expression in rat nasal SCCs, other genetic endpoints such as UDS, DNA repair inhibition, etc), and clastogenic effects in human buccal, nasal and peripheral blood lymphocytes and chromosomal changes (monosomy and trisomy) in lymphocytes.

Formaldehyde forms predominantly DPXs that are detected in cell-free systems and single cells in vitro. DPXs are also formed in nasal epithelia but not in extranasal passages of rodents, which are completely removed within a day after formation. In vivo data in human and mammalian cells demonstrate that formaldehyde is genotoxic at the site of first contact, including cells of the mouth or the nose. DPXs are also detected in nasal and extranasal tissues of monkeys, suggestive of direct effects of formaldehyde in tissues that correspond to observed tumor sites (nasal and nasopharynx) in humans. In addition, this is used as a basis for cross-species comparison with humans. Formaldehyde-DNA adducts are labile and constitute a minor fraction of the DNA-reaction products and are less likely to play an important role in the genotoxicity of formaldehyde. There is limited literature on the formation of DDXs after formaldehyde exposure, but its role in genotoxicity is not clear.

Formaldehyde clastogenicity has been demonstrated by the induction of SCEs, SSBs, MNs, and CAs in cultured mammalian cells. Formaldehyde induces mutations in salmonella and escherichia bacterial strains that contain an AT base pair at the primary reversion site that is used to detect oxidative compounds and cross-linking agents without metabolic activation by exogenous enzyme-activating systems. Formaldehyde induces mutations in cultured mammalian cells at levels that do not cause significant toxicity.

Formaldehyde exposure causes differential induction of MNs in human nasal epithelial and buccal epithelial cells, which is significant in industrial exposure workers and students working in anatomy or mortuary science, respectively. However, recent data and data from larger studies support a finding of increased MNs in blood lymphocytes, although the issue remains controversial because of issues relating to study design, exposure regimen, and

confounding factors, including MN levels in nasal and buccal cells well below established background levels, reports limited by the number of cells observed, variation in standard techniques, and nonconcordance between buccal and nasal findings (Speit and Schmid, 2006). Several clastogenic effects, such as induction of MNs, SCEs, and CAs, were seen in human peripheral blood lymphocytes; also a recent study showed increased monosomy 7 and trisomy 8 in the lymphocytes of formaldehyde exposed workers. Formaldehyde exposure also caused *p*53 mutations in rat nasal carcinomas with the expression of mutant *p*53 protein.

Overall, induction of DPXs as a predominant lesion in vitro and in vivo, clastogenicity, and mutagenicity with locus-specific mutations in nonhuman and human cells supports the concept of genotoxic action of formaldehyde at the POE.

A summary of the genotoxicity of formaldehyde in humans is presented in Table 4-89.

4.4. SYNTHESIS AND MAJOR EVALUATION OF NONCARCINOGENIC EFFECTS

The adverse health effects due to formaldehyde exposure have been extensively studied in humans and in animal models. Studies of human exposure include occupational exposures, environmental exposures, and clinical studies of intentionally exposed subjects (see Section 4.1). Occupational exposures are primarily due to inhalation and dermal contact. Animal studies are available for a variety of routes of exposure, including inhalation, oral, dermal, and intravenous and I.P. injections (see Section 4.2). Additionally, as discussed in Chapter 3, in vitro studies address biological activity and the metabolic fate of formaldehyde.

Taken together, the human and animal studies support numerous health effects, not only at the POE as expected for a reactive gas but also on pulmonary function, neurobehavioral function, reproduction, development, immunomodulation, and sensitization (atopy, asthma). The discussion below provides a description of the adverse effects seen in each area, summarizing the data for both human and animal studies. MOA data are discussed where information regarding formaldehyde's biological activity may be linked to the observed adverse health effects.

4.4.1. Sensory Irritation

Sensory irritation of the eyes, nose, and throat is reported in humans upon direct contact with formaldehyde gas during inhalation exposures (Holmström and Wilhelmsson, 1988; Ritchie and Lehman, 1987) and includes irritation resulting from acute exposures (Lang et al., 2008; Yang et al., 2001; Krakowiak et al., 1998; Kulle, 1993; Green et al., 1989, 1987; Kulle et al., 1987; Sauder et al., 1987, 1986; Schachter et al., 1987, 1986; Witek et al., 1987; Day et al., 1984; Bender et al., 1983; Weber-Tschopp et al., 1977). Controlled exposures in inhalation chambers confirm the specificity of these responses to formaldehyde exposure and allow for assessment of these symptoms through both subjective and objective measures (Kulle, 1993;

Table 4-89. Summary of human cytogenetic studies

Study population	*N*	Exposure time (years)		Formaldehyde concentration (ppm)		Cytogenetic observations			Reference
		Range	Mean	Range	Mean (TWA)	CAs	SCEs	MNs	
Analyses of nasal and/or buccal cells									
Plywood workers Age and sex matched controls	15 15	2−19	6.8	0.32−0.83	(1)			+ nasal	Ballarin et al. (1992)
Male mortuary science students Female mortuary science students	22 7	Buccal and nasal swabs taken before and after first 9 weeks of embalming course		0.1−4.3	1.4			+ buccal − nasal − buccal − nasal	Suruda et al. (1993)
Mortuary science students[a]	28	Buccal and nasal swabs taken before and after first 9 weeks of embalming course		0.1−4.3	1.4			+buccal[b] − nasal	Titenko-Holland et al. (1996)
Female anatomy faculty Male anatomy faculty Controls (Females)	8 5 7	NA	23.6 25.6	NA	NA			+ buccal − buccal	Kitaeva et al. (1996)
Anatomy students	25	Buccal and nasal swabs taken before and after 8-week anatomy course		0.06−1.06	(0.508)			+ buccal + nasal	Ying et al. (1997)
Anatomy/pathology staff Controls (University staff)	28 18	1−13	4.70	2−4	NA			+ buccal	Burgaz et al. (2002)
Workers at a formaldehyde plant Controls	18 23	1−15	8.5		0.8			+ nasal	Ye et al. (2005)
Volunteers	21	10 days		13.5 ppm-hours				− buccal	Speit et al. (2007b)
Analyses of peripheral lymphocytes									
Manufacturing workers Age and sex matched controls	15 15	23−35	28		<5 1971 <1 later	−			Fleig et al. (1982)

Table 4-89. Summary of human cytogenetic studies (continued)

Study population	*N*	Exposure time (years)		Formaldehyde concentration (ppm)		Cytogenetic observations			Reference
		Range	Mean	Range	Mean (TWA)	CAs	SCEs	MNs	
Pathology workers Controls	6 5	4−11			0.9−5.8			−	Thomson et al. (1984)
Anatomy students[c]	8	10-week class		1.08−1.99[d] 0.08−0.6[e]	1.2 0.3		+		Yager et al. (1986)
Papermakers Controls	20 20	2−30	14.4	<3	NA	+[f]	−		Bauchinger and Schmid (1985)
Wood workers Controls	25 19	<5 to <16		0.45−8.6	NA	−			Vargová et al. (1992)
Male embalming students Female embalming students	22 7	Blood sampled before and after first 9 weeks of embalming course		0.15−4.3	1.4		− −	+ −	Suruda et al. (1993)
Manufacturing workers Anatomy faculty Controls	15 8 6		10 17	Up to 4 NA	NA	+ ND		+	Kitaeva et al. (1996)
Medial students Controls	30 30	Sampled near end of 15-month term		<1	NA -	−			Vasudeva and Anand (1996)
Anatomy students Controls (students)	13 10	12-week class			2.37[g]	+	+	+	He at al. (1998)
Physicians Technicians Controls (age matched/unexposed)	6 7 20	2-24 2-25	10 15	3.1-2.8	1.6		+ +		Shaham et al. (1997)
Anatomy students	23-25	Blood samples taken before and after 8-week anatomy course		0.06−1.06	(0.508)	-	−	−	Ying et al. (1999, 1997)

Table 4-89. Summary of human cytogenetic studies (continued)

Study population	*N*	Exposure time (years)		Formaldehyde concentration (ppm)		Cytogenetic observations			Reference
		Range	Mean	Range	Mean (TWA)	CAs	SCEs	MNs	
Female anatomy/pathology lab workers	10	1−16	8.9	1.2−15.1	NA			+	Sari-Minodier et al. (2001)
Controls (Women)	27								
Hospital pathology workers[h]	90	1−39	15.4	0.04−0.7[i]	0.4		+[j]		Shaham et al. (2002)
Controls	52			0.72-5.6	2.24		+		
Workers at a formaldehyde plant	18	1−15	8.5		0.8		+	−	Ye et al. (2005)
Controls	23								
Workers at two plywood factories	151	ND			0.08−6.42			+	Yu et al. (2005)
Controls	112								
Pathology or anatomy workers	59	ND		<0.1−20.4[k]	2[k]			+	Orsière et al. (2006)
Controls	37								
Pathologists	18	ND		0.4−7.0[k]	2.3[k]			+	Iarmarcovai et al. (2006a, b)
Controls	18								
Pathological anatomy lab workers	30	0.5−27	11	0.04−1.58	0.44		+	+	Costa et al. (2008)
Controls (21 females and 9 males)	30								
Plasticware workers	97	2 mo to 25 yrs		0.5-0.9 mg/m^3		+			Lazutka et al.,1999
Controls (nonexposed donors)	90								
Wood workers	40					+			Chebotarev et al. (1986)
Controls	22	NR		NR					
School children (1984)	20				0.26	+			Neri et al. (2006)
School children (1985)	16				0.11	+			
School children (1986)	18				0.03				
Controls (1984)	17				0				

Table 4-89. Summary of human cytogenetic studies (continued)

Study population	*N*	Exposure time (years)		Formaldehyde concentration (ppm)		Cytogenetic observations			Reference
		Range	Mean	Range	Mean (TWA)	CAs	SCEs	MNs	
Preschool controls (1984)	24				0				
Preschool children (1984)	13			0.17-0.3					
Phenolformaldehyde resin workers	31	0.33-30 yr			0.41	+			Suskov and Sazonova (1982)
Controls	74				0				

[a]Same population in Suruda et al. (1993) but different slides used. Nineteen complete slide sets for buccal analysis and 13 complete slide sets for nasal epithelial cell analysis.
[b]Not dose related; both low- and high-exposure groups had same SCE increase.
[c]Each student sampled before and after 10-week anatomy class.
[d]Breathing zone samples.
[e]Room air samples.
[f]Increase only in 11 supervisors. See text for details.
[g]Average breathing zone during dissection procedure.
[h]Exposed and controls from 14 hospitals.
[i]Low- and high-exposure groups established but numbers not provided.
[j]Not dose related; both low and high groups had same SCE increase.
[k]Described as "mean concentrations for sampling times of 15 minutes."
CAs = chromosomal aberrations; SCEs = sister chromatid exchanges; MNs = micronuclei; TWA = time-weighted average; ND = not determined; NA = not applicable.

Holness and Nethercott, 1989; Green et al., 1987; Kulle et al., 1987; Sauder et al., 1986; Weber-Tschopp et al., 1977). Eye irritation may be reported as itching, burning, and general discomfort. Tearing, redness of the eyes, and increased blink frequency are observed and may be quantified in exposure under controlled conditions (Lang et al., 2008; Yang et al., 2001; Andersen and Molhave, 1983; Weber-Tschopp et al., 1977; Schuck et al., 1966). Eye irritation appears to be the most sensitive endpoint in most individuals and may be observed after short exposures (195 minutes at 0.5 ppm: Lang et al. [2008]; 30 seconds at 1.65 ppm: Yang et al. [2001]).

Itching, burning, and discomfort of the nose, which may be accompanied by increased mucous production (runny nose), are reported by individuals exposed via inhalation (Krakowiak et al., 1998; Kulle, 1993; Green et al., 1987; Kulle et al., 1987; Weber-Tschopp et al., 1977). Throat irritation may also be described subjectively as itching and burning and is often accompanied by a cough (Krakowiak et al., 1998). Symptoms of eye and mucous membrane irritation are also reported in numerous rodent studies and support the health effects reported in humans (see Section 4.1.1.1). Although dermal contact may result in dermatitis and an apparent hypersensitivity reaction, symptoms do not present upon contact as sensory irritation. There are no human or animal data that assess sensory irritation from oral exposures.

The time to onset of sensory irritation symptoms and severity of the sensory irritation are a function of both the air concentration and duration of exposure. Additionally, nose and throat irritation becomes more prominent at higher exposures and longer duration of exposure (Kulle, 1993; Kulle et al., 1987). Controlled human laboratory exposures (Yang et al., 2001; Kulle, 1993; Kulle et al., 1987; Cain et al., 1986; Andersen and Molhave, 1983) provide more direct exposure-response evidence for sensory irritation. These studies are limited to healthy nonsmoking individuals. Two studies (Cain et al., 1986; Andersen and Molhave, 1983) document discomfort and irritation of the eye in response to acute exposures as low as 0.25 ppm. Dose-response relationships are reported in a number of different ways: as an incidence of the reported symptom among subjects, as a score for severity of the symptom, or in some cases as a subjective measure, such as blink frequency for eye irritation.

Symptoms of sensory irritation, including eye irritation (burning watering, increased blinking), nasal irritation (rhinitis, itching/burning), throat/respiratory tract irritation (wheezing, coughing, phlegm production), have been reported in numerous worker cohorts. Occupational exposure environments include hospital and medical settings, students, and industrial workers (Takahashi et al., 2007; Takigawa et al., 2005; Krakowiak et al., 1998; Akbar-Khanzadeh et al., 1994; Uba et al., 1989; Horvath et al., 1988; Schachter et al., 1987). Formaldehyde levels often vary in a work environment and peak as well as average exposures may be used to report occupational exposures. Although sensitive individuals often remove themselves from an

irritating workplace (the healthy worker effect), eye, nose, and throat symptoms are still reported in this environment. Among workers in a plant where formaldehyde resins were used, those exposed to an average of 210 ppb formaldehyde (range of 40-400 ppb) reported increased symptoms, including nasal and eye irritation, above those in the control population (Holmström and Wilhelmsson, 1988).

These effects have been noted in students, particularly medical students, who are exposed to formaldehyde in cadaver labs. In a study of 24 formaldehyde-exposed anatomy students (personal breathing zone samples 0.73 ppm, range 0.49−0.93) (Kriebel et al., 1993), eye, nose, and throat irritation was present when comparing rates of irritation from the end or middle of class to before the start of class. Takahashi et al. (2007) showed that 143 medical students reported various symptoms (including eye and throat irritation) and that the percentage of students reporting symptoms increased between the beginning (measured after the first day of class) and the end of the course (2 months later). After the first day of class, approximately 35% of students reported eye soreness and about 15% reported throat irritation.

Sensory irritation has also been reported in occupational settings, including particleboard manufacturing, woodworking, and embalming, with average formaldehyde concentrations of 300-400 ppb. Symptoms of irritation were observed at a frequency of 40-65% among exposed workers compared to 6-25% among unexposed comparison groups (Horvath et al., 1988; Alexandersson and Hedenstierna, 1988; Holmström and Wilhelmsson, 1988; Holness and Nethercott, 1989).

Eye, nose and throat irritation also were observed in association with residential exposure to formaldehyde. Studies with formaldehyde measurements and analyses that adjusted for potential confounders (e.g., age, gender and smoking status) observed a clear exposure-response relationship for average formaldehyde concentrations or cumulative exposure (Ritchie and Lehnen, 1987; Hanrahan et al., 1984; Liu et al., 1991).

Ritchie and Lehnen (1987) surveyed residents in 2,000 homes classified as having formaldehyde concentration <0.1 ppm, 0.1−0.3 ppm, and >0.3 ppm. Increases in the prevalence of eye irritation of 1-2%, 12-32%, and 86-93%, respectively were observed with increasing categories of formaldehyde exposure. Liu et al. (1991) report irritant effects associated with formaldehyde exposure in mobile homes, where formaldehyde concentrations ranged from the 0.01 ppm detection limit to 0.46 ppm. Eye irritation (60%), nose/throat irritation (10−20%), or headache (<10%) were reported in residents.

MOA

The mucosae of the URT, oral cavity pharynx, and upper airways are complex tissues, where epithelial and goblet cells predominate. In addition, the nasal mucosa is highly enervated. The main nerves include the trigeminal nerve and olfactory sensory cells (olfactory epithelium, the vomeronasal organ, and the organ of Masera) (Feron et al., 2001). A possible MOA for sensory irritation includes formaldehyde-induced stimulation of the trigeminal nerve (though whether formaldehyde acts as a direct agonist is unknown). Trigeminal nerve stimulation in the nasal passages transmits signals to the CNS, which then sends efferent signals back to the nasal tissues, causing sensory irritation, and possibly systemically via vagal nerve stimulation, resulting in more systemic effects.

Animal studies are potentially useful models for understanding mechanisms of toxicity, especially where sufficient human data do not exist. While experimental animal studies provide a model of secondary effects, rodents also demonstrate RB, an effect not seen in humans. Thus, species that exhibit bradypnea (like mice and rats) may not be appropriate for assessing respiratory endpoints. The mechanism underlying RB includes formaldehyde binding to the sensory nerve endings of the trigeminal nerve, where signals travel to the CNS. The vagus nerve transmits the efferent signal to produce smooth muscle contraction. The animals become inactive, their core temperatures decrease by several degrees C, and their respiratory rates and minute volumes decrease. However, this is not to say that trigeminal nerve stimulation is not an appropriate potential mechanism of action in other species or in humans. Since trigeminal nerve stimulation has been independently confirmed in species without RB, this mechanism may be a viable explanation for the observed effects.

4.4.2. Pulmonary Function

Several adequately conducted and reported studies evaluated chronic effects of occupational (Malaka and Kodama, 1990; Herbert et al., 1994; Horvath et al., 1988; Holness and Nethercott, 1989; Alexandersson et al., 1982; Alexandersson and Hedenstierna, 1989) or residential exposure to formaldehyde (Krzyzanowski et al., 1990; Franklin et al., 2000). Small decreases in preshift lung function relative to unexposed groups were reported in several different occupational settings including plywood, oriented strand board and carpentry (Malaka and Kodama, 1990; Herbert et al., 1994; Alexandersson et al., 1982; Alexandersson and Hedenstierna, 1989). The studies included appropriate comparison groups, formaldehyde measurements, detailed reporting of methods, and adjustment for potential confounding variables, including age and smoking status (in some studies weight and ethnicity). Lung function values were presented as percent of expected based on age, height, and sex or adjusted

for these variables in the analysis. Studies of long-term exposure also reported increased respiratory symptoms such as cough, increased phlegm, asthma, chest tightness and chest colds in exposed workers (Malaka and Kodama, 1990; Herbert et al., 1994; Pourmahabadian et al., 2006, Alexandersson et al., 1982; Alexandersson and Hedentierna 1989).

Other studies evaluated pulmonary function among occupational groups with exposure to formaldehyde but the results of these studies are less informative because spirometric values were not adjusted for age, height or gender, statistical analyses were not reported, or the studies suffered from small sample size, methodological, analytical or reporting deficiencies (Khamgaonkar and Fulare, 1991; Pourmahadadian et al. (2006); Kilburn et al., 1985; Main and Hogan, 1983; Ostojic et al., 2006; Holmström and Wilhelmsson, 1988; Nunn et al., 1990). Also, occupationally exposed groups compared to nonoccupational referent groups may have exhibited a healthy worker effect masking any formaldehyde related lung function decrements (Holness and Nethercott, 1989).

A longitudinal study by Alexandersson and Hedenstierna (1989) documented an association of lung function decrements between 1980 and 1984 with time-weighted average occupational formaldehyde concentrations of 0.42−0.5 mg/m^3 (340−400 ppb). Statistically significant annual decreases in FEV_1/FVC and $FEF_{25–75\%}$ were noted over 5 years in nonsmokers after correction for normal aging and reference lung function spirometry values. The decrease in $FEF_{25–75\%}$ was 0.212 ± 0.066 L/second (mean ± SD) for each year of exposure and was significant ($p < 0.01$). The decrease in FEV1/FVC was 0.4 ± 0.2 ($p < 0.01$). A cross-sectional study with a participation rate of 93% among carpentry workers observed statistically significant decrements in FEV_1, FEV_1/FVC, and $FEF_{25–75\%}$ associated with an 8-hour time-weighted average formaldehyde exposure of 1.13 ppm (Malaka and Kodama, 1990). In multiple regression models adjusting for age, height, weight, cigarettes/day, and dust, formaldehyde as a continuous variable was a significant predictor for FEV_1, FEV_1/FVC, and $FEF_{25–75\%}$. Each unit increase in formaldehyde (ppm-years) was associated with a decrease of 0.015 liters, 0.347%, and 0.043 l/s in FEV_1, FEV_1/FVC, and $FEF_{25–75\%}$, respectively. The strongest response was for $FEF_{25–75\%,}$ which showed a 12% drop in observed function compared with expected function in the unexposed.

Kryzanowski et al. (1990) described a well-designed and executed cross-sectional study of residential formaldehyde exposure in a large, representative sample that provided clear evidence of a linear relationship between increased formaldehyde exposure and decreased peak expiratory flow rate (PEFR) among children. A statistically significant linear relationship between increased household mean formaldehyde exposure and decreased PEFR was reported in children (ß = -1.28 ± 0.46 L/minute per ppb formaldehyde). The average formaldehyde

concentration was 26 ppb, with a maximum sample value of 140 ppb. Decrements in PEFR associated with increasing formaldehyde concentrations also were observed among adults beginning at an average concentration of 40 ppb. While Franklin et al. (2000) did not observe an association between a one-time measurement of FVC or FEV_1 among children aged 6−13 years and indoor concentrations of formaldehyde, levels of exhaled nitric oxide (NO) were higher in children exposed to average formaldehyde levels ≥0.05 ppm compared to < 0.05 ppm. These findings indicate that formaldehyde may increase lower airway inflammation at concentrations associated with effects on pulmonary function.

The pulmonary function measures associated with formaldehyde exposure are consistent with bronchial constriction, inflammation, or chronic obstructive lung disease. Decreases in spirometric values, including vital capacity (VC), forced expiratory volume (FEV), forced vital capacity (FVC) and FEV/FVC have been documented. Decreases in lung volume (FEV_1, FVC) indicate possible pulmonary obstruction (narrowing of the airways during exhalation) (Pellegrino et al., 2005). Early changes in small airways are observed as reductions in expiratory flow in the terminal portion of the spirogram (PEF, $FEF_{75\%}$, $MEF_{25\%-75\%}$). These changes may be observed even if FEV_1 is not affected.

Lung function deficits have been reported in pre- versus postshift measurements among occupational groups (Herbert et al., 1994; Horvath et al., 1988). Students have also shown decrements in lung function that are associated with exposure to formaldehyde in laboratories Kriebel et al., 1993; Kriebel et al., 2001. Kriebel and colleagues (1993) observed a 2% cross-lab decrement in PEF among anatomy students during the first two weeks of a 3-hour laboratory that they attended once per week . The cross-lab pulmonary response was attenuated over the 10-week duration of the course (formaldehyde geometric average concentration of 0.73 ppm). While the acute effects of formaldehyde exposure appeared to diminish after several weeks of exposure, the effect on prelaboratory PEF across 10 weeks was a 2.7 ± 1.1 L/minute per week drop that was statistically significant ($p < 0.01$) in a model adjusting for random person effects, asthma, interaction between time and asthma, and eye as well as nose symptoms of irritation. Prevalence of eye and nose symptoms was associated with decreased PEF ($p < 0.02$).

Overall, acute formaldehyde exposures (0.5−3 ppm) have not induced significant pulmonary deficits in healthy, nonexercising volunteers in controlled human exposure studies (Kulle et al., 1987; Schachter et al., 1986; Schachter et al., 1987; Witek et al., 1986; Day et al., 1984; Andersen and Molhave, 1983). However, it is unclear whether the data analysis in these reports had the statistical power to substantiate the small deficits reported in occupational and student studies. The studies exposed small numbers of diverse individuals, often including males and females of varying age, and some included current smokers. Some studies report the

absolute values of the lung function parameters without adjustment to individual expected function or the unexposed baseline for each individual (Kulle et al., 1987; Andersen and Molhave, 1983; Day et al., 1984). In other studies that reported lung function values as a percent of baseline, the variation of the mean change in lung function parameters is quite large, nearly equaling the reported value and exceeding it in several cases (Witek et al., 1986; Schachter et al., 1986; 1987). The absence of normalized raw data, combined with large individual variation, limit the interpretation of these studies.

Small but statistically significant deficits in pulmonary function (e.g., decreased FEV_1, FVC_1, FEV_3, specific airways conductance) due to acute formaldehyde exposure (2 or 3 ppm) have been reported in healthy volunteers in controlled human exposure studies using exercise (Green et al., 1987, 1989; Sauder et al., 1986;). Although changes in lung function parameters averaged over experimental groups were generally small, some individuals exhibited clinically significant deficits, even after only 2 hours of exposure (Green et al., 1987). This differential response suggests susceptibility in certain subjects (Green et al., 1987). Other studies that included an exercise component did not report a difference in response among healthy volunteers (Schachter et al., 1986; Kulle et al., 1987). Acute controlled studies that evaluated responses among asthmatics reported no changes in pulmonary function associated with formaldehyde exposure (Sheppard et al., 1984, Ezratty et al., 2007; Harving et al., 1990; Green et al., 1987, 1989; Sauder et al., 1987; Witek et al., 1987, 1986; Krakowiak et al., 1998). These findings suggest that a brief exposure to formaldehyde may not trigger a response in the airways of asthmatic individuals in the absence of allergen. However, the large variation in pulmonary response among the individuals (healthy and asthmatic) that participated in the experimental exposure studies suggests that some individuals may be more sensitive to formaldehyde.

Several animal studies document increased airway resistance and bronchial constriction following inhalation exposure to formaldehyde (Nielson et al., 1999; Swiecichowski et al., 1993; Biagini et al., 1989; Amdur et al., 1960). A study using cynomolgus monkeys (Biagini et al., 1989) demonstrated that methacholine-induced bronchial constriction can be similarly induced by acute formaldehyde exposure (10 minutes at 2.5 ppm). Thus, formaldehyde exposure simulated bronchial constriction observed after methacholine challenge, but these effects may not occur by a similar MOA. Similar results were reported in guinea pigs (Swiecichowski et al., 1993; Amdur et al., 1960), rats (Ohtsuka et al., 1997), and mice (Nielson et al., 1999).

Taken as a whole, studies of occupational exposure to formaldehyde, as well as residential exposure to low indoor formaldehyde concentrations support an association with deficits in pulmonary function among adults and children. Respiratory symptoms also were reported at the same exposure levels. A longitudinal study that documented a progressive

decrease in pulmonary function among nonsmokers over 5 years (Alexandersson and Hedenstierna, 1989) supports the conclusion that formaldehyde exerts a progressive chronic effect on pulmonary function. Formaldehyde exposures used in controlled human chamber studies have not caused functional deficits among healthy, nonexercising volunteers or among asthmatics, although wide variability in responses suggests that some individuals may be sensitive responders. A few studies that incorporated exercise reported small decreases in pulmonary function related to formaldehyde exposure. These effects have been corroborated in animal studies exposed to formaldehyde.

MOA

Formaldehyde-induced inflammation of the airways may contribute to observed decreases in measures of pulmonary function. Even short-term inflammatory reactions could reduce the effective diameter of the conductive airways, resulting in lower respiratory volumes in a number of functional tests. Formaldehyde-induced trigeminal nerve stimulation contributes to airway inflammation, which in turn would reduce airway function. Chronic exposures may result in increased sensitization or chronic inflammatory responses, which could contribute to the effects seen in the worker and residential populations.

Formaldehyde-induced pulmonary function deficits may also be in part a result of smooth muscle contraction in repose to trigeminal nerve stimulation. Trigeminal nerve stimulation transmits signals to the CNS. The resulting efferent signal from the vagal nerve produces smooth muscle contraction and may result in decreased pulmonary function. Efferent signaling has also resulted in release of substance P and other neuromodulatory compounds, which may contribute to BC and sensitization of pulmonary responses (asthma, atopy).

4.4.3. Hypersensitivity and Atopic Reactions

A large number of studies have investigated the potential association between formaldehyde exposure and a continuum of adverse health effects ranging from decrements in pulmonary function to asthma. In general, epidemiologic studies of adults have reported varied results between null findings and positive findings but have not consistently distinguished between studies in which formaldehyde may be causing an increase in the incidence of asthma (e.g., phenotypic switching), increasing the prevalence of asthma, initiating an asthma attack or worsening the severity of an attack. Formaldehyde may itself be an allergen or it may potentiate the ability of other allergens to cause phenotypic switching or increase the sensitivity of atopic individuals. Thus formaldehyde exposure among nonatopic individuals could theoretically cause phenotypic switching in the presence or absence of allergens possibly resulting in a diagnosis of

asthma. Formaldehyde could also cause an asthma attack or potentiate the influence of other stimuli on the risk of asthma attacks.

Asthma is a specific manifestation of IgE-mediated hypersensitivity, characterized by BHR and airway inflammation, resulting in lower airway obstruction (Fireman, 2003; Kuby, 1991). A variety of hypersensitivity reactions have been reported following exposure to formaldehyde. Rashes and skin reactions have been reported in some individuals after dermal exposures to formaldehyde. Increased expression of Th-2 cytokines in the lymph nodes of mice given dermal applications of formaldehyde does indicate the involvement of an immune component to the observed sensitization (Dearman et al., 2005; Hilton et al., 1998; Arts et al., 1997). However, the response does not appear to be IgE mediated (Arts et al., 1997; Lee et al., 1984). Gorski et al. (1992) observed an increase in formaldehyde-mediated neutrophil burst in dermatitis patients exposed in a controlled chamber study and suggests a putative role of oxidative stress and reactive oxygen species (ROS). Some case reports of bronchial asthma in occupational settings suggest direct respiratory tract sensitization to formaldehyde gas (Lemiere et al., 1995; Burge et al., 1985; Hendrick et al., 1982; Hendrick and Lane, 1977, 1975; Stenton and Hendrick, 1994, Nordman et al., 1985). However, the development of an allergic asthmatic response to formaldehyde appears to be a rare occurrence.

The few studies that reported an association of formaldehyde exposure with a new diagnosis of asthma are consistent with the notion that formaldehyde may be an allergen or that formaldehyde exposure may result in sensitization to common allergens among nonatopic individuals, referred to as phenotypic switching (Rumchev et al., 2001; Smedje and Norback, 2001). Rumchev et al. (2002) reported that residential formaldehyde exposure was associated with an increased risk of incident asthma in a population-based case-control study of 192 children aged 6 months to 3 years. The asthma diagnosis was based on a recent discharge from the emergency department of a children's hospital in Perth, Australia, with a primary diagnosis of asthma. The assessment of formaldehyde exposure was based on in-home measurements (8-hour passive sampler) in two seasons and associations with asthma were adjusted for several potential confounding factors including, other indoor air pollutants, house dust mite concentrations, humidity and temperature, family history of asthma and allergy, smoking, and other risk factors and demographic variables. Multiple regression models indicated a 3% increased risk with each 10 $\mu g/m^3$ increase in formaldehyde concentration in the home (OR reported per unit formaldehyde increase 1.003 [95% CI:1.002-1.003]). When formaldehyde was categorized into four exposure groups, odds ratios were increased at 50-50 $\mu g/m^3$ and above 60 $\mu g/m^3$ compared to 10-29 and 30-49 $\mu g/m^3$. A 39% increased risk was observed at the highest exposure category ($p < 0.05$). Asthma incidence also was evaluated in a four year follow-up of

1732 students, aged 7-13 years, from 39 schools in Sweden who completed a mailed questionnaire in 1993. Among the 1347 students who responded in 1997 (78%), 56 (4.5) were diagnosed with asthma by a physician. While asthma incidence was not associated with formaldehyde exposure in the entire cohort (OR 1.2 [95% CI: 0.8-1.7]), among 22 students with no history of atopy at baseline, a higher concentration of formaldehyde was associated with an odds ratio for incident asthma of 1.7 (95% CI: 1.1-2.6). Mold concentration also was associated with incident asthma. The authors did not discuss the correlation of formaldehyde and mold concentrations and do not appear to have adjusted for mold concentrations in the formaldehyde analyses. However, when the units for both mold and formaldehyde are standardized to the geometric mean standard deviation, the magnitude of the formaldehyde effect is larger. Therefore, it is unlikely that the association with formaldehyde was the result of uncontrolled confounding by mold concentrations.

Other studies of asthma prevalence have observed an association with indoor formaldehyde concentrations among children exposed at home (Krzyzanowski et al., 1990; Garrett et al., 1999) and at school (Zhao et al., 2008). A large, representative study of 202 households (mean formaldehyde level of 26 ppb) found that among children aged 6−15 years old and exposed to environmental tobacco smoke, the prevalence of physician-diagnosed asthma was 45.5% for those with measured levels of formaldehyde in the kitchen >60 ppb (N = 11). The prevalence of asthma dropped to 0% for levels 41−60 ppb (N = 12) and 15.1% for levels ≤40 ppb (N = 106) (chi-squared trend test $p < 0.05$). No trend in asthma prevalence was seen for children who were not exposed to environmental tobacco smoke (Krzyzanowski et al., 1990). Krzyzanowski et al. also reported a statistically significant linear relationship between increased household mean formaldehyde exposure and decreased peak expiratory flow rates among children. PEFR is a diagnostic tool used to identify new asthma cases. Two studies of self-reported asthma reported null results (Gee et al., 2005; Tavernier et al., 2006; Palczynski et al., 1999). However, Tavernier et al. did not report the range of formaldehyde concentrations evaluated so the variation of the concentration of formaldehyde is not known. Among the 187 children aged 15 years or less studied by Palczynski et al., only 9 were defined as having asthma.

Ambient formaldehyde concentrations outdoors on school grounds also were associated with cumulative asthma in a cross-sectional study of students at 10 schools in China (Zhao et al., 2008). Ambient formaldehyde concentrations of 5.8 $\mu g/m^3$ were associated with an odds ratio for cumulative asthma of 4.6 (95% CI:1.1-19.5). The odds ratio was adjusted for age, gender, parental asthma or allergy and home factors including environmental tobacco smoke. Another study that evaluated ambient formaldehyde exposure reported an increase in the severity of asthma symptoms in a panel of 22 Hispanic children, 10-16 years old, with a minimum one year

history of doctor diagnosed asthma (Delfino et al., 2003). A 24-hour average formaldehyde concentration at a central site monitor of 7.21 ppb (range 4.27-14.02 ppb) was associated with an increase in asthma symptom scores on the next day (OR 1.37 (95% CI:1.04-1.89).

A recent meta-analysis of formaldehyde exposure supports an association with asthma in children (McGwin et al., 2010). Of the seven studies that were included in the meta-analysis, six reported increased risks of asthma associated with exposure to formaldehyde. The results of the random-effect model results showed an overall effect estimate of OR = 1.17 (95% CI: 1.01-1.036). The three studies with the highest statistical weights based on the inverse of the variance of the study ORs were for the studies by Rumchev et al. (2002), Garrett et al. (1999) and Krzyzanowski et al. (1990).

A cross-sectional study of residential exposure to formaldehyde among 88 adults from Sweden reported an increase in reports of nocturnal breathlessness in association with mean bedroom formaldehyde concentrations of 29 $\mu g/m^3$ compared to 17 $\mu g/m^3$ (Norback et al., 1995). The adjusted odds ratio was 12.5 (95% CI:2.0-77.9) per a 10-fold increase in the indoor concentration, higher in magnitude than the odds ratios for toluene, terpenes and volatile organic compounds. This finding is supported by the observation by Krzyzanowski et al. (1990) of decreased morning peak expiratory flow rate among adult smokers exposed to >40 ppb formaldehyde in their homes. Another study of pregnant women found no association with asthma prevalence for personal exposure to ≥ 47 ppb compared to < 18 ppb (Matsunaga et al., 2008). However, the number of women with a diagnosis of asthma was small (N = 21) and the confidence limits were wide (OR 2.65 [95% CI: 0.63-11.11]).

The epidemiologic literature also indicates that formaldehyde may increase the prevalence of allergic sensitization to common allergens, as well as the severity of responses (Garrett et al., 1999; Delfino et al., 2003; Cassett et al., 2006).

Garrett et al. (1999) reported that the prevalence and severity of allergic sensitization to 12 common allergens was increased in relation to formaldehyde levels in the homes of 148 children, aged 7-14 years, living in Victoria, Australia based on the highest of four seasonal 4-day formaldehyde measurements in the home ($p < 0.001$). The proportion of atopic children between groups of the highest recorded formaldehyde level was 0.33, 0.64, and 0.75 for <20 $\mu g/m^3$, 20-50 $\mu g/m^3$, and >50 $\mu g/m^3$, respectively (test for trend, $p < 0.001$). The differences in prevalence were statistically significant ($p = 0.001$). In logistic regressions, the crude association for atopy with an increase in bedroom formaldehyde concentration per 10 $\mu g/m^3$ was OR = 1.34 which increased when adjusted for parental asthma and gender to an odds ratio of 1.40 per 10 $\mu g/m^3$ (95% CI: 0.98-2.00). Thus, parental asthma was not a confounder of the association between formaldehyde and prevalence of atopy. The adjusted odds ratio for atopy with an

increase in highest recorded formaldehyde per 20 μg/m^3 was 1.42 (95% CI: 0.99−2.04). Passive smoking, the presence of pets, indoor nitrogen dioxide concentrations, airborne fungal spores and house-dust-mite allergens also did not influence the effect estimates and were unlikely to be confounders. The authors reported that mean respiratory symptom scores adjusted for parental asthma and parental allergy increased with increasing formaldehyde exposure categories (<20 μg/m^3, 20-50 μg/m^3, >50 μg/m^3, $p < 0.05$). Delfino et al. (2003) also reported an association of the severity of respiratory symptoms with ambient formaldehyde concentration on the previous day among asthmatic children. The association between formaldehyde concentrations and severity of allergic sensitization was analyzed using two measures, number of positive skin prick tests and the ratio of wheal diameters after skin pricks with allergens compared to the histamine wheal size. Average levels of both measures of severity were higher in the two higher formaldehyde groups compared to the lowest group ($p < 0.05$). Further, both measures were linearly related to increasing formaldehyde categories in regression models controlling for parental asthma and allergy and sex. The role of formaldehyde in exacerbating allergic responses to common allergens among atopic individuals was demonstrated in a controlled human exposure study by Cassett et al. (2006) using formaldehyde concentrations of 100 μg/m^3 and a 30 minute cross-over exposure protocol. Another study using different allergens, dosimeters, and study protocol did not report an effect of formaldehyde on allergic responses (Ezratty et al., 2007).

Exacerbation of response after formaldehyde exposure has been demonstrated in animal studies as well. Sadakane et al. (2002) demonstrated that formaldehyde exposure exacerbated sensitization and challenge with Der f and suggested that formaldehyde exposure may aggravate eosinophilic infiltration and goblet cell proliferation that accompanies allergic responses. Several animal studies report increased airway resistance and BC due to inhalation exposures to formaldehyde (Nielsen et al., 1999; Swiecichowski et al., 1993; Biagini et al., 1989; Amdur, 1960). Changes in pulmonary resistance were observed as early as 10 minutes after exposure (Biagini et al., 1989), and reported effect levels ranged from 0.3 to 13 ppm. BHR is commonly associated with allergic Type I hypersensitivity reactions but is not sufficient to demonstrate that an agent induces Type 1 hypersensitivity.

In conclusion, the epidemiologic studies of formaldehyde exposure among children support the finding that low indoor and outdoor concentrations result in increased asthma incidence and prevalence. Formaldehyde exposure is associated with increased prevalence of allergic sensitization to common allergens and with increased severity of asthmatic symptoms and the allergic response to allergen challenge. The studies of formaldehyde exposure among

adults is sparse but suggest that exposure increases the prevalence of respiratory symptoms. These effects are supported by findings of experimental animal studies.

MOA

The MOA underlying this response has not been elucidated. Formaldehyde-induced IgE production has been reported in some studies (Vandenplas et al., 2004; Wantke et al., 1996a). Other studies suggest that this effect does not appear to be immunogenic in nature (Fujimaki et al., 2004; Lee et al., 1984). Although formaldehyde exposure has been reported to alter cytokine levels and immunoglobulins in some experimental systems (Fujimaki et al., 2004a; Ohtsuka et al., 2003), these immunomodulatory effects do not support immunogenically mediated type 1 hypersensitivity.

These decrements may be mediated via neurogenic potentiation (Sadakane et al., 2002; Riedel et al., 1996; Tarkowski and Gorski, 1995). Tarkowski and Gorski (1995) suggest that formaldehyde may increase permeability of respiratory epithelium and destruction of immunologic barriers. Tachykinin NK1 receptor and various neuropeptides (NGF and substance P) have been implicated in formaldehyde-induced sensitization and lend weight of evidence to a neurogenic MOA (Van Schoor et al., 2000; Ito et al. 1996).

4.4.4. Upper Respiratory Tract Histopathology

Several studies in occupational workers have reported increased squamous cell metaplasia and reduced mucociliary clearance in nasal and buccal swabs from humans occupationally exposed to formaldehyde (Holmström et al., 1989; Holmström and Wilhelmsson, 1988). Evidence of genotoxic effects include increased MNs and CAs in nasal and buccal epithelial cells from both workers and students exposed to formaldehyde (Ying et al., 1997; Titenko-Holland et al., 1996; Suruda et al., 1993) and suggest a potential association between genotoxicity and altered histopathology.

Numerous animal experimental studies in multiple strains of rats, mice, hamsters, rabbits, and monkeys describe formaldehyde-induced URT pathology (Fló-Neyret et al., 2001; Roemer et al., 1993; Reuzel et al., 1990; Monticello et al., 1989; Zwart et al., 1988; Wilmer et al., 1987; Morgan et al., 1986b, 1983; Swenberg et al., 1986; Buckley et al., 1984). Effects are first observed in the anterior respiratory mucosa and progress through the nasal passages with increasing exposure concentration and time. The first observed effect includes damage to the mucociliary apparatus of the nasal passages in response to formaldehyde. Studies conducted both in vivo and in vitro demonstrate that formaldehyde disrupts mucus flow and ciliary beat that are dependent on concentration and duration of exposure. Mucociliary apparatus deficits have

been recorded even after 18 hours of recovery following formaldehyde exposure. The breakdown of the mucociliary apparatus may allow for increased infection and allow the underlying epithelium to come into contact with exogenous chemicals.

Formaldehyde is highly reactive and may impact all cells in the nasal mucosa, including epithelial cells (ciliated, columnar, and cuboidal), goblet cells, sensory neurons, and intraepithelial lymphocytes. The histologic changes of these processes have been described in all laboratory animals examined and progress from the anterior nares to the posterior regions of the nasal passages, including the ETs and olfactory epithelium if the concentration and duration of exposure are great enough.

Humans and nonhuman primates have significantly less complex nasal passages than rodents. Formaldehyde has lower peak flux in human nasal tissues compared with rodents, which are obligate nose breathers, but will penetrate more deeply into the human respiratory tract than in rodents, since humans lack the autonomic RA response. Additionally, humans may switch to mouth breathing in the presence of an irritant gas, thus bypassing the sensitive nasal passages and increasing the tissue dose in the mouth and throat. These differences have been demonstrated by using nonhuman primates where, at comparable concentrations, tissue pathology and increased cell proliferation progressed further into the respiratory tract than in rodents (Monticello et al., 1989). Nonhuman primates share common structural respiratory components and patterns of breathing and do not have a reflex autonomic apnea response.

Despite the anatomical and physiological differences in breathing patterns and different exposure parameters between humans and rodents, similar toxic effects are reported in tissues at the POE in humans and laboratory animals. Several occupational studies have reported increased squamous cell metaplasia in nasal and buccal samples in response to formaldehyde exposure (Ballarin et al., 1992; Boysen et al., 1990; Holmström et al., 1989), paralleling the histologic effects seen in experimental animal studies. A few human epidemiology studies suggest increased NPCs (see Section 4.5) as well as oral/buccal tumors in response to formaldehyde exposure (Shangina et al., 2006; Laforest et al., 2000).

The observed formaldehyde-induced URT toxicity is related to its high reactivity and solubility. Moreover, additional interspecies differences in the surface area and configuration of the nasal passages and upper airways will influence the areas of high formaldehyde flux in POE tissues.

MOA

Formaldehyde-induced damage to the mucociliary apparatus of the nasal passages may occur because formaldehyde may disrupt mucus flow and ciliary beat that is dependent on

concentration and duration of exposure. Formaldehyde reacts with the mucosal glycoproteins and thus may contribute directly to the breakdown of the mucus layer. As formaldehyde reaches the cells of the pseudostratified epithelium in the nasal passages, it exerts a range of effects from direct damage to cell membrane, intracellular proteins, and DNA to alterations in GSH pools and increased ROS. Adaptive effects include increased mucus flow and goblet cell proliferation as well as the transition of respiratory epithelium to more insensitive cuboidal cells. With continued exposure at sufficient concentration, squamous metaplasia develops, creating a protective layer of keratinized cells. Gradually, this damage exceeds the cell's ability to compensate for and repair damage; chronic nasal lesions develop, and the cells die both through general necrosis as well as programmed cell death, depending on the severity of the cellular damage (Monticello et al., 1989; Swenberg et al., 1983).

Genotoxic effects have been reported in nasal and buccal lesions taken from both workers and students exposed to formaldehyde (Ying et al., 1997; Titenko-Holland et al., 1996; Suruda et al., 1993). MN formation occurs in the more sensitive pseudostratified epithelium of the nasal passages, nasopharynx, and upper airways, since there is only one layer of epithelial cells that are constantly regenerating. However, the genotoxicity observed in buccal cells is more difficult to explain, since buccal basal cells are usually covered by protective keratinized cell layers. Cuts, sores, or other buccal lesions would increase basal epithelial cells' vulnerability to direct exposure to formaldehyde.

4.4.5. Toxicogenomic and Molecular Data that may Inform MOAs

Over the past several years, studies have begun to examine the effects of formaldehyde exposure on gene and protein expression. These include studies on in vivo and in vitro changes in the global expression of mRNA (transcriptomics) and proteins (proteomics) in the tissues and cells of humans and rodents exposed to formaldehyde. Currently, nine "-omics" studies from five research groups are available. These studies are summarized in Section 5.2 and are evaluated and discussed in the context of their relevance to informing MOAs and the dose-response characterization briefly here.

In 2002, EPA released the *Interim Policy on Genomics* (U.S. EPA, 2002c), which addresses how to use genomic data in regulatory decision making. Although the policy encourages research in genomics, it places limits on its use, stating that genomic data alone are not sufficient as a basis for decision making. These data thus cannot currently be utilized as the critical effect in a chemical risk assessment but can be utilized in a weight-of-evidence approach on a case-by-case basis. The Science Policy Council developed a white paper entitled *Potential Implications of Genomics for Regulatory and Risk Assessment Applications at EPA* (U.S. EPA,

2004). This report described three areas where genomic data might be applied in risk assessment at EPA: MOA analysis, susceptible population, and mixtures assessments. The genomic data on formaldehyde thus may be applied to a discussion of MOA.

Toxicogenomics studies have investigated the gene and protein expression changes resulting from formaldehyde exposure in a variety of respiratory tissues, including nasal tissue (Andersen et al., 2008; Thomas et al., 2007; Hester et al., 2005, 2003), and, in lung tissue (Lee et al., 2008; Li et al., 2007; Sul et al., 2007, 2006; Im et al., 2006) used human tracheal cell lines to study genomic changes after exposure to formaldehyde in vitro. Unfortunately, these studies are not directly comparable because different gene chip technology platforms were used in different tissues, in both in vivo and in vitro study designs. In general, the gene and protein expression changes reflect changes in apoptotic pathway genes, oxidative stress, and tissue remodeling. Andersen et al. (2008) concluded that there was a threshold level where exposure to formaldehyde (6 ppm) does not elicit changes in nasal epithelium of F344 rats. Overall, Andersen et al. (2008) concluded that genomic changes were no more sensitive than tissue responses and that formaldehyde, being an endogenous chemical, is well handled until some threshold is achieved when toxicity rapidly ensues with genomic and histologic changes. At about 6 ppm, this largely involves tissue remodeling (and protection), but regenerative hyperplasia occurs at higher doses. Andersen et al. (2008) conclude that there is a threshold where exposure to formaldehyde does not elicit changes in F344 nasal epithelial tissue over the duration examined in this study (i.e., 15 days). Andersen et al. (2008) argue that this is consistent with bioassays that indicate no tumor formation in rodents below 6 ppm formaldehyde.

The primary conclusion in the Andersen et al. (2008) paper is that genomic changes, including those suggestive of mutagenic effects, did not temporally precede or occur at lower doses than phenotypic changes in the tissue. The authors stated as follows:

> "The genomic signatures related to these transitions are for cell membrane and extracellular components, then inflammation and cellular stress, and eventually apoptosis. Importantly, these hierarchical models indicate that the tissue responses at low dose concentrations are qualitatively different from those at high concentrations and linear extrapolations or extrapolations that specify similar modes of action at high and low doses would be inappropriate."

4.4.6. Noncancer Modes of Actions

Noncancer health effects of interest span numerous organ systems and include reproductive and developmental effects, neurological/neurobehavioral effects, and a complex interaction between inflammation and immune and adverse pulmonary function. To date, no

-omics studies have examined changes in reproductive tissue or altered gene expression in developing animals. In regard to neurological/behavioral effects, one study (Lu et al. [2008], described in Section 4.1.1.6) has reported elevations in the mRNA for NMDA receptor subunits in brain homogenates following exposure to 2.4 ppm. Hester et al. (2003) reported a significant increase in NMDA receptor subunit transcripts, along with other neuropeptide genes, in nasal tissue of rats instilled into nostril with 400 mM formaldehyde (Described in Section 4.2.1.2.2.1). Together, these changes may relate to formaldehyde-induced sensory irritation and, perhaps, changes throughout the brain.

In regard to inflammatory, immune, and pulmonary effects, transcript and protein changes in rodent tracheal tissue and lung tissue indicate that exposure to 3 to 38 ppm formaldehyde results in genes involved in oxidative stress and cell proliferation and may additionally increase airway ADH3 levels (Lee et al., 2008; Sul et al., 2007; Yi et al., 2007; Im et al., 2006; Yang et al., 2005). Together, these data provide evidence for adverse pulmonary effects that may exacerbate or facilitate asthma.

In lung tissue, Yang et al. (2005) identified three proteins up regulated and one protein down regulated following 15 days of exposure to about 28 ppm formaldehyde. None of the proteins corresponded with transcript changes reported by Sul et al. (2007). Interestingly, Sul et al. (2007) reported that only two transcripts were significantly up regulated in the lung in response to 5−10 ppm formaldehyde, while 19 were down regulated. In this regard, it is worth considering that changes in proteins may not relate to their regulation but rather to their overall percent composition in a cell (relative to other protein changes) before and after exposure. In addition to transcript changes, Sul and colleagues (2007) reported DNA damage and lipid peroxidation and noted that the observed down regulation of GR would facilitate oxidative stress, while the down regulation of phospholipase A2 (PLA2) might represent a mechanism for mitigating lipid peroxidation. It is worth noting that an increase in either of these genes could also be argued to support similar conclusions (i.e., that GR is up regulated to increase GSH levels and that PLA2 up regulation explains lipid peroxidation); this highlights the problem with interpreting these data. Nevertheless, these studies indicate adverse effects (e.g., oxidative stress, lipid peroxidation, cell proliferation etc.) in the rodent lung in response to 5−30 ppm formaldehyde.

In a hypothesis-driven study by Yi et al. (2007), formaldehyde exposure was shown to increase lung ADH3 levels. Several studies indicate that allergic responses and hyperreactivity are uncoupled and may relate to ADH3 expression and activity. Que et al. (2005) has shown that, in a rodent asthma model, ADH3 knockout mice exhibit similar signs of inflammation but are protected from bronchoconstriction. Lino dos Santos Franco et al. (2006) provided evidence,

in rodents, that formaldehyde may induce inflammatory responses (e.g., leukocyte infiltration in the lung) through neurogenic mechanisms but that bronchial tone is mediated by NO. The latter effect is likely to be mediated by S-nitrosoglutathione GSNO and thus influenced by ADH3. Interestingly, the single nucleotide polymorphisms (SNPs) that Wu et al. (2007) reported as linked to other polymorphisms in the promoter region was the one demonstrating protection against asthma. Hedberg et al. (2001) reported that at least one SNP in the promoter region reduced ADH3 expression. Together, these data suggest that reduced ADH3 expression might lower GSNO turnover and bronchial tone, thereby reducing signs of asthma. In this regard, it is conceivable that wheezing and bronchoconstriction are the symptoms that lead to medical intervention and not the inflammation per se. Thus, while ADH3 polymorphisms may not cause asthma, ADH3 polymorphisms may influence hyperresponsivity and the likelihood of asthma diagnosis. This is discussed further in Section 4.6 on susceptible populations. Formaldehyde has been shown to accelerate GSNO breakdown (Staab et al., 2008a; Yi et al., 2007); thus, pulmonary responses to formaldehyde may represent a balance between mechanisms that induce NO (i.e., inflammation) and those that terminate GSNO (i.e., ADH3).

In regard to -omics changes in blood samples, the apparent limited distribution of formaldehyde may suggest that these changes are secondary to effects at sites of contact but could also indicate systemic distribution. As noted elsewhere in this report, bradypnea can induce changes in dosimetry as well as changes in core body temperature and blood gases (hypoxia itself induces hypothermia in rodents, and thus the reduction in minute volume and gas changes may both contribute to hypothermia). These physiological responses (hypothermia and hypoxia) surely induce changes in gene expression. Observed gene and protein changes in the blood following formaldehyde exposure could also relate to irritation and inflammation at sites of contact. In this regard, Im et al. (2006) reported changes in cytokines indicative of Th-2 responses. Altogether, the authors identified 32 proteins altered in the plasma of rats exposed to formaldehyde. Although no coherent mechanisms are apparent from these changes, the authors posited that they could serve as biomarkers for formaldehyde exposure. The concordance of such changes across species remains to be demonstrated.

Li et al. (2007) identified dose-response relationships for six genes in human blood samples that were putatively associated with formaldehyde exposure. Three of these genes are reported to inhibit apoptosis and were posited as supporting in vitro data by Tyihak et al. (2001); however, Li and colleagues (2007) did not report any increase in blood cell count or in Hs 680.Tr cell counts in vitro (i.e., these changes were not phenotypically linked to changes in cell kinetics or hematology). However, these findings are not inconsistent with those of Hester et al. (2003) that indicated no significant increase (or decrease) in nine genes involved in apoptotic pathways.

Finally, serum and glucocorticoid-induced protein kinase 1 (SGK1) was elevated in blood samples and was posited to relate to possible inflammatory and immune responses.

4.4.7. Immunotoxicity

Results from studies that evaluated the immunotoxicity of formaldehyde are mixed. For example, most human studies that investigated systemic immune effects by measuring increases in formaldehyde-specific IgE are negative (Doi et al., 2003; Kim et al., 2001; Palcynski et al., 1999; Krakowiak et al., 1998; Wantke et al., 1996; Grammer et al., 1990). Vandenplas et al. (2004) reported a transiently positive increased formaldehyde-specific IgE titer in occupationally exposed workers. In contrast, Thrasher et al. (1990, 1988, 1987) reported positive formaldehyde-specific IgE titers in small (six to eight person) case studies of exposed workers, and Carraro et al. (1997) reported elevated IgE titers in smokers. Grammer et al. (1990) did not report any differences in albumin IgE in formaldehyde-exposed workers compared with controls. In a residential study, Pross et al. (1986) found that formaldehyde insulation in homes had no effect on tested human immunologic parameters.

One study suggests that immune system parameters are perturbed by formaldehyde exposure. Lyapina et al. (2004) reported decreased immune resistance in all 29 workers exposed to formaldehyde. This effect was associated with decreased neutrophil respiratory burst activity. A LOAEL of 700 ppb was established.

Results from animal studies are mixed as to whether formaldehyde causes immunotoxicity. Leach et al. (1983) reported systemic immunomodulation in F344 rats that was attributed to formaldehyde exposure, but the formaldehyde effects on measures of humoral and cell-mediated immunity were not confirmed in B6C3F1 mice (Dean et al., 1984). Jakab et al. (1992) detected no differences in phagocytic ability of alveolar MPs from mice after formaldehyde exposure. Formaldehyde-exposed rats that were injected with pneumococcus antigen or tetanus toxoid produced antibodies in amounts similar to nonexposed, infected control animals (Holmström et al., 1989b).

However, specific immune parameters appear to be affected by formaldehyde exposure. For example, increased host resistance and hydrogen peroxide release from peritoneal MPs were reported and confirmed (Adams et al., 1987; Dean et al., 1984) and suggest a putative role for ROS. Increased host resistance may be mediated by formaldehyde-induced chronic inflammation and respiratory mucosal damage that causes an up regulation in MPs and therefore increases host immunity. Jakab et al. (2002) reported reduced pulmonary bacterial resistance in mice after exposure to formaldehyde, as determined by increased bacterial loading. This result

contrasts with Dean et al. (1984) and is attributed to differential exposure regimens and experimental design.

Mode of Action

Circulating immune cells present in the mouth and upper airways, such as intraepithelial lymphocytes, direct a local inflammatory response but may also contribute to systemic responses through secreted cytokines and soluble factors released into the bloodstream (Togias, 1999).

Altered host resistance and hydrogen peroxide release from peritoneal MPs were reported and confirmed (Adams et al., 1987; Dean et al., 1984) and suggest a putative role for ROS. Indeed, increased neutrophilic ROS have been associated with formaldehyde-induced dermatitis (Gorski et al., 1992), and, conversely, decreased neutrophil respiratory burst activity has been shown in workers with history of formaldehyde-induced respiratory tract inflammation (Lyapina et al., 2004). Oxidative stress may occur directly as a result of formaldehyde exposure or as a secondary consequence to inflammatory responses.

4.4.8. Effects on the Nervous System

There is considerable evidence that formaldehyde exposure causes adverse effects on the nervous system following inhalation at relatively low exposure levels (see Sections 4.1.1.6 and 4.2.6) but little or no information regarding a possible mechanism of action for these effects. Data regarding adverse effects on the nervous system following oral exposure are very limited, reflecting a data gap in this area. Relevant data in animals and humans for several types of neurological endpoints, following inhalation exposure, are summarized below.

4.4.8.1. *Irritant Threshold Detection*

Humans are exquisitely sensitive to the irritant properties of formaldehyde, as has been discussed previously (see Section 4.1.1.1). Animal data confirm the irritant properties of this compound at very low concentrations (Wood and Coleman, 1995).

4.4.8.2. *Behavioral Effects*

Limited data in humans, as well as more robust data in animals, provide evidence of behavioral changes following exposure to formaldehyde at levels as low as 0.1 ppm. Studies in animals have found effects that persist for days to weeks after termination of exposure. In spite of significant limitations, the available human data are consistent with the animal findings.

Several types of behavior have been evaluated in animals following formaldehyde exposures. The most consistent findings, demonstrated by multiple laboratories and in multiple

species, have been changes in motor activity, habituation, and learning/memory task performance. Motor activity and habituation have been evaluated under a variety of exposure conditions, using both rats and mice. Consistent decreases in activity have been seen in adult animals (Malek et al., 2004, 2003 a, b; Usanmaz et al., 2002). Senichenkova (1991) and Sheveleva (1971) also found changes in motor activity in offspring following in utero exposure, including decreased habituation in juvenile rats exposed in utero. Decreased performance in learning and/or memory paradigms have been seen in multiple laboratories, also in both rats and mice (Lu et al., 2008; Malek et al., 2003c; Pitten et al., 2000).

Data from controlled human exposures are very limited, but studies have shown decreased performance in addition tasks and reaction time tasks following acute exposures to formaldehyde (Lang et al., 2008; Bach et al., 1990). In contrast, Andersen and Molhave (1983) indicated they found no change in performance on several types of tasks (including addition, multiplication, and card punching) following acute exposure to volunteers, but supporting data were not provided.

Data for humans are also available from epidemiology studies of individuals who were occupationally exposed to formaldehyde. A variety of neurobehavioral deficits, including lack of concentration and loss of memory, disturbed sleep, impaired balance and dexterity, and changes in mood, were identified (Kilburn et al., 1987, 1985). However, most of the individuals evaluated in these studies were also occupationally exposed to other solvents, raising questions regarding possible confounding of the results due to multiple exposures. In addition, the formaldehyde exposure information provided in the studies is not sufficient to permit a reliable dose-response assessment for the effects identified. The types of effects seen in humans in the available epidemiology studies are, however, consistent with those seen in available animal studies.

In general (and noting the differences in exposure paradigms and types of tasks), behavioral effects in animals and humans appear to occur at similar exposure levels. Animal studies demonstrated LOAELs as low as 100 ppb following acute or repeated exposures (Malek et al., 2003b, c); human controlled exposure studies have found effects in that same range, with LOAELs of approximately 300 ppb following acute exposures (Lang et al., 2008; Bach et al., 1990).

4.4.8.3. *Neurochemistry, Neuropathology, and Mechanistic Studies*

Limited data are available regarding neurochemical and neuropathological sequelae of formaldehyde exposure. Studies from one laboratory (Sorg et al., 2004, 2001) have suggested that behavioral sensitization to formaldehyde is linked to alterations in HPA control of

corticosterone and changes in mesolimbic dopamine pathways. Neurochemical changes in response to formaldehyde exposure have also been documented in other laboratories (Fujimaki et al., 2004b; Hayashi et al., 2004). Some of these data appear to be conflicting, and there are no definitive data supporting a specific mechanism for formaldehyde effects on the nervous system at this time. Neuropathological data are also limited, although data from one laboratory (Sarsilmaz et al., 2007; Aslan et al., 2006) suggest a concern for changes in brain structure in neonatal rats following exposure at 6 or 12 ppm. No human data are available that address these endpoints. However, a prospective cohort study of nearly one million people followed for 15 years reported strongly significant dose-response associations between death from ALS and exposure to formaldehyde of a known duration, with longer exposures associated with greater risk (Weisskopf et al., 2009). This large, well-designed prospective cohort study strongly supports the causal association of neuropathological effects in humans following long-term formaldehyde exposure.

4.4.8.4. *Summary*

Overall, there is strong evidence that formaldehyde exposure via inhalation may cause adverse effects on nervous system function in experimental animals at relatively low levels of exposure (LOAELs as low as 100 ppb). Although human data regarding neurotoxicity following formaldehyde inhalation are limited, available data provide support that the types of effects seen in humans are similar to those found in animal studies. Evidence from available human controlled inhalation exposure studies indicates that humans may be affected at doses similar to those used in animal studies; however, the human data are extremely limited.

There are insufficient data to evaluate the potential for neurotoxicity following oral exposure to formaldehyde. Limited evaluations of brain weight or histopathology in available chronic or subchronic oral studies found no evidence of formaldehyde-induced changes (Til et al., 1989, 1988; Tobe et al., 1989; Johannsen et al., 1986). However, reliable studies examining nervous system function or focused studies of neuropathology following oral exposure to formaldehyde are not available.

4.4.8.5. *Data Gaps*

Major data gaps were found regarding the evaluation of changes in nervous system structure or function following formaldehyde exposure by both the inhalation or oral routes.

With respect to inhalation exposure, none of the available human studies resulted in data sufficient to conduct a reliable dose-response assessment for changes in nervous system function. Most of the available animal inhalation studies used short exposure durations (acute or short-

term), precluding a reliable evaluation of neurotoxicity following chronic exposure. Available data for neurodevelopmental exposures are also quite limited, consisting of evaluation of neuropathology in only one brain region and functional evaluations focused only on changes in motor activity.

Major data gaps also exist regarding neurotoxicity following oral exposure, with no relevant human data and extremely limited animal data. Available oral exposure studies were insufficient to permit a reliable evaluation of the potential for neurotoxicity following oral exposure to formaldehyde.

4.4.9. Reproductive and Developmental Toxicity

A number of studies have been identified that indicate an effect of formaldehyde exposure on reproductive and developmental outcomes. Human data are described in Section 4.1.1.7, and animal studies are addressed in Section 4.2.7 of this document.

4.4.9.1. *Spontaneous Abortion and Fetal Death*

Several epidemiologic studies reported increases in risk of spontaneous abortion following maternal occupational formaldehyde exposure (Taskinen et al., 1999, 1994; John et al., 1994; Seitz and Baron, 1990; Axelsson et al., 1984). While these finding have been questioned (Collins et al., 2001b), upon careful examination, none of the principal biases in epidemiologic studies, including information bias, selection bias, and confounding, appear to be more likely causes of these reported findings than the conclusion that they may reflect an underlying causal process. While each of these occupational studies focused on women who were coexposed to formaldehyde and other chemicals, the occupational groups were quite different and had different sets of coexposures. The woodworkers in the Taskinen et al. (1999) study were potentially coexposed to organic solvents related to painting and lacquering, dusts, and phenols, none of which was shown to be an independent predictor of adverse risk. The cosmetologists studied by John et al. (1994) were coexposed to hair dyes, bleach, alcohol-based disinfectants, and chemicals specific to services, such as fingernail sculpturing, but, in analyses that were specifically adjusted for other work exposures and their potentially confounding effects, the investigators reported an increased risk for the use of formaldehyde-based disinfectants. The laboratory workers studied by Axelsson et al. (1984) were potentially coexposed to a wide range of solvents, but the miscarriage rate was highest among those exposed to formaldehyde. For a potential confounder to entirely explain an observed effect of another exposure, it must be more strongly associated with the adverse outcome. It does not appear that the collective results of

formaldehyde exposures associated with increased risk of spontaneous abortion—often in spite of exposures being crudely measured—can be explained by information bias or confounding.

Taken together, these findings are consistent with an adverse effect of formaldehyde exposure on the risk of pregnancy loss. The single study with the strongest quantitative assessment of that risk is Taskinen et al. (1999), and the results presented are of sufficient quality to support quantitative risk assessment by using the LOAEL/NOAEL approach.

This study was a well-designed population-based case-control study that appears to have been well executed and appropriately analyzed. The study population of Finnish women was well defined and adequately selected to allow for meaningful comparisons of health effects between individuals with different levels of exposure to formaldehyde. The participation rate was 64%, which is low enough to raise a concern about the potential for selection bias. However, the authors noted that selection bias has not influenced the results of other reproductive epidemiology studies reporting results on smoking, irregular menstruation, and earlier miscarriages, which are known to lengthen the time to pregnancy (Bolumar et al., 1996; Sallmén et al., 1995; Baird and Wilcox, 1985). Furthermore, there is no evidence to support conjecture that an individual's decision to participate in this study would be differential with respect to their workplace formaldehyde exposures while being nondifferential with respect to the other exposures of interest, including organic solvents, wood dust, and phenols. Since the women who chose to participate in this study were not likely to be aware of the specific hypotheses under investigation nor could they have known the formaldehyde exposures that were independently estimated by an industrial hygienist, selection bias is not a likely explanation for the findings of adversity.

Data on pregnancy history, including spontaneous abortions, were collected by questionnaire. Spontaneous abortion is the most common adverse outcome of pregnancy (Klein et al., 1989), and retrospective self-report of spontaneous abortion has been found to match well with prospectively collected reproductive histories (Wilcox and Horney, 1984). Many spontaneous abortions, however, are missed with self-reporting, with the magnitude likely exceeding 25%, but only rarely do women self-report false positive events (Wilcox and Horney, 1984). The effect of such an undercount is to cause a bias towards the null when the likelihood of undercounting is unrelated to formaldehyde exposure. The implication is that the observed association of increased risk of spontaneous abortion associated with occupational exposure to formaldehyde may be an underestimation of the true risk.

The findings by Taskinen et al. (1999) of reduced fertility and increased risk of spontaneous abortion are internally consistent and coherent with other reports of increased risk of pregnancy loss associated with exposure to formaldehyde (John et al., 1994; Taskinen et al.,

1994; Seitz and Baron, 1990; Axelsson et al., 1984). Absent evidence of alternative explanation for these findings, it is concluded that exposure to formaldehyde is associated with pregnancy loss and diminished fertility.

In animal studies, Sheveleva (1971) noted an increase in preimplantation loss in rats exposed to 0.04 and 0.4 ppm formaldehyde by inhalation on GDs 1−19, and Kitaev et al. (1984) observed evidence of degeneration in harvested embryos on GD 2, following 4 months of maternal inhalation exposure to 0.41 ppm formaldehyde in rats. In a second series of tests reported in Kitaev et al. (1984), female rats were exposed to 0.41 and 1.22 ppm formaldehyde for 4 months to test the hypothesis that the embryo degeneration could have been the result of disrupted reproductive hormone levels in the dams. Ovarian weight and blood levels of LH were increased at 0.41 ppm (but not at 1.22 ppm), and significantly increased levels of FSH were observed at 1.22 ppm. Kitaev et al. (1984) proposed that effects at the 0.41 ppm might be related to disruption of the hypothalamic-pituitary axis and that at the higher exposure level (1.22 ppm) frank toxic effects to the embryo were observed. The increased FSH levels at 1.22 ppm may also be indicative of hormonal perturbations induced by formaldehyde exposure that could affect pregnancy maintenance in humans. The finding of treatment-related increased preimplantation loss in rats appears to support the evidence of spontaneous abortion in the epidemiologic data. In addition, a dominant lethal study in rats by Odeigah (1997) identified significant postimplantation loss following premating I.P. formaldehyde exposures to males, suggesting a potential MOA involving germ cell toxicity. Nevertheless, a number of developmental toxicity studies in rats did not report treatment-related embryolethality following gestation exposures to formaldehyde. These included inhalation studies by Martin (1990), Saillenfait et al. (1989), and Kilburn and Moro (1985), a series of studies by Gofmekler and Bonashevskaya (1969), Gofmekler (1968), and Pushkina et al. (1968), and studies by Senichenkova and Chebotar (1996) and Senichenkova (1991). It is noted, however, that, to the extent that these studies evaluated embryonic or fetal death, the observations were conducted late in gestation and the studies may not have been designed to detect the preimplantation losses as observed in Kitaev et al. (1984) and Sheveleva (1971). Additionally, a number of the reports for these studies did not include sufficient details to engender a high degree of confidence in the reported results. Fetal death was also not observed in oral studies with formaldehyde in beagle dogs (Hurni and Ohder, 1973) and rats (Seidenberg and Becker, 1987).

4.4.9.2. *Congenital Malformations*

The effect of occupational exposures to formaldehyde on the incidence of congenital malformations was examined by Dulskiene and Gražulevičiene (2005), Taskinen et al. (1994), and Hemminki et al. (1985). Results were mixed.

In animal studies, the most frequently observed structural anomaly noted following inhalation exposures to formaldehyde during gestation was a delay in fetal (i.e., 1st stage) testes descent (Senichenkova and Chebotar, 1996; Senichenkova, 1991; Kilburn and Moro, 1985), although similar findings were not reported by Saillenfait et al. (1989) or Martin (1990) in what appear to be well-conducted prenatal developmental toxicity studies. No study in the available database specifically examined the 2nd stage of postnatal testes descent in pups. Thus, there is no evidence to determine if the observed effect represented a developmental delay or if it was related to disruptions in male reproductive tract ontogeny, which is dependent on normal levels of fetal testicular testosterone and on the expression of insulin-like hormone-3 (insl3) in fetal Leydig cells (Klonisch et al., 2004). Senichenkova (1991) observed an increased incidence of other organ anomalies following formaldehyde exposure during gestation; however, the anomalies are not characterized in the report. Alterations on fetal organ weights and/or size were noted in several studies (Kilburn and Moro, 1985; Gofmekler, 1968), but it is difficult to ascertain if these findings represented agenesis, hypoplasia, or evidence of systemic organ toxicity. Histopathologic evaluation of pup tissues following maternal gestational exposures to 0.01 and 0.81 ppm formaldehyde was conducted by Gofmekler and Bonashevskaya (1969), revealing reduced glycogen content in the myocardium, the presence of iron in hepatic Kupffer cells, and a positive Schiff reaction in the basement membrane (indicating functional alterations in the renal tubule) at both exposure levels.

4.4.9.3. *Low Birth Weight and Growth Retardation*

A population-based study (Gražulevičiene et al., 1998) reported an association between atmospheric formaldehyde exposure and low birth weight, with an adjusted OR of 1.37 (95% CI: 0.90−2.09).

A number of inhalation studies in rats identified reduced fetal weight as an adverse outcome of in utero formaldehyde exposure and are supportive of the association noted in humans. In a study that exposed pregnant rats to formaldehyde during GDs 6−20, Saillenfait et al. (1989) observed significantly decreased male and female fetal rat weights (78 and 81% of control values, respectively) at 40 ppm formaldehyde. In a study that exposed the dams from GDs 6−15, Martin (1990) found decreased fetal weights at exposure levels of 5 and 10 ppm. In both studies, observations of reduced or delayed skeletal ossification (i.e., the thoracic vertebrae

in Saillenfait et al. [1989] and the pubes and ischial bones in Martin [1990]) were consistent with the fetal weight deficits. Kilburn and Moro (1985) also reported fetal body weight decreases in rats at an inhalation exposure level of 30 ppm. Conversely, increased fetal body weight as compared with controls (generally considered to be nonadverse) was noted by Gofmekler (1968) and Pushkina et al. (1968) at maternal formaldehyde exposure levels of 0.1 and 0.81 ppm administered for approximately 2−3 weeks prior to mating and then throughout gestation. Increased fetal weight was also noted in rats by Senichenkova (1991) and Senichenkova and Chebotar (1996) following maternal exposures to 0.41 ppm formaldehyde throughout gestation.

Studies that assessed the effects of oral administration of formaldehyde on development are quite limited. The only oral study identified that found a treatment-related effect on offspring growth was a study using beagle dogs (Hurni and Ohder, 1973). In this study, formaldehyde was administered at doses of 3.1 or 9.3 mg/kg-day in the feed during gestation, and pup weight decrements at postnatal week 8 were 6.3 and 12% in the low- and high-dose groups, respectively.

4.4.9.4. *Functional Developmental Outcomes (Developmental Neurotoxicity)*

Indications of effects on the developing nervous system were observed in several rodent studies, although no similar epidemiologic findings in children were identified. These studies (Sarsilmaz et al., 2007; Aslan et al., 2006; Weiler and Apfelbach, 1992; Senichenkova, 1991; Sheveleva, 1971) are described in detail in Section 4.2.6. In the studies by Aslan et al. (2006) and Sarsilmaz et al. (2007), neonatal rats were exposed to formaldehyde for 30 days at 6,000 or 12,000 ppb. Decreases in the volume of discrete areas of the brain were observed at both exposure levels in both studies, and, additionally, decreased cell numbers were noted in a region of the hippocampus in the Sarsilmaz et al. (2007) study. Weiler and Apfelbach (1992) exposed juvenile rats to 0.25 ppm formaldehyde for 130 days or adult rats to 0.5 ppm formaldehyde for 90 days. Olfactory thresholds measured in this study were significantly higher in the rats that had been exposed as juveniles than in those that had been exposed only as adults. Sheveleva (1971) observed alterations in spontaneous mobility in 1- and 2-month-old pups from dams that had been exposed to formaldehyde at 0.04 or 0.4 ppm throughout gestation. In the Senichenkova (1991) study, maternal rats were exposed to 400 ppb formaldehyde during GDs 1−19, and functional observational testing was conducted on the juvenile offspring. Changes in open-field motor activity, exploratory activity, and habituation were observed in the offspring.

4.4.9.5. *Male Reproductive Toxicity*

A number of laboratory animal studies have reported effects of formaldehyde exposure on male reproductive system endpoints. These effects include decreased testes weight, changes

in Leydig cell quantity and quality, degeneration of seminiferous tubules, decreased testosterone levels, alterations in biomarkers of toxicity in the testes, and alterations in sperm measures (Galilapour et al., 2007; Xing et al., 2007; Zhou et al., 2006; Özen et al., 2005, 2002; Sarsilmaz et al., 1999; Odeigah, 1997; Majumder and Kumar, 1995; Chowdhury et al., 1992; Til et al., 1989, 1988; Tobe et al., 1989; Johanssen et al., 1986; Maronpot et al., 1986; Cassidy et al., 1983; Appelman et al., 1982; Guseva, 1972). Following concurrent exposures to formaldehyde in air and drinking water for 6 months, Guseva (1972) found decreases in testicular nucleic acid levels. In a study conducted by Chowdhury et al. (1992), rats were administered I.P. injections of formaldehyde for 30 days, and evidence of decreased testes weight, serum testosterone levels, and Leydig cell quality was observed. Sarsilmaz et al. (1999) followed up on these findings (exposing male rats to formaldehyde via inhalation at 10 and 20 ppm for 4 weeks) and found decreases in Leydig cell quantity and the percentage of cells with normal nuclei. Hypothesizing that the reported decreases in Leydig cell quality may have been the result of oxidative stress and damage, Özen et al. (2002) evaluated biomarkers of such changes and found that testicular zinc and copper levels were decreased and iron levels were increased following exposures of adult male rats to 10 and 20 ppm formaldehyde for 4 or 13 weeks. Additionally, relative testes weight was decreased in a dose- and duration-dependent manner, although this effect had not been observed by Sarsilmaz et al. (1999). Özen et al. (2005) noted decreased serum testosterone levels, decreased seminiferous tubule diameters, and increased levels of heat shock protein in spermatogonia, spermatocytes, and spermatids of rats following 91 days of exposure to 10 ppm formaldehyde. A study by Golalipour et al. (2007) observed decreased numbers of testicular germ cells, altered spermatogenesis, and reduced seminiferous tubular diameter and epithelial height in rats following 18 weeks of formaldehyde inhalation exposure; the severity of the seminiferous tubule pathology was positively correlated to the number of hours/week of exposure. Zhou et al. (2006) found decreased testis weight, atrophy of seminiferous tubules, edematous interstitial tissue, and alteration of epididymal sperm count, morphology, and motility in rats after 2 weeks of formaldehyde exposure at 8 ppm. Abnormal sperm were also observed in mice by Xing et al. (2007) after 13-weeks of inhalation exposure at 16.9 ppm, and Cassidy et al. (1983) reported sperm abnormalities in rats following a single oral dose of 200 mg/kg. Additionally, Majumder and Kumar (1995) observed significantly reduced sperm count, motility, and viability following 30 days of I.P. injection of 10 mg/kg-day formaldehyde to male rats. Also in this study, the ability of formaldehyde to affect sperm parameters was confirmed with in vitro testing. A study conducted by Odeigah (1997) demonstrated epididymal sperm count and morphology abnormalities following five I.P. doses of $\geq$0.125 mg/kg formaldehyde and

additionally identified dominant lethal effects (decreased live embryos and increased dead implants) following mating of treated male rats with untreated females.

Although Til et al. (1989) reported low incidences of Leydig cell tumors in formaldehyde-treated rats in a chronic drinking water study, no alterations in testes weight or histopathologic lesions of the testes were observed in subchronic inhalation studies conducted by Appleman et al. (1982) or Maronpot et al. (1986) or in subchronic or chronic oral studies by Johanssen et al. (1986), Til et al. (1988), or Tobe et al. (1989).

No epidemiologic studies have identified an association between formaldehyde exposure and alterations in the male reproductive system (e.g., see Ward et al. [1984]).

4.4.9.6. *Female Reproductive Toxicity*

The available database for the assessment of the effects of formaldehyde exposure on the female reproductive system was limited. In addition to the findings of spontaneous abortions, as described above, Taskinen et al. (1999) examined fecundability in a cohort of healthy female wood-processing industry workers and found that conception was significantly delayed in women who were occupationally exposed to formaldehyde. The FDR, a ratio of average incidence densities of pregnancies for exposed female employees compared with unexposed female employees, was lower in women exposed to mean formaldehyde levels of approximately 0.33 ppm (range: 0.15−1.00 ppm) compared with controls (adjusted FDR = 0.64 [95% CI: 0.28-0.92]). An FDR <1.0 is indicative of delayed conception, which is an indicator of reduced fertility. The subfertility observed in this study is supportive of the association observed between formaldehyde exposure and spontaneous abortion, since subclinical pregnancy losses are increased in women with compromised fertility (Gray and Wu, 2000; Hakim et al., 1995), and both spontaneous abortion and subfertility can be related to exposure to environmental toxicants (Correa et al., 1996).

As described above, formaldehyde exposures to female rats resulted in decreased ovarian weight and altered LH and FSH levels (Kitaev et al., 1984). Maronpot et al. (1986) reported endometrial hypoplasia and lack of ovarian luteal tissue in female mice exposed to 40 ppm formaldehyde for 13 weeks. Additionally, it is noted that, in developmental toxicity studies that included repeated exposures of dams before mating and/or during gestation, reports of adverse pregnancy outcomes were few. Gofmekler (1968) reported an increase in pregnancy duration and decrease in litter size; however, this finding was not observed in other studies.

With the exception of spontaneous abortion and increased time to pregnancy, associations of formaldehyde exposure with adverse female reproductive system outcomes were not observed in the available epidemiologic data.

4.4.9.7. *Mode of Action*

A strong case cannot be made for any one MOA that explains one or more of the reproductive and developmental outcomes observed in formaldehyde epidemiologic or toxicology studies. This is due to a number of issues, including the following: (1) inconsistencies in study findings for the toxicology studies, which may be explained by study quality issues (see detailed descriptions of studies in Sections 4.1 and 4.2); (2) few studies that allow for comparisons because no study was performed with the same study design (e.g., stage of exposure, dose, species, and strain); (3) few mechanistic studies available to test hypothesized MOAs; and (4) a bias that is pervasive in the formaldehyde literature that outcomes observed beyond the POE (the nose) are not expected from inhalation exposure, which is the route of exposure for most of the developmental and reproductive studies. This discussion presents putative MOAs that have been hypothesized by study authors and the studies that support the hypothesized MOAs. The four hypothesized MOAs are not mutually exclusive. They could be acting alone for certain endpoints (in which case the others are not operable) or in concert for certain endpoints.

The focus of this discussion is on analyzing possible MOAs for the developmental and reproductive outcomes that were noted most consistently, across toxicology studies and, in some cases, across human and animal studies. These outcomes include developmental delays, fetal loss, and sperm quality and quantity effects.

An endocrine-disrupting MOA is supported by some of the reproductive and developmental epidemiology and toxicology studies. For example, the decreases in fetal body weight (Martin, 1990; Saillenfait et al., 1989), delayed ossifications (Senichenkova and Chebotar, 1996; Senichenkova, 1991; Martin, 1990; Saillenfait et al., 1989), and delayed eruption of incisors (Senichenkova, 1991) noted in rats after gestational exposure to formaldehyde are consistent with developmental delays. In turn, developmental delays can result from effects on the hypothalamic-gonadal-pituitary axis in the dam that cause hormonal level changes in the pup; however, hormone levels in pups were not measured. Kilburn and Moro (1985) also observed organ size changes and undescended testes after developmental formaldehyde exposure. These outcomes can also be explained by an endocrine MOA. There are three studies that directly tested for changes in hormones after formaldehyde exposure. Kitaev et al. (1984) observed ovarian weight and serum LH and FSH increases after inhaled formaldehyde in adult female rats. Chowdhury et al. (1992) assessed serum testosterone levels in adult rats after formaldehyde IP exposure and found significant decreased testosterone and testes weights and a decrease in 3-β,Δ-5-hydroxy steroid dehydrogenase in Leydig cells, suggesting that formaldehyde affects steroidogenesis. Özen et al. (2002) also reported

significant serum testosterone level decreases as well as decreased mean seminiferous tubule diameters. Furthermore, the steroidogenesis MOA leading to reduced testosterone is also consistent with the sperm quality and quantity decrements observed in the studies by Özen et al. (2002), Sarsilmaz et al. (1999), and Odeigah (1997) studies.

In human studies, delayed time to pregnancy and increased incidence of spontaneous abortion (Taskinen et al., 1999), consistent with some study findings from the toxicology literature, could also be explained by an endocrine MOA. Alterations in hormone levels could lead to pregnancy maintenance problems. Extrapolating the Chowdhury et al. (1992) results of the steroidogenesis MOA to females, formaldehyde exposure could affect progesterone levels required for pregnancy. However, progesterone levels were unchanged in the female rat in the one study that assessed progesterone (Kitaev et al., 1984). Consistent with an endocrine mediated MOA, Maronpot et al. (1986) observed endometrial hypoplasia and lack of ovarian luteal tissue in females exposed to formaldehyde.

A second hypothesized MOA for some of the developmental and reproductive outcomes is genotoxicity of the gametes. Such an MOA could explain pregnancy loss in humans (Taskinen, et al., 1999) and preimplantation loss in animal studies (Xing et al., 2007; Kitaev et al., 1984; Sheveleva, 1971) and fetal viability (e.g., litter size decreases) after formaldehyde exposure. Consistent with male gamete genotoxicity, Odeigah (1997) and Xing et al. (2007) observed reduced fertile matings and live embryos, and increased dead implants in a dominant lethal study.

Oxidative stress/damage is another MOA that is consistent with testicular toxicity, sperm effects, and reduced embryo viability. Özen et al. (2002) investigated the mechanism of oxidative stress being responsible for the testes quality effects by assessing testicular iron, copper, and zinc levels. Zinc and copper levels were reduced in the rat testes, consistent with an oxidative stress MOA. Özen et al. (2002) also reported increased iron levels and decreased zinc levels in the lung, consistent with oxidative stress. Another study (Zhou et al., 2006) that investigated the oxidative stress MOA in the testes observed significant changes in oxidative stress biochemical markers (decreases in SOD, GPX, GSH, and an increase in MDA levels). The authors also assessed the protective effect of coadministration with vitamin E, an antioxidant, on decreased testes weight, biochemical alterations, histopathologic effects, and on sperm count, motility, and morphology. The study of Pushkina et al. (1968) found reduced levels of Vitamin C, another antioxidant, in the fetus and maternal liver after formaldehyde exposure.

The MOAs proposed are not mutually exclusive and in fact could interact with one another. For example, an endocrine MOA could lead to oxidative stress, and that oxidative stress could lead to genotoxicity.

4.4.9.8. *Data Gaps*

The inhalation developmental toxicity studies conducted on formaldehyde and described in Section 4.2.7 comprise an adequate assessment of prenatal developmental toxicity for application to inhalation reference concentration determination. The assessments of postnatal developmental toxicity and of reproductive function following inhalation of formaldehyde are less complete. It is notable that, although the database contains some studies that assess various aspects of reproductive organ system toxicity, particularly in males, there is no assessment of multigenerational reproductive function, such as would be evaluated in a two-generation reproductive toxicity study, nor is there an assessment of potential reproductive effects of formaldehyde exposure in human males.

Adequate assessments of developmental and reproductive toxicity following oral exposures to formaldehyde have not been conducted.

4.5. SYNTHESIS AND EVALUATION OF CARCINOGENICITY

4.5.1. Cancers of the Respiratory Tract

Epidemiologic studies of formaldehyde-exposed workers provide sufficient evidence of a causal association between formaldehyde exposure and nasopharyngeal cancer (see Section 4.1.2.1.1) as well as nasal and paranasal cancers (see Section 4.1.2.1.2). The epidemiologic evidence of association between formaldehyde exposure and other upper respiratory tract cancers (see Section 4.1.2.1.3) is consistent with, and supportive of, a causal association but insufficient on its own to reach a causal conclusion. However, taken together with the causal evidence of an association between formaldehyde and nasopharyngeal cancer and sinonasal cancer in neighboring tissues of the upper respiratory tract and sites of first contact with inhaled formaldehyde, along with the strongly supportive evidence of association in animals, the evidence is sufficient to conclude that formaldehyde is causally related to cancers of the upper respiratory tract as a group.

The observational evidence from epidemiologic studies reporting associations between formaldehyde exposure and increased risk of nasopharyngeal cancer supports a conclusion of a causal association for this specific cancer. However, epidemiologic studies of rare outcomes such as nasopharyngeal cancer, which has an expected incidence of 1 per 100,000 people per year in the United States, do not typically have great statistical power to rule out the null

hypothesis (i.e., no association). However, the weight of evidence of the several studies reviewed in Section 4.1.2.1.1 provide an accumulation of consistent observational evidence sufficient to exclude chance as an explanation for the association. Additionally, there is insufficient evidence of consistent confounding or other bias across the studies that were considered; thus, confounding and bias were also ruled out as explanations for the observed association. The lack of a convincing and consistent alternative hypothesis of causation − in spite of repeated examinations − further supports the conclusion that the association between formaldehyde exposure and increased risk of nasopharyngeal cancer is causal.

The single strongest cohort study, Hauptmann et al. (2004), shows a statistically significant exposure-response relationship between formaldehyde exposure and upper respiratory tract cancers. Hauptmann et al. (2004) demonstrated significant excess risk of nasopharyngeal cancer in exposed workers based on U.S. population death rates (standardized mortality ratio [SMR] = 2.1; 95% confidence index [CI] 1.05−4.21) in a large cohort of 25,619 industrial workers. In addition to the SMR based on an external comparison population, relative risks (RRs) were presented based on internal comparisons of workers in order to minimize potential selection bias due to the well known healthy worker effect. Statistically significant exposure-response relationships between increased formaldehyde exposure and increased risk of nasopharyngeal cancer were reported for two different metrics of exposure (peak and cumulative exposure). Relative risks for nasopharyngeal cancer were also elevated for increased duration of exposure to formaldehyde and for the average intensity of exposure. These analyses controlled for potential confounders including calendar year, age, sex, race, and pay category. While exposure measurement error is likely to be present in any epidemiologic study, there was no evidence of any differential measurement error that could have produced the observation of a spurious association. Any nondifferential measurement error would likely have attenuated the effect of formaldehyde was smaller than that which would otherwise have been observed in the absence of measurement error.

The case-control studies similarly also report associations between formaldehyde exposure and cancer mortality for nasopharyngeal cancer. Although other risk factors for nasopharyngeal cancer (e.g., Epstein-Barr Virus) and the predominant nasopharyngeal cancer histological subtype (SCC versus undifferentiated) vary significantly across the world, case-control studies consistently provide evidence of an association between occupational exposure to formaldehyde and nasopharyngeal cancer (Vaughn et al., 1986a; Vaughn et al., 2000; Roush et al., 1987; Hildesheim et al., 2001; West et al., 1993). In their more recent study, Vaughn et al. (2000) used worker histories to estimate each individual worker's formaldehyde exposure. Workers with more than 1.10 ppm-years of cumulative exposure were found to be at

significantly higher risk of nasopharyngeal cancer, with an odds ratio (OR) of 3.0 (95% CI: 1.3-6.6) (Vaughn et al., 2000). Two different exposure metrics, duration of exposure and cumulative exposure, were positively associated with increased risk of nasopharyngeal cancer, with a significant test for trend (p = 0.014 and 0.033, respectively). The OR also increased in magnitude as the probability of "Ever" having occupational exposure increased, from OR = 1.6 among those whose exposure was judged to be "Possible, probable or definite" to OR = 13.3 among those with "Definite" exposure ($p-$trend < 0.001).

Nasopharyngeal cancer histological subtype analysis indicates that these associations held for both SCC and epithelial nasopharyngeal cancer, but not for the undifferentiating and nonkeritinizing nasopharyngeal cancer (Vaughn et al., 2000). However, formaldehyde exposure is also associated with risk of nasopharyngeal cancer in Taipei, Taiwan, where greater than 90% of the cases had nonkeritinizing and undifferentiated carcinomas and less than 10% of the cases were diagnosed as having SCCs (Hildesheim et al., 2001). These reported associations were strengthened by considering higher probability of exposure (RR = 2.6; 95% CI: 1.1-6.3), greater intensity of exposure (RR = 2.1; 95% CI: 1-4.2) and EBV seropositive cases (RR = 2.7; 95% CI: 1.2-5.9) (Hildesheim et al., 2001). Case-control studies have also linked residential exposure to formaldehyde, specifically for years of residence in mobile homes (Vaughn et al., 1986b) and the use of mosquito coils in the Philippines (West et al., 1993). Independent testing of 6 brands of East Asian mosquito coils evaluated the emission rates of carbonyl compounds in the mosquito smoke and reported that formaldehyde and acetaldehyde had the highest emission rates. Among the three experiments on each of the six brands, the range of formaldehyde concentrations was from 0.87 $\mu g/m^3$ (1 ppb) to 25 $\mu g/m^3$ (31 ppb).

As a group, other URT sites of direct contact with formaldehyde upon inhalation (i.e., salivary gland, mouth, nasal cavity and larynx) also showed evidence of a trend in increasing relative risks with increasing average intensity and peak exposure in the Hauptmann et al. (2004) cohort study, although these trends did not reach the level of statistical significance. The results from other cohort studies and case-control studies are mixed (between positive associations and null findings) for associations between formaldehyde exposure and specific cancers of the URT (IARC, 2006). For rare cancers, extremely large cohorts would be needed to have the statistical power to detect an association for tumors defined by individual sites (e.g., mouth, salivary gland, hypopharynx). Results vary in the smaller cohort studies, where a single case may result in an elevated risk but taken together the evidence is considered suggestive (see Section 4.1.2). Case-control studies have been useful to better understand potential associations between formaldehyde exposure and rare cancers of the URT. Luce et al. (2002) evaluated pooled data from 12 case-control studies and demonstrated a statistically significant increased risk between

formaldehyde exposure and sinonasal cancer. A case-control study by Gustavsson et al. (1998) suggested an association between formaldehyde exposure and oral squamous cell carcinoma (SCC), esophageal, and laryngeal cancers, with odds ratios (ORs) of 1.28, 1.90, and 1.45, respectively. However, the individual ORs were not statistically significant. Hypopharyngeal cancer was linked with formaldehyde exposure with an OR of 3.78 (95% CI: 1.50-9.49) in another case-control study (Laforest et al., 2000). While the data on site-specific cancers of the URT is somewhat sparse, they are consistent with a carcinogenic hypothesis and in their large cohort study, Hauptmann and colleagues (2004) concluded that in spite of the small numbers of deaths from cancers of the URT, the positive associations with average intensity and peak exposure were consistent with the carcinogenicity of formaldehyde at these sites of first contact.

4.5.1.1. *Supporting Animal Evidence*

Animal studies, primarily rodent bioassays, strongly support the causal relationship between of formaldehyde exposure and URT carcinogenicity. Formaldehyde-induced cancers are primarily seen in the nasal passages (Kerns et al., 1983; Monticello et al., 1996; Tobe at al., 1985; Kamata et al., 1997; Sellakumar et al., 1985), but it should be noted that rodents, unlike humans, are obligate nose breathers and have convoluted nasal turbinates. Chronic animal studies report tumor incidence in a variety of rodent models. Study descriptions are provided above in detail (see Section 4.2.2, Table 4-42).

In rodent studies of the respiratory tract, only nasal tumors are considered to be induced by formaldehyde. Repeated exposures to 10-15 ppm formaldehyde result in gross nasal lesions and high incidence of nasal tumors (see Table 4-42, Section 4.2.1). Although increased cell proliferation, squamous metaplasia, dysplasia, and focal necrotic lesions have been noted in the larynx and trachea in some studies, no tumors in these locations have been reported in the rodent studies. The majority of studies were conducted using rats (F344, Wistar, or Sprague-Dawley), and all studies of 18 months or greater in mice and rats show evidence of formaldehyde-induced nasal carcinogenicity. The nasal tumors are primarily SCCs, although papillomas, polypoid adenomas, adenocarcinomas, fibrosarcomas, and esthesioneuroepitheliomas have been reported (Kamata et al., 1997; Monticello et al., 1996; Morgan et al., 1986a, b; Takahashi et al., 1986; Sellakumar et al., 1985; Kerns et al., 1983; Albert et al., 1982). Although hyperplasia, dysplasia, and squamous metaplasia of the respiratory epithelium have been observed beyond the nasal cavity, other respiratory tract tumors have not been reported to be significantly increased by formaldehyde exposure alone.

Increased tumor incidence and decreased latency are correlated with increasing formaldehyde exposure concentration. Reviewing data from the only lifelong inhalation study

(i.e., until "natural death") with multiple exposure groups, nasal SCCs occurred much earlier in the high-exposure animals. For example, tumors are first noted at 8 and 9 months following exposure for high-exposed (15 ppm) female and male F344 rats versus tumors not arising until 24 months in low-exposed rats (2 ppm) (Kerns et al., 1983). In a follow-up study by Monticello et al. (1996), the incidence of SCC in rats exposed at 15 ppm was 47%, with the first tumor noted at 12 months. The incidence of SCC in male rats exposed at 10 ppm was 22%, with the first tumors observed at 18 months after exposure. Moreover, of the 90 rats exposed at 6 ppm for 20 months, only one SCC was noted. No SCCs were detected in rats exposed to 0.7 or 2 ppm formaldehyde. These incidence rates are not mortality-adjusted (see Chapter 5, Section 5.3.4) and include animals from each scheduled sacrifice (3, 6, 12, and 18 months). In a lifelong study of male Sprague-Dawley rats exposed to 10 ppm formaldehyde, the cumulative nasal tumor incidence was calculated as a function of time of exposure (Sellakumar et al., 1985). After 2 years of exposure, the adjusted probability of nasal carcinoma was greater than 60%.

There is some evidence that less-than-lifetime exposure to formaldehyde can induce nasal tumors over an extended observation period. Two studies, both in male Wistar rats, report nasal tumors in response to less-than-lifetime exposures (Woutersen et al., 1989; Feron et al., 1988). A 13-week exposure at 20 ppm followed by an observation period of 30 months (inclusive of exposure) in Wistar rats resulted in six nasal tumors including three nasal SCCs, one cystic SCC of the nasolacrimal duct, one carcinoma in situ and an ameloblastoma, while no tumors were noted in the corresponding air-exposed controls (Feron et al., 1988). A limited number of formaldehyde-related tumors were noted from 4 or 8 weeks of exposure followed by 30 months of observation. Although the tumor incidence of these less-than-lifetime exposures is low, this is consistent with the 2-year bioassays in Wistar rats. Wistar rats are more resilient to formaldehyde-induced nasal toxicity than F344 or SD rats (see Section 4.2.1), and only 1 of 26 (4%) Wistar rats exposed at 10 ppm for 28 months developed SCC (Woutersen et al., 1989) versus 22% in F344 rats (Monticello et al., 1996).

The specificity of formaldehyde-induced tumors in the nasal passages of rodents is believed, at least in part, to be a function of tissue dose. Computational fluid dynamics (CFD) modeling used to predict formaldehyde tissue flux during inhalation exposures suggests that at comparable concentrations, tissue flux in the nasal passages of rodents is more intense than for nonhuman primates and humans. Modeling predicts a different pattern of formaldehyde flux into URT tissues of rodents compared to humans, where formaldehyde penetrates more deeply into the respiratory tract of primates than rodents even considering nose-only breathing for primates (see Section 3.4). Humans will generally switch to mouth breathing when sensing an irritating smell and during physical exertion, resulting in direct exposures to the mouth and greater tissue

flux in tissues beyond the bypassed nasal passages. Therefore, species differences in tissue dose may contribute to formaldehyde-induced tumors in humans beyond the nasal passages, which are not evident in rodent bioassays.

4.5.2. Lymphohematopoietic Malignancies

4.5.2.1. *Background*

Lymphohematopoietic (LHP) cancers include neoplasms of both lymphoid and myeloid cell origins. Cancers of the immune system are described as leukemia if they primarily involve cells from peripheral blood and bone marrow at diagnosis and lymphomas if they constitute a solid tumor (Robbins, 2004). Some forms of leukemia which present as an immature immune cell phenotype are believed to arise from lymphomyeloid stem cells or progenitor cells normally found in the bone marrow (e.g., acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML)) (Greaves, 2004). However, multiple myeloma, lymphomas and some leukemias may arise from mature functional lymphocytes present outside of the bone marrow (Greaves, 2004; see Figure 4-32).

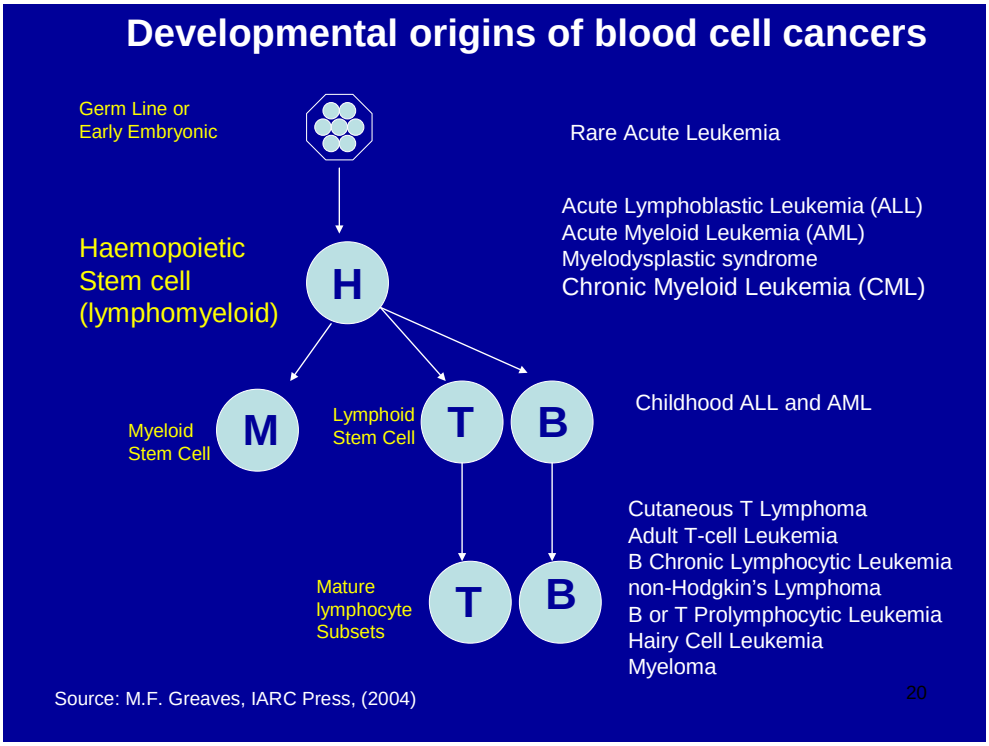

Figure 4-32. Developmental origins for cancers of the lymphohematopoietic system (Adapted from Greaves (2004).

4.5.2.2. *All LHP Malignancies*

Epidemiologic studies involving formaldehyde-exposed workers provide sufficient evidence of a causal association between formaldehyde exposure and all LHP malignancies (see Section 4.1.2.2.1, summarized in Section 4.1.2.2.1.4). Positive associations between formaldehyde exposure and LHP cancers have been reported for chemical workers (Wong et al., 1983; Bertazzi et al., 1986), embalmers (Walrath and Fraumeni, 1983, Walrath and Fraumeni, 1984; Hayes et al., 1990), anatomists and pathologists (Harrington and Shannon 1975; Hall et al., 1991; Levine at al 1984; Stroup et al., 1986; Matanoski et al., 1989) (see Table 4-90). However, clear associations (in terms of overall standardized mortality ratios [SMRs] or proportional mortality ratios [PMRs]) were not reported in analyses for garment workers, iron-foundry workers, and a large U.S. industrial cohort (Pinkerton et al., 2004; Andjelkovich et al., 1995; Beane-Freeman et al., 2009; Marsh et al., 1996), although associations were observed in some of these studies when exposure-response relationships were considered. Several published meta-analyses are available which more formally assess the strength of association between formaldehyde exposure and mortality from all LHP cancers. Pooled SMRs indicate stronger associations for professional workers (embalmers, anatomists, and pathologists) than industry workers (see Table 4-91). Bosetti et al. (2008) found similar relationships, with a pooled SMR of 1.31 (95% CI: 1.16-1.47) for 'professionals' (i.e., embalmers, anatomists and pathologists) versus a pooled estimate of 0.85 (95% CI: 0.74-0.96) for industrial workers. A recent meta-analysis by Zhang et al. (2009) reports a summary relative risk (RR) of 1.25 (95% CI: 1.09-1.43) for both professional and industry workers for all LHP cancers (ICD 9 codes 200-209). These researchers identified 19 cohort study analyses, including cohort study updates. Zhang et al. (2009) used the reported RR from the highest exposure category to increase statistical power and reduce uncertainty regarding confounding or other bias. The criteria for study inclusion and exclusion applied by Zhang et al. (2009) appear to be appropriate and the methodology for using myeloid-specific results where possible also appears to be appropriate. This meta-analysis is supportive of a causal association between formaldehyde and LHP malignancies.

The apparent differences by industry/profession may reflect many influences, including exposure potential and demographic characteristics. External analysis (use of the general population for comparison) relies on the assumption that cancer incidence rates are expected to be similar between the general population and the study population in the absence of exposure. The 'healthy worker effect' is well known, and there may be differences in the magnitude of this selection bias by industry or profession. For instance, LHP cancer incidence and mortality have many risk factors including socioeconomic status. Therefore, the consistent positive findings in professional workers versus mixed results in industrial workers could be influenced by the

Table 4-90. Summary of cohort and case-control studies which evaluated the incidence of all LHP cancers in formaldehyde-exposed populations (ICD-8 Codes: 200-209) and all leukemias (ICD-8 Codes: 204-207). (See Table 4-9 for complete study details and findings)

Study population	Study details	All LHP cancers	Leukemia	Reference
SMR Analysis[1]				
Pathologists and technicians (n = 2,079)	Years of study 1955-1973	2.0 ($p < 0.01$) {pathologists} 0.5 {technicians}	0.6 {pathologists} 0.5 {technicians}	Harrington and Shannon, 1975
Pathologists and technicians (n = 2,720)	1974-1980	NR	0.91 (0.05-4.29) men 9.26(0.47-43.9) women	Harrington and Oakes, 1984
Pathologists (n = 4,512)	Years of study 1974-1987	1.44 (0.69-2.63)	1.52 (0.41-3.89)	Hall et al., 1991
Ontario Undertakers (n = 1,477)	Mortality from 1950-1977	1.24	1.60	Levine et al.,1984
Male Anatomists (n = 2,327)	Mortality from 1925-1979	1.20 (0.7-2.0)	1.5 (0.7-2.7)	Stroup et al., 1986
Male pathologists (n = 4,485)	Mortality through 1977	NR	1.06	Logue et al., 1986
Male pathologists (n = 6,111)	Participants from 1912-1950 membership rolls. Mortality followed through 1978.	1.25 (0.95-1.62)	1.35 (0.92-1.92)	Matanoski et al., 1989
Chemical industry workers, men (n = 14,014)	Mortality from 1941-2000	NR	0.91(0.62-1.39)	Coggon et al., 2003
Chemical workers (n = 2,026)		1.36 (0.5−2.95)		Wong et al., 1983
Industrial workers (n = 25,619)	Mortality followed through 2004	0.94 (0.84-1.06)	1.02 (0.85-1.59)	Beane-Freemen et al., 2009
Industrial workers (n = 7,328)		0.89		Marsh et al., 1996 {Subset of NCI cohort reported by Hauptmann et al., 2003}
Garment workers (n = 11,098)	Mortality followed through 1998	0.97 (0.74-1.26)	1.09 (0.70-1.62)	Pinkerton et al., 2004

Table 4-90. Summary of cohort and case-control studies which evaluated the incidence of all LHP cancers in formaldehyde-exposed populations (ICD-8 Codes: 200-209) and all leukemias (ICD-8 Codes: 204-207). (See Table 4-9 for complete study details and findings) (continued)

Study population	Study details	All LHP cancers	Leukemia	Reference
SMR Analysis[a]				
Resin plant workers (n = 1,330)	Employed between 1959−1980 Mortality through 1986	2.01	NR	Bertazzi et al., 1986
Plastic manufacturing (n = 5,932)		1.69 (salaried workers) 0.93 (hourly workers)	1.98 (salaried workers) 0.98 (hourly workers)	Dell and Teta, 1995
Embalmers, New York (n = 1,132)	Licensed between 1925−1980	1.15	1.32	Walrath and Fraumeni, 1983
Embalmers, CA (n = 1,007)	Licensed between 1925−1980	1.22	1.75 ($p < 0.05$) 1.24 (<20 years) 2.21 ($p < 0.05$) (>20 years)	Walrath and Fraumeni, 1984
Embalmers, U.S. (n = 4,046)		1.39 (1.15−1.63) White 1.31 (1.06−1.59) Nonwhite 2.41 (1.35−3.97)	1.52[b] (0.98−2.35) White 1.44 ($p < 0.05$) Nonwhite 2.72 ($p < 0.05$)	Hayes et al., 1990
Case-Control Studies[a]				
American cancer Society Cancer Prevention Study II: (n = 362,828 men)	Results for men reporting formaldehyde exposure, and occupations related to formaldehyde exposure	1.22 (0.84−1.77) (formaldehyde exposed) 3.44 (1.11−10.68) {formaldehyde exposure and occupation}	0.96 (0.54−1.71) (formaldehyde exposed) 5.79 (1.44−23.25) {formaldehyde exposure and occupation}	Stellman et al., 1998
White men diagnosed with leukemia (Iowa and Minnesota) (n = 622)	Recruited in 1980−1983	NR	1.0 (0.7−1.4) Low 0.7 (0.2−2.6) High	Blair et al., 1993

[a]Relative risk estimate (SMR or OR) presented with 95% confidence intervals, where available.
[b]SMR for leukemia for the total group calculated from the published data for lymphatic leukemia (204, myeloid leukemia (205), and other/unspecified (206, 207).

Table 4-91. Secondary analysis of published mortality statistics to explore alternative disease groupings within the broad category of all lymphohematopoietic malignancies

	ICD-8 Codes	U.S. embalmers (Whole cohort) (Hayes et al., 1990) SMR (95% CI)	U.S. industry (peak exposure metric: >4 ppm vs. >0 to ≤2 ppm) (Beane-Freeman et al., 2009) Relative risk (95% CI)
All lymphohematopoietic malignancies	200-209	1.39 [a] (1.15-1.67)	1.37 (1.03-1.81) [c]
Alternative Disease Groupings			
Exclude myeloid leukemia	200-204, 206-209	1.35 [a] (1.13-1.72) [b]	1.31 [d] (0.97-1.75) [d,e]
Solid tumors of lymphoid origin (Lymphosarcoma and reticulosarcoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma and multiple myeloma)	200-203	1.24 [a] (0.94-1.61) [b]	1.33 [d] (0.93-1.90) [d,e]

[a] $SMR = \frac{Obs}{Exp}$.

[b] Fischer's exact confidence intervals.

[c] See Table 2 of Beane-Freeman et al. (2009).

[d] $RR = \frac{Deaths_{Comparison\ Group} / Person-Time_{Comparison\ Group}}{Deaths_{referent\ Group} / Person-Time_{referent\ Group}} = \left[\frac{108-19}{103-14}\right] \times \left[\frac{PT_{referent\ Group}}{PT_{Comparison\ Group}}\right]$.

$where \left[\frac{PT_{referent\ Group}}{PT_{Comparison\ Group}}\right] \cong \left[\frac{108^c}{103^c \times 1.37^c}\right] = 0.765$

[e] $Var(\log RR) = \frac{1}{Deaths_{Comparison\ Group}} + \frac{1}{Deaths_{referent\ Group}}$.

appropriateness of the comparison to the general population − that is, a differential extent of selection bias. Interestingly, salaried workers, but not the hourly workers, in an Italian plastic manufacturing plant had elevated SMRs for LHP cancers (1.69 (95% CI: 1.07-2.53) and 0.93(95% CI: 0.62-1.35), respectively) (Dell and Teta, 1995). Without knowledge of which worker group is most similar to the comparison population with respect to LHP cancers mortality, one cannot discern if this potential effect of demographic variability accentuates effects in professional/salaried workers or obscures the effects in industrial/hourly-wage workers.

The only study which has data to inform the effects of either exposure level or the appropriateness of an external comparison group on the association between formaldehyde exposure and all LHP cancer mortality is the National Cancer Institute (NCI) cohort study of industrial workers (Blair et al., 1986; Beane-Freeman et al., 2009), which presents relative rates based on internal comparisons for 3 different exposure metrics. Although SMR analysis with an external comparison group did not indicate increased mortality from all LHP cancers (0.94, CI: 0.84-1.06, for the exposed workers), internal analysis using the low-exposed workers as the comparison group demonstrates positive exposure-response relationships for increased mortality from all LHP malignancies cancers and peak exposure across the study periods (1965-2004) (see Figure 4-7 and 4-8) (Beane-Freeman et al., 2009), with a statistically significant trend ($p < 0.05$) for every year since 1977. These results, indicating a positive exposure-response relationship among plant workers, who most likely have similar demographic characteristics, are noteworthy given the apparent lack of association when SMRs for the same cohort are calculated against mortality rates for the general population. The lack of an apparent association with SMRs may be attributable to the healthy worker effect and/or some other difference between the exposed workers and the general population.

Although the association between formaldehyde exposure and all LHP cancer mortality in industrial and professional cohorts is mixed, the strength of the internal analysis of the NCI cohort, in the absence of positive SMRs compared to the general population, suggests that SMR analyses may not be the most appropriate methodology for assessing LHP cancer mortality. Given the potential for demographic differences between an industrial workforce and the general population, the results of the internal analysis of the NCI industrial cohort provide a higher quality analysis—and therefore should be given significantly more weight than SMR analyses of industrial workers that could not distinguish their findings from the null. Given the consistency and strength of the positive associations for all LHP cancers cancer mortality in professional cohorts (embalmers, anatomists and pathologists) taken together with the strong positive results of the NCI cohort, human epidemiologic evidence are sufficient to conclude that there is a causal association between formaldehyde exposure and mortality from all LHP malignancies (as a group).

4.5.2.3. *All Leukemia*

Epidemiologic studies involving formaldehyde-exposed workers provide sufficient evidence of a causal association between formaldehyde exposure and all leukemia as a group (see Section 4.1.2.2.1, summarized in Section 4.1.2.2.1.4). Like the analysis of all LHP cancers, an analysis of all leukemia combines diseases which differ significantly in cell of origin and

etiology, including acute and chronic forms of both myeloid and lymphatic leukemia. This class also includes other leukemia (e.g., erythraemia) and a general category of 'other and unspecified leukemia' (ICD-8 207). Regardless, there is some utility in evaluating the all leukemia mortality data because many studies provided results for this grouping. Also, the diagnosis of leukemia versus solid LHP tumors is fairly distinct thereby limiting misclassification of the endpoint.

Although results are mixed across the studies (see Table 4-90), an association between formaldehyde exposure and leukemia mortality is supported by cohort analyses of embalmers, pathologists and anatomists (Hayes et al., 1990; Walrath and Fraumeni, 1983; Walrath and Fraumeni 1984; Hall et al., 1991; Levine et al., 1984; Stroup et al., 1986; Matanoski et al., 1989). Formaldehyde exposure and formaldehyde-related occupation are associated with leukemia diagnosis in a case-control study (RR = 5.79 (95% CI: 1.44-23.25), but not formaldehyde exposure alone (RR = 0.96; 95% CI: 0.54-1.71) (Stellman et al., 1998) (see Section 4.1.2.2.1 for complete study summaries).

In contrast, SMR analyses of the industrial cohorts do not indicate a similar association (Coggon et al., 2000; Beane-Freeman et al., 2009, Pinkerton et al., 2004). Although the SMR analysis provided for the NCI cohort does not indicate a positive association for all leukemia using an external reference group (Beane-Freeman et al., 2009), the SMR for exposed versus unexposed workers within the cohort suggests all leukemia is elevated 2.1-fold with this internal comparison (95% CI: 0.99-4.56)[6]. A positive exposure-response relationship further strengthens the association of formaldehyde exposure to leukemia mortality (Beane-Freeman et al., 2009). Where the referent group is defined as 'low exposed' individuals, leukemia is elevated in the highest peak exposure category (RR = 1.42; 95% CI: 0.92-2.18) compared to both the referent group and the unexposed category (RR = 0.59; 95% CI: 0.25-1.36), and there is a statistically significant trend across all groups (p = 0.02). Categorical analysis for the average intensity and cumulative exposure metrics suggests greater mortality in the high-exposure groups versus the 'low exposed' individuals (RR = 1.10 [95% CI: 0.68-1.78] and 1.11 [0.7-1.74]. respectively), but analysis of individual results across the exposure-response range indicates cumulative exposure is a better predictor (p = 0.08 for trend across all exposed and unexposed.)

Several meta-analyses have been conducted for formaldehyde exposure and leukemia which indicate a positive association (see Section 4.1.2.2.1.3). Collins et al. (2004) report an overall RR for 18 available studies of 1.1 (CI: 1.0-1.2), suggesting an association of leukemia with formaldehyde exposure. This association was stronger for both pathologists/anatomists

[6] $Var\left(\ln\left(\frac{SMR_{Exposed}}{SMR_{Non\ exposed}}\right)\right) = \frac{1}{Obs_{Exposed}} + \frac{1}{Obs_{Non\ exposed}}$.

(1.4; CI: 1.0-1.9) and embalmers (RR = 1.6; 1.2-2.0), but there was no association when considering only industrial workers (RR = 0.9; 0.8-1.0). Study design also impacted the apparent strength of association, with stronger associations seen in case-control studies (RR = 2.4; 0.9-6.5) versus cohort studies (RR = 1.0; 0.9-1.2). Bosetti et al. (2008) reported an association between formaldehyde exposure and leukemia mortality with a pooled RR of 1.39 (95% CI: 1.15-1.68) for 8 groups of professional workers. In the same analysis, the pooled RR for the 4 industrial cohorts was 0.90 (0.75-1.07). Zhang et al. (2009) reported a pooled RR of 1.54 (95% CI: 1.18-2.00) for all cohorts identified in their meta-analysis, although this pooled RR should be considered with some caution, as myeloid leukemia alone was included in the analysis where available (Zhang et al., 2009).

While the epidemiologic evidence for a causal association between formaldehyde and all leukemia as a group is not at strong as for all LHP as a group, the repeated identification of an association in multiple meta-analyses taken together with the clear causal association between myeloid leukemia demonstrated by Hauptmann et al. (2009) and the consistent evidence reported by Beane-Freeman et al. (2009) are sufficient to conclude that there is a causal association between formaldehyde exposure and mortality from all leukemia as a group (see Section 4.1.2.2.1.4).

4.5.2.4. *Subtype Analysis*

Given the associations discussed above between formaldehyde exposure and both all LHP cancers and all leukemia, further analysis is needed to examine if the observed increase in all LHP cancers is primarily a reflection of increased leukemia, or if other types of LHP cancers may be elevated as well. Although analysis of mortality data by subtype may provide a better understanding of the specific disease associations, there are potential pitfalls as well. Chief among these concerns are the potential for disease misclassification (especially in studies with older mortality data) and lack of statistical power as the number of observed cases is reduced by considering subtypes. Case control studies by design address specific diseases and are well-suited for subtype analysis, but often provide little exposure information. The following analysis will draw from the available data to examine which forms of LHP malignancies may be associated with formaldehyde exposure.

There has been speculation that the association between formaldehyde exposure and increases in all LHP cancers and all leukemia are driven by increased myeloid leukemia (Pyatt et al., 2008; Heck and Casanova, 2004; Golden et al., 2006). If this were the case, then mortality from LHP cancers other than myeloid should not be elevated, once the excess mortality from myeloid leukemia is accounted for. Only 2 studies provide the data to evaluate this hypothesis—

both conducted by the NCI (Hayes et al., 1990; Beane-Freeman et al., 2009). From the published data, crude mortality statistics can be calculated for alternative disease groupings (see Table 4-91). In the NCI embalmer study (Hayes et al., 1990), only myeloid leukemia was statistically elevated in the subtype analysis. For the NCI industrial cohort (Beane-Freeman et al., 2009), elevations were also seen for Hodgkin's lymphoma relative to the referent group. In both cases, the association between formaldehyde exposure and LHP malignancies remains when myeloid leukemia is dropped from the analysis. Further, similar associations are found when all leukemia and myeloproliferative diseases are dropped from the analysis and only solid tumors of lymphoid origin are included (lymphosarcoma and reticulosarcoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma and multiple myeloma). These reanalyses illustrate the need for a more careful subtype analysis to assess the potential for associations between formaldehyde exposure and various forms of LHP cancers.

4.5.2.5. *Myeloid Leukemia*

The associations between myeloid leukemia and formaldehyde exposure are positive and consistent (see Table 4-92, Section 4.1.2.2.4 for summary and evaluation of studies). Of the six studies which formally assess myeloid leukemia mortality by SMR analysis, five are positive, including cohorts of both professional and industrial workers although only two reach statistical significance (Pinkerton at al., 2003; Stroup et al., 1986; Hayes et al., 1990; Walrath and Fraumeni, 1984; Walrath and Fraumeni, 1983). Myeloid leukemia mortality was not increased in the NCI industrial worker cohort by SMR analysis, but, there is evidence in the internal analyses that myeloid leukemia is elevated by peak exposure (1.78 (0.97−3.64), and when exposed are compared to unexposed within the cohort (1.38 (0.65−2.97). The strongest association is seen in the recent update of US embalmers (11.2 (1.3−95.6 for ever exposed). Hauptmann et al. (2009) provide several alternative exposure metrics which all show positive associations (number of embalmings, average formaldehyde intensity, cumulative formaldehyde exposure) (see Section 4.1.2.2.1 for study details).

Further subtype analysis to distinguish between acute and chronic myeloid leukemia is problematic. Although Walrath and Fraumeni (1983, 1984) note that AML is prominent in their analyses of New York and California licensed embalmers; they do not provide PMR (actually SMR) analyses for CML. Walrath and Fraumeni (1983 and 1984) report leukemia cell types—for both studies the majority of myeloid leukemia are acute (5/6 and 4/6, respectively, for New York State and California embalmers). However, SMRs cannot be calculated for AML versus CML in this paper, as comparison rates are not available from the 1920's through the 1960's—the timeframe with the majority of deaths. The authors do contrast the observed rate of AML in the cohort to the background rate for AML in white men in the 1970s—but given the potential

Table 4-92. Summary of studies which provide mortality statistics for myeloid leukemia subtypes

Study population	Myeloid Leukemia	Acute Myeloid Leukemia	Chronic Myeloid Leukemia	Reference
SMR Analysis[a]				
Garment workers (*n* = 11,098)	1.44 (0.08-2.37)	1.34 (0.61-2.54)	1.39 (0.38-3.56)	Pinkerton at al., 2003
Anatomists (*n* = 2,317)	NR	NR	8.8[b]	Stroup et al., 1986
Industrial workers (*n* = 25,619)	0.90 (0.67-1.21) SMR Ratio 1.38 (0.65-2.97) (exposed/unexposed)	NR	NR	Beane-Freeman et al., 2009
Embalmers, U.S. (*N* = 4,046)	11.2 (1.3-95.6)	NR	NR	Hauptmann et al., 2009
Embalmers, U.S. (*N* = 4,046)	1.57 (1.01-2.34)	1.52 (0.85-2.52)	1.84 (0.79-3.62)	Hayes et al., 1990
Embalmers (NY) (*n* =	1.46 (0.54-3.19)	NR	NR	Walrath and Fraumeni,1983,
Embalmers (CA) (*n* =	1.50 (0.55-3.26	NR	NR	Walrath and Fraumeni, 1984
Case-Control Studies[a]				
White men diagnosed with leukemia (Iowa and Minnesota) (*n* = 622)	NR	Low: 0.9 (0.5-1.6) High: NR	Low: 1.3 (0.6-3.1) High: 2.9 (0.3-24.5)	Blair et al., 1993

[a]Relative risk estimate (SMR, or OR) presented with 95% confidence intervals, where available.
[b]Leukemia SMR 1.5 (0.7-2.7) {5 of 10 deaths due to myeloid}; Chronic Myeloid Leukemia (CML) SMR of 8.8.

misclassification of late stage CML as AML, especially historically, this may not be an appropriate comparison. Therefore, although these studies support an association between formaldehyde exposure and myeloid leukemia in general (Walrath and Fraumeni, 1983; 1984), the reported AML and CML subtype information does not allow a satisfactory subtype analysis for myeloid leukemia.

Several studies do present a formal subtype analysis within myeloid leukemia. Similar SMRs are reported for AML 1.34 (0.61−2.54) and CML (1.39(0.38−3.56)) in garment workers (Pinkerton at al., 2003). The initial NCI study of US embalmers indicates a slightly stronger association of formaldehyde exposure to CML (1.84 (0.79−3.62) compared to AML (1.52

(0.85−2.52) (Hayes et al., 1990.) Stroup et al. (1986) report that all of the myeloid leukemiaobserved in anatomists was CML with an SMR of 8.8. Finally, a population-based case-control study of white men in Iowa and Minnesota provides positive SMRs for CML based on estimated formaldehyde exposure (Low: 1.3 (0.6−3.1); High: 2.9 (0.3−24.5)), but no association for AML (Low exposed: 0.9 (0.5−1.6)) (Blair et al., 1993). Therefore, although few cases exist for further subtype analysis, the available data indicate either no differences in SMRs for acute myeloid leukemia (AML) versus chronic myeloid leukemia (CML) (Hayes et al., 1990; Pinkerton et al., 2003) or suggest CML is more prominent (Blair et al., 2000; Stroup et al., 1986).

The meta-analysis by Zhang et al. (2009) evaluated the studies of formaldehyde exposure and myeloid leukemia available at the time including Hauptmann et al. (2003), Pinkerton at al., 2003; Hayes et al., 1990; Stroup et al., 1986; Walrath and Fraumeni, 1984, 1983. While the findings of Hauptmann et al. (2003) on the NCI cohort have been recently updates by those of Beane-Freeman et al. (2009) who updated the cohort, the Zhang et al. (2009) analysis provide the only formal meta-analysis specific to myeloid leukemia. Zhang et al. (2009) reported a statistically significant summary RR of 1.90 (95% CI: 1.31−2.76).

The study by Hauptmann et al. (2009) stands out among the studies of embalmers and professionals in the funeral industry based on the strength of the quantitative exposure data and the demonstration of exposure-response relationships which provide causal evidence of an association between formaldehyde exposure and increased risk of myeloid leukemia. These results were internally consistent and demonstrated statistically significant associations that were unlikely the result of chance. As this nested case-control study was based on the cohorts of Hayes et al. (1990) and those of Walrath and Fraumeni (1983, 1984), the potential for selection bias is considered to be low. Further, the controls in Hauptmann et al. (2009) were carefully selected to avoid individuals who died of any causes that were thought to even possibly be related to formaldehyde exposure. Confounding is also unlikely to be an alternative explanation for the observed results as there were clear and convincing exposure-responses and the magnitude of the effect estimates were extremely large.

Given the consistency of the positive associations for formaldehyde with myeloid leukemia cancer mortality across five of the six studies (Hauptmann et al., 2009; Pinkerton at al., 2003; Hayes et al., 1990; Stroup et al., 1986; Walrath and Fraumeni, 1984, 1983; but not Beane-Freeman et al., 2009), the statistically significant meta analysis by Zhang et al. (2009) and the convincing results from Hauptmann et al. (2009), the human epidemiologic evidence is sufficient to conclude that there is a causal association between formaldehyde exposure and mortality from myeloid leukemia (also see Section 4.1.2.2.4 for further evaluation).

4.5.2.6. *Solid Tumors of Lymphoid Origin*

Multiple myeloma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, lymphosarcoma, reticulosarcoma, and other lymphomas may all be derived from immune cells outside of the bone marrow compartment, in peripheral blood, in the gut and respiratory mucosa and immune tissues at the POE (e.g., lymph nodes, mucosa-associated lymphoid tissue (MALT), gut-associated lymphoid tissue (GALT) (Greaves, 2004). The only meta-analysis to specifically address lymphoid malignancies found evidence for increased lymphoma (Hodgkin's lymphoma (pooled RR = 1.23; 95% CI: 0.67-2.29) and multiple myeloma (1.31; 1.02-1.67), but not for non-Hodgkin's lymphoma (1.08; 0.86-1.35) (Zhang et al., 2009). As seen in Table 4-93 below, individual study results are mixed for these lymphoid cell-line malignancies, as they are for all LHP cancers and all leukemia above. Although these tumors are from mature lymphocytes, there is still variability in the etiology, natural history and risk factors for the many subtypes which are included in these categories.

4.5.2.6.1. *Hodgkin's lymphoma.*

The only meta-analysis to specifically address Hodgkin's lymphoma was conducted by Zhang et al. (2009) and included eight studies (Anjelkovich et al., 1995; Coggon et al., 2003; Harrington and Shannon, 1975; Hauptmann et al., 2003; Hayes et al., 1990; Pinkerton et al., 2004; Walrath and Fraumeni, 1983, 1984; and Wong, 1983). Zhang et al. (2009) reported a summary RR = 1.23 (95% CI: 0.67-2.29). This elevated, but nonstatistically significant finding is consistent with the large variance on reported results among the individual studies as well as the wide confidence intervals of the results which were based on small numbers of cases—even from the large cohort studies. Six of the eight studies observed three or fewer deaths from Hodgkin's lymphoma. Coggon et al. (2003) reported 6 deaths from Hodgkin's lymphoma against 8.5 expected for an SMT = 0.70 (95% CI: 0.26-1.53) and Hauptmann et al. (2003) reported 21 observed deaths with 20 deaths among the exposed workers who has an SMR = 1.26 (95% CI: 0.81-1.95). However, the Beane-Freeman et al. (2009) update of the Hauptmann et al. (2003) study had the largest number of observed cases (n = 27) and was not included in the Zhang et al. (2009) meta-analysis. In fact, the Beane-Freeman et al. (2009) study describes more deaths from Hodgkin's lymphoma than all the other studies in Zhang et al. (2009) combined. Excluding the Hauptmann et al. (2003) results from the list of studies in the meta-analysis leaves 19 cases.

Table 4-93. Summary of mortality statistics for Hodgkin's lymphoma, lymphoma and multiple myeloma from cohort analyses of formaldehyde exposed workers

Study population	Hodgkin's Lymphoma	Non-Hodgkin's Lymphoma	Multiple myeloma	Reference
SMR Analysis[a]				
Pathologists (*n* = 2,079)	1.4	2.0 ($p < 0.05$)	NR[b]	Harrington and Shannon, 1975
Pathologists (*n* = 4,512)	1.21 (0.03-6.71)	1.44 (0.69-2.63)	NR	Hall et al., 1991
Male Anatomists (*n* = 2,327)	– [c]	0.7 (.1-2.5) [d] 2.0 (0.7-4.4) [e]	NR	Stroup et al., 1986
Male pathologists (*n* = 6,111)	0.36 (0.04-1.31)	1.31 (0.66-2.35) [d] 1.54 (0.82-2.63)[e]	NR	Matanoski et al., 1989
Chemical workers (*n* = 2,026)	2.94 (0.33-10.63)	NR	NR	Wong et al., 1983
British Chemical plants (*n* = 14,014)	0.36 (0.01-2.01)	0.89 (0.41-1.70)	1.18 (0.48-2.44)	Coggon et al., 2003
Swedish workers- abrasive production plant (*n* = 911)	NR	2.0 (0.2-7.2)	4.0 (0.5-14)	Edling et al., 1987a
Industrial workers (*n* = 25,619)	1.42 (0.96-2.10)	0.86 (0.70-1.05)	0.94 (0.71-1.25)	Beane-Freemen et al., 2009
Embalmers, New York (*n* = 1,132)	0.87 ($p < 0.05$)	1.08[d] 1.22[e]	NR	Walrath and Fraumeni, 1983
Embalmers, CA (*n* = 1,007)	– [c]	3.10[d] 1.33[e]	NR	Walrath and Fraumeni, 1984
Embalmers, U.S. *(n* = 4,046)	0.72 (0.15-2.10)	1.26 (0.87-1.76) 1.12 (0.58-1.96)[d] 1.35 (0.84-2.01)[e]	1.37 (0.84-2.12	Hayes et al., 1990
Case-Control Studies[a]				
Women in Connecticut (*n* = 601)	NR	1.3 (1.0-1.7)	NR	Wang et al., 2009
White men, Iowa and Minnesota (*n* = 622)	NR	1.2 (0.9-1.7)	NR	Blair et al., 1993
ACS Cancer Prevention Study II (*n* = 128)	NR	NR	1.8 (0.6-5.7)	Boffetta et al., 1999
Men, ACS Cancer Prevention Study II (*n* = 45,399)	NR	0.92 (0.5-1.68) 2.88 (0.40-10.5)[f]	0.74 (0.27-2.02)	Stellman et al., 1998
Danish workers (*n* = 1,098)	NR	NR		Heineman et al., 1992
Danish women (607)	NR	NR	1.6 (0.4-5.3)	Pottern et al., 1992

[a]Relative risk estimate (SMR or OR) presented with 95% confidence intervals, where available.
[b]NR is not reported.
[c]"—" no cases observed.
[d]Lymphosarcoma and reticulosarcoma only.
[e]"other lymphoma."
[f]Formaldehyde exposure in a wood-related occupation. RR for wood-related occupation alone was not elevated 0.97 (0.55−1.73).

There is evidence for an exposure-response relationship for Hodgkin's lymphoma in the NCI industrial cohort among exposed workers (Beane-Freeman et al., 2009). Clear exposure response relationships for Hodgkin's lymphoma are defined with all three metrics of exposure, peak average intensity and cumulative exposure ($p = 0.01$, $p = 0.05$ and $p = 0.08$ respectively for mortality through 2004). These associations have been evident from first follow-up through the current publication, and statistically significant for the majority of the follow-up period demonstrating that this is a strong and consistent finding in the NCI cohort (see Figures 4-3 and 4-4) (Beane-Freeman et al., 2009).

As the majority of the studies reported specific data on Hodgkin's lymphoma report on just three or fewer cases, the best epidemiologic evidence is obtained from the most recent evaluation of the NCI cohort by Beane-Freeman et al. (2009). This cohort study, on its own, reported on more deaths from Hodgkin's lymphoma that the remainder of the epidemiologic literature. Hodgkin's lymphoma was both shown to be at increased risk associated with peak exposure concentrations. Peak exposures in the highest exposure category were associated with a significant increase in Hodgkin's lymphoma deaths comparing death rates among workers with peaks of ≥4 ppm to those with >0 to 2.0 ppm (RR = 3.96, 95% CI: 1.31-12.02). Across the three categories of peak exposure, there was a statistically significant exposure-response trend ($p = 0.01$). The exposure-response trend including the never-exposed workers was also statistically significant ($p = 0.004$). The RR was also elevated for average intensity of formaldehyde exposure with RR = 2.48 (95% CI: 0.84-7.32) and there were significant tests for trend among only the exposed workers ($p = 0.05$) and all workers ($p = 0.03$). Similarly, there were nearly significant tests for trend with cumulative exposure among only the exposed workers ($p = 0.08$) and all workers ($p = 0.06$).

The majority of the studies reporting on Hodgkin's lymphoma did not have sufficient statistical power describe any potential association with formaldehyde as the numbers of observed and expect cases were small and the resulting effects estimates were imprecise. As the Beane-Freeman et al. (2009) study reported on the largest number of cases and was the individual study with the most detailed and objectively ascertained exposure assessment and demonstrated significant exposure-response gradients, it is judged that this epidemiologic evidence is supportive of an association between formaldehyde and Hodgkin's lymphoma (slso see Section 4.1.2.2.4 for further evaluation).

4.5.2.6.2. *Non-Hodgkin's lymphoma.*

The only meta-analysis to specifically address non-Hodgkin's lymphoma was conducted by Zhang et al. (2009) and included eleven studies. Zhang et al. (2009) reported a summary

RR = 1.08 (95% CI: 0.86-1.35). Hauptmann et al. (2009) did not specifically report on non-Hodgkin's lymphoma. Beane-Freeman et al. (2009) did report on 106 deaths from non-Hodgkin's lymphoma but did not identify any significant association in their categorical analyses or in their tests for trend for either peak exposure, average intensity of exposure or for cumulative exposure.

Wang et al. (2009) assessed the effect of formaldehyde exposure on the risk of non-Hodgkin's lymphoma in a population-based case-control study. Semiquantitative exposure metrics included average exposure intensity and average exposure probability which were evaluated individually and together. Analyses use unconditional logistic regression and controlled for age, family history of hematopoietic cancers, alcohol consumption, and race. For the low average exposure intensity category the OR = 1.4 (95% CI: 1.0-1.8), while for the Medium-High category the OR = 1.2 (95% CI: 0.8-1.7). For the Low average exposure probability category the OR = 1.3 (95% CI: 1.0-1.7), while for the Medium-High category the OR = 1.4 (95% CI: 0.9-2.3). The investigators also examined the risk of non-Hodgkin's lymphoma among major subtypes. The risk of follicular lymphoma and chronic lymphocytic leukemia/Small lymphocytic lymphoma was slightly elevated but the risk of diffuse large B-cell lymphoma was OR = 1.9 (95% CI: 1.3-2.6) for ever having been exposed to formaldehyde. For Low average intensity exposure, the risk was OR = 2.1 (95% CI: 1.3-2.6) while for Medium-High average intensity exposure, the risk was OR = 1.5 (95% CI: 0.9-2.4). Even so, an exposure-response relationship was demonstrated using the continuous parameterization of average intensity rather that the categorical ($p = 0.03$). Likewise, an exposure-response relationship was demonstrated using the continuous parameterization of average probability of exposure rather that the categorical ($p = 0.01$).

The findings of Wang et al. (2009) provide some support for an association between formaldehyde exposure and non-Hodgkin's lymphoma. It should be noted that a population-based case-control study, where incidence rather than mortality defines the case—may be more appropriate for cancers with relatively low mortality (i.e., CCL, large B-cell lymphoma, Small lymphocytic lymphoma).

Aside from the semiquantitative study by Wang et al. (2009), non-Hodgkin's lymphoma does not appear to be associated with formaldehyde exposure. There is not sufficient evidence of a causal association between formaldehyde exposure and non-Hodgkin's lymphoma (also see Section 4.1.2.2.4 for further evaluation).

4.5.2.6.3. *Multiple myeloma.*

The only meta-analysis to specifically address Hodgkin's lymphoma was conducted by Zhang et al. (2009) and included nine studies (Boffetta et al., 1989; Coggon et al., 2003; Dell and Teta, 1995; Edling et al., 1987; Hauptmann et al., 2003; Hayes et al., 1990; Heineman et al., 1982; Pottern et al., 1992; Stellman et al., 1998). Zhang et al. (2009) reported a summary RR = 1.31 (95% CI: 1.02-1.67). This statistically significant finding is consistent with the findings of Beane-Freeman et al. (2009) who reported that peak exposures in the highest exposure category were associated with a significant increase in multiple myeloma deaths comparing death rates among workers with peaks of ≥ 4 ppm to those with >0 to 2.0 ppm (RR = 2.04, 95% CI: 1.01-4.12). Across the three categories of peak exposure, there was also some evidence of an exposure-response trend (p = 0.08); however, there was no evidence of an exposure-response trend including the never-exposed workers. The association of multiple myeloma with formaldehyde exposure was also shown throughout the cohort experience (see Figure 4-3 and 4-4) which adds strength to this finding.

The epidemiologic evidence for a causal association between formaldehyde and all multiple myeloma as described by the statistically significant increased risk identified in the meta-analysis of Zhang et al. (2009) and the most recently updated analysis of the NCI cohort by Beane-Freeman et al. (2009) are considered to be supportive of an association between formaldehyde exposure and mortality from multiple myeloma (also see Section 4.1.2.2.4 for further evaluation).

4.5.2.7. *Supporting Evidence from Animal Bio-Assays for Formaldehyde-Induced Lymphohematopoietic Malignancies*

Chronic animal studies provide limited supporting evidence for formaldehyde-induced leukemia and lymphoma. Although the majority of chronic animal bioassays do not report either leukemia or lymphoma, many studies focused primarily on the respiratory tract and did not provide routine examination of other tissues, limiting the detection of leukemia and lymphoma (Horten et al., 1963; Holmström et al., 1989; Wouterson et al., 1989; Appleman et al., 1988; Monticello et al., 1996; Dalbey, 1982). Kamata et al. (1986) did examine additional tissues, but there were only 5 animals at each sacrifice. Drinking water studies were similarly limited, where Takahashi et al. (1986) only examined tissues from the stomach and intestines, and the study by Tobe et al. (1989) only examined 20 Wistar rats per sex per exposure group including interim sacrifices. Therefore, few studies have adequately evaluated the carcinogenic potential of formaldehyde with respect to leukemia and lymphoma. [Complete study descriptions for all experiments evaluated are found in Section 4.2.2.]

The two-year drinking water study by Til et al. (1989) in male and female Wistar rats, did have adequate examination of tissues outside of the portal of entry, but found no increase in leukemia or lymphoma, with only 4 tumor-bearing animals in all treatment groups sacrificed at 24 months (n = 200). Sellakumar et al. (1985) conducted a lifelong inhalation study in male Sprague-Dawley rats exposed at 15 ppm formaldehyde. Some tissues outside of the respiratory tract were routinely examined (liver, kidneys, and testes), including any organ exhibiting gross pathology. However, spleen, thymus, and lymph nodes were not routinely examined, limiting detection of leukemia and lymphoma, especially smaller lesions. Although Sellakumar et al. (1985) was a lifelong study, there was a high mortality rate at 2 years (>80% from the figure), again limiting the power of this study to detect late-in-life malignancies. Although, this study did not indicate formaldehyde-induced lymphoma or leukemia, the limitations of the study design should be acknowledged when interpreting these results.

The largest and most comprehensive cancer bioassay from formaldehyde inhalation exposures is the study conducted at the Battelle Columbus Laboratory (1981). Although the summary reports of this study do not discuss leukemia or lymphoma rates (Swenberg et al., 1980; Kerns et al., 1983), mouse lymphoma and rat leukemia were selected by the study pathologist and biostatistician for analysis and presented in the final laboratory report (Battelle Columbus Laboratories, 1981).

Unadjusted leukemia incidence in male and female rats was similar between control and formaldehyde exposed rats (9% and 6% in female rats; 9% and 4% in male rats respectively: $p > 0.05$) (Battelle Columbus Laboratories, 1981). However, both male and female rats at the highest exposure (15 ppm) exhibited significant early deaths due to nasal lesions (see Figure 4-33). Additionally, the unadjusted leukemia rates included animals from all scheduled sacrifices, with early time points representing 30% of the experimental animals (6, 12 and 18 months). Statistical analysis performed by Battelle (Tyrone extension of the Cox Test), which accounted for time to lesion and survivorship rates, indicated a significant increase leukemia for female rats but not male rats exposed at 15 ppm (p = 0.0003 and p = 0.6891 respectively) (Battelle Columbus Laboratories, 1981). Since only gross pathology was performed on tissues outside of the respiratory tract of mid-dose animals, trend analysis was not performed (Battelle Columbus Laboratories, 1981). As the first leukemia in unexposed rats was noted at 21 months, the early deaths prior to that time in formaldehyde-exposed rats, reduced the number of animals in which the leukemia could have been observed. Leukemia incidence in Fischer 344 rats surviving at least 21 months are shown in Table 4-94. Although the histopathology was not as rigorous in the mid-dose animals, leukemia was reported in these animals at a similar or greater incidence than in control animals.

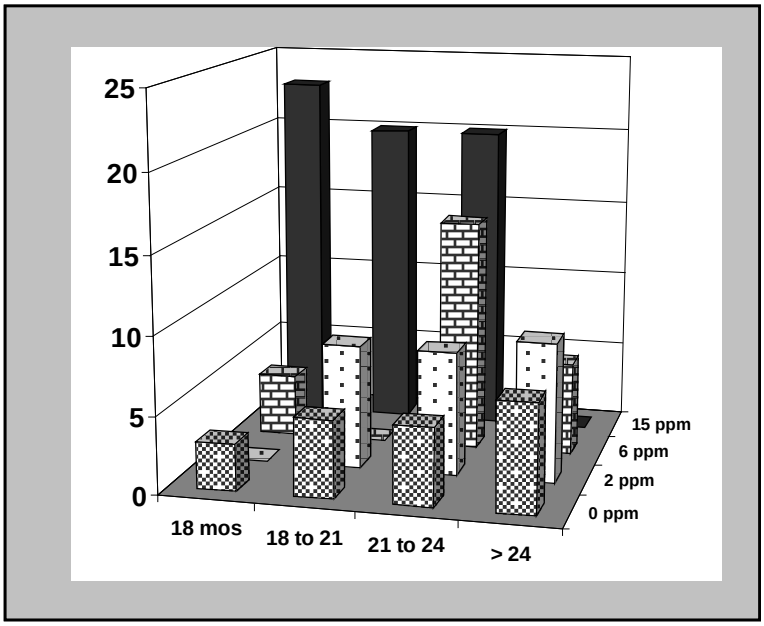

Figure 4-33. Unscheduled deaths in female F344 rats exposed to formaldehyde for 24 months.

Source: Data extracted from the Final Report by Battelle Columbus Laboratories (1981).

Table 4-94. Leukemia incidence in Fischer 344 rats surviving at least 21 months

Formaldehyde exposure (8 hrs/day, 5 days a week)	F344 male rats	F344 female rats
0 ppm	8*/72 (11%)	11/72 (15%)
2 ppm	10/72 (14%)	17/72 (24%)
6 ppm	5/35 (14%)	16/76 (21%)
15 ppm	4/39 (10%)	6/34 (18%)

[a]Leukemia incidence is given as the number of tumor bearing animals for the total number of animals examined for each dose group.
Source: Data extracted from the Final Report by Battelle Columbus Laboratories, 1981

Male and female B6C3F1 mice exposed to formaldehyde for 24 months in the Battelle Laboratory study did not experience the same rate of formaldehyde-related mortality as formaldehyde-exposed rats (Kerns et al., 1983). However, significant early deaths were

observed in male mice due to infighting and are not further considered here (Battelle Columbus Laboratories, 1981). Statistical analysis performed by Battelle, which accounted for time to lesion and survivorship rates, suggest an increase in lymphoma in female mice ($p = 0.06$, Tyrone extension of the Cox Test) (Battelle Columbus Laboratories, 1981). However, this analysis included animals from the 6 month sacrifice where tissues outside of the respiratory tract were not examined. When these animals are removed from the analysis, the cumulative incidence of lymphoma in formaldehyde-exposed female B6C3F1mice is (28%) versus controls (22%) ($p < 0.05$) (see Figure 4-34). Results for the mid-dose groups are not shown here as tissues outside of the respiratory tract were not routinely examined (e.g., spleen, liver, thymus, and lymph nodes).

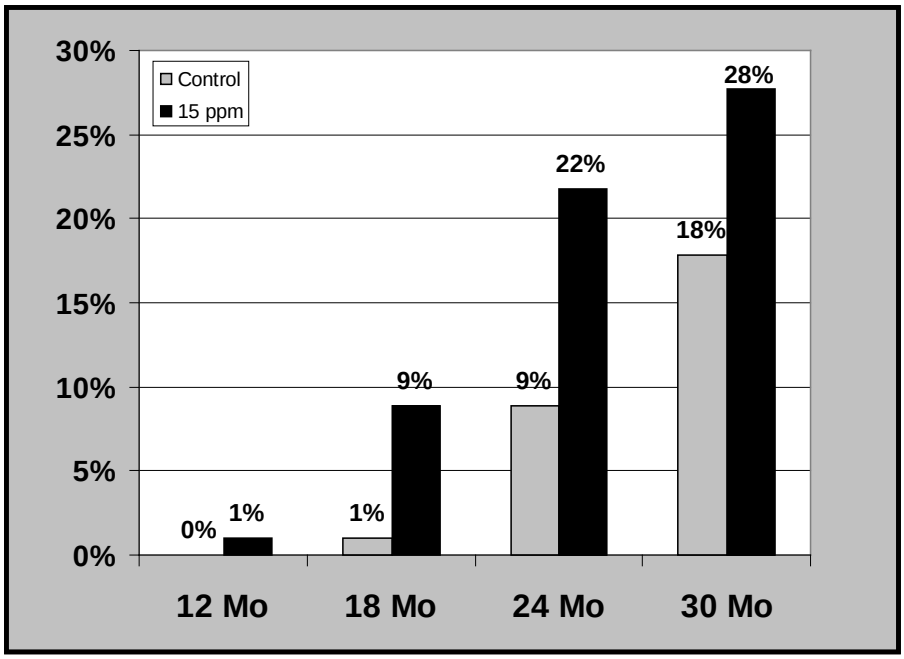

Figure 4-34. Cumulative incidence of tumor bearing animals for lymphoma in female B6C3F1 mice exposed to formaldehyde for 24 months ($p < 0.05$).

Note: Mice from the 6-month interim sacrifice are not included since only nasal passages were examined.

Source: Data extracted from the Final Report by Battelle Columbus Laboratories (1981).

Summary

There have been numerous chronic animal bioassays assessing lesions from inhalation exposure to formaldehyde: however, few have adequate tissue pathology or study design (study size and duration) to assess leukemia and lymphoma incidence. Of the available studies, the study conducted at Battelle Columbus Laboratories (1981) provides the only evidence of formaldehyde-induced leukemia or lymphoma. Although there were significant early deaths in some of the exposure groups, formaldehyde exposure slightly increased leukemia incidence in female but not male rats (Battelle Columbus Laboratories, 1981). Lymphoma incidence was also elevated in female B6C3F1 mice exposed at 15 ppm versus control mice (see Figure 4-6).

Although one chronic inhalation study which examined tissues outside of the respiratory tract did not find increased leukemia in Wistar rats (Sellakumar et al., 1985), this study did not have the comprehensive histopathology, study size, or duration as the study conducted at Battelle Columbus Laboratories (1981). Thus, the negative results in the Sellakumar et al. (1985) study do not contradict the positive findings in fisher 344 Fisher female rats or B6C3F1 mice. Therefore, the available evidence for increased Fisher 344 female rat leukemia and mouse lymphoma from the Battelle study do provide limited support for the plausibility of formaldehyde-induced leukemia and lymphoma.

4.5.3. Carcinogenic Mode(s) of Action

The EPA 2005 *Guidelines for Carcinogen risk Assessment* recommend a mode of action (MOA) analysis when data are available for evaluation. The purpose of this MOA analysis is to determine if sufficient data exist to adequately inform the exposure-response relationship for cancer below the range of observed data in either human or animal studies. Since the majority of the data supporting the carcinogenicity of formaldehyde comes from animal bio-assays and epidemiological studies of workers, EPA must extrapolate from the observed risk of cancer mortality/incidence in those studies to levels considered protective of human health for lifelong environmental exposures. In this context, the EPA *Cancer Guidelines* provide a framework to review MOA information for relevant data to establish an MOA informing appropriate low-dose extrapolation.

The supporting data for the MOA evaluation of formaldehyde are complex, and presented across multiple sections of a large document; therefore, this section includes a brief summary of the biological actions of formaldehyde and key mechanistic data which are believed to be relevant to the MOA evaluation (see Section 4.5.3.1). This information is not intended as a stand-alone description of the evidence for a particular mechanism, but is intended to highlight the major supporting arguments and direct the reader to text providing more detailed discussion.

The summary of data discussed below combines what is known about the human cancer of concern (nasopharyngeal cancer, sinonasal cancer, leukemia and other lymphohematopoietic cancers) with the potential formaldehyde-specific mechanisms of action to postulate carcinogenic modes of action for each cancer or group of cancers (see Sections 4.5.3.2 and 4.5.3.2). The resulting evaluation provides multiple possible MOAs for formaldehyde-induced cancers where some key mechanistic events may be commonly at work in different tissues, and some key events may be more relevant to a specific tissue/cancer type. Each of these MOAs is evaluated with respect to its relevance to human cancer, and the overall weight of evidence for its relevance to formaldehyde-related human cancer.

Overall, multiple MOAs considered relevant to humans are presented for each cancer type. Although some MOAs may have a greater level of supporting evidence, this reflects in part how well a particular mechanism or key event may have been studied. For example, there are a large number of studies across many testing systems, and levels of biological organization to support the mutagenicity of formaldehyde. In contrast other likely MOAs, such as viral reactivation, have little direct mechanistic evidence, but the available evidence is supportive.

The MOAs considered most relevant to upper respiratory tract cancers (e.g. nasopharyngeal cancer and sinonasal cancer) are: (1) direct mutagenicity; (2) inhibition of DNA repair mechanisms; (3) formaldehyde-induced cell proliferation; (4) cytotoxicity-induced cell proliferation; (5) tumor promotion activity; and 6) localized immunosuppression/viral reactivation (see Section 4.5.3.2). The majority of these MOAs would apply equally to immune cells present at the site of first contact and may also contribute to those lymphohematopoietic cancers which arise from peripheral immune cells (e.g., Hodgkin's lymphoma, multiple myeloma and some forms of leukemia). Additional MOAs are considered, specifically for formaldehyde-induced leukemia are: (1) damage of a circulating hematopoietic stem cell or progenitor cell at the site of first contact; and (2) bone marrow toxicity (see Section 4.5.3.3).

In summary—no single MOA is singled out as the best explanation for cancer resulting from formaldehyde exposure. Only one MOA—cytotoxicity induced cell proliferation—suggests an exposure threshold below which the MOA would not be active. However this MOA is the least applicable to humans and other MOAs are considered operative at exposures below exposures associated with cytotoxicity-induced cell proliferation. Therefore, multiple MOAs are considered supported by formaldehyde-specific mechanistic information which provide biological plausibility for the cancers observed in formaldehyde exposed populations.

4.5.3.1. *Mechanistic Data for Formaldehyde*

4.5.3.1.1. *DNA reactivity/genotoxicity/mutagenicity.*

An agent's genotoxic potential and ability to induce mutations is a key consideration in assessing a carcinogenic MOA, as cancer results from a series of genetic and epigenetic alterations affecting genes that control cell growth, division, and differentiation (Hanahan and Weinberg, 2000; Vogelstein et al., 1988; Kinzler and Vogelstein, 2002). The EPA *Cancer Guidelines* suggest several lines of evidence which are key to evaluating a mutagenic MOA: (1) Is the chemical under study DNA-reactive and/or has the ability to bind to DNA; (2) Does the chemical generate positive results in in vitro mutagenic test systems (specifically gene mutations and chromosomal aberrations); (3) Does the chemical induce manifestations of genetic damage in in vivo tests (specifically gene mutations and chromosomal aberrations), and (4) Does the chemical have properties and structure-activity relationships (SAR) similar to known mutagens (U.S. EPA, 2005). As reviewed in Section 4.3 above, there is adequate evidence for formaldehyde-induced genotoxicity and mutagenicity for consideration of these key events in formaldehyde's carcinogenic MOA.

Formaldehyde, as a reactive chemical, forms DPX or DPC, DNA adducts and DDX or DDC and may act to form adducts between other chemicals and DNA (Brutlag et al., 1969; Donecke, 1978; Ohba et al., 1979; Fennel, 1999; Casanova-Schmitz and Heck, 1983, 1984; Heck and Casanova, 1987; Casanova et al., 1989). The high reactivity of formaldehyde results in little specificity indicating that a range of adducts and crosslinks might be expected. Formaldehyde induces a variety of genotoxic and mutagenic events when tested both in vitro and in vivo systems including DNA-protein crosslinks (DPC or DPX), point mutations, DNA single strand breaks (SSB) and chromosomal aberrations (CAs) (see Section 4.3).

Numerous studies have shown that formaldehyde induces genotoxic and mutagenic effects under a variety of experimental conditions (see Section 4.3 for a detailed discussion, also reviewed by IARC 2006; Ma and Harris 1988; Auerbach et al., 1977). As discussed, formaldehyde is known to directly react with DNA forming DPC and DNA adducts. A dose-response in DPX formation has been described at exposure levels of 2-30 ppm formaldehyde in rodent tissues (Casanova-Schmitz and Heck, 1983). Dose-dependent increases were also observed for SSBs in peripheral blood lymphocytes and livers of rats (Im et al., 2006), and lung epithelial cells of rats exposed to formaldehyde by inhalation (Sul et al., 2007), and for MN and comet assay parameters in workers occupationally exposed to formaldehyde (Yu et al., 2005) have also been reported. All these studies suggest that formaldehyde can induce dose-dependent increase in the genotoxicity in both animals and humans. The DPX induced by formaldehyde are bulky adducts which may induce distortion of the DNA helix and are likely to induce mutations.

Mutations may occur during repair of formaldehyde-induced DNA damage, or as a result of replication errors during mitogenesis. Additionally, there is some evidence that DNA single strand breaks (SSB) may be induced directly by formaldehyde reactivity (Grafstrom et al., 1984). Clastogenic effects including increased micronuclei (MN), chromosomal aberrations (CAs) and sister chromatid exchanges (SCEs) are also reported in a range of in vitro study systems.

Formaldehyde caused a concentration-dependent increase in mutations at the *tk* locus of human lymphoblastoid cells (Craft et al., 1987) and clastogenicity (e.g., MN) in human cell lines deficient in either DNA nucleotide excision repair (NER) or DDC repair systems even though there is no change seen in DPC induction or removal between these cell lines (Speit et al., 2000). These data suggest that alteration of DNA repair, not DPC removal, contributes to formaldehyde-induced clastogenicity. Since DPC repair involves proteolytic removal of proteins from the DNA, it has been hypothesized that single peptides or small peptide chains cross-linked to the DNA as in the case of DPC are critical to formaldehyde-induced mutations.

Formaldehyde-induced MN and CAs are associated to concentration-dependent mutagenic effects in L5178Y mouse lymphoma cells (Speit and Merk, 2002). Detailed analysis of both spontaneous and formaldehyde-induced lesions indicate that recombination or deletion of DNA from the thymidine kinase (*tk*) locus was primarily responsible for the loss of heterogeneity leading to the observed mutant phenotype. Therefore, it is believed that formaldehyde is mutagenic by a clastogenic mechanism, rather than through point mutations in the L5178Y mouse lymphoma cell system. This finding is consistent with Craft et al. (1987) who demonstrated formaldehyde-induced mutagenicity in the *tk* locus of TK6 human lymphoblastoid cells, while Grafström et al. (1984) demonstrated increased SSBs in formaldehyde-exposed human cell lines. The elegant series of experiments by Speit and Merk provide the possible links between DPC, clastogenicity and locus-specific mutations firmly demonstrating formaldehyde-induced mutations in the in vitro mouse lymphoma testing system.

Formaldehyde is genotoxic at the portal of entry (POE) in animal studies, resulting in increased DPC formation in the nasal mucosa as discussed above. However, there are no animal studies which directly examine the mutagenicity in nasal or respiratory epithelial cells in the early stages of exposure. It is likely that the mutations are seen in advanced stage of tissue transformation with formaldehyde exposure. With weak positive results in pulmonary lavage cells (Dallas et al., 1992) and clastogenicity demonstrated in gastro-intestinal epithelial cells of rats (Migliore et al., 1989), below exposure levels which trigger regenerative cell proliferation, the existing evidence, although thin, supports clastogenic effects of formaldehyde.

Clastogenic effects are consistently reported in humans exposed to formaldehyde in the industrial workplace or during anatomy or mortuary classes (see Section 4.3 for a full

discussion). Increased micronuclei have been reported in nasal epithelial cells from industry workers (Ballarin et al., 1992; Ye et al., 2005), buccal epithelial cells from anatomy and mortuary science students and/or staff (Kitaeva et al., 1996; Titenko-Holland et al., 1996; Burgaz et al., 2001, 2002) compared to corresponding controls. Comparisons of micronuclei in nasal and buccal cells of anatomy students before and after classes where they are exposed to formaldehyde indicate an exposure-related increase in clastogenicity (Ying et al., 1997). An examination of exfoliated buccal and nasal cells in mortuary students indicates greater increases in centromere-negative micronuclei, suggesting the effects are due to chromosome breakage or clastogenicity rather than aneuploidy (Titenko-Holland et al., 1996). Micronuclei were also increased in a dose-dependent manner in buccal cells as well as peripheral blood lymphocytes (PBLs) in mortuary students during the course of an embalming class; however, SCEs were reduced in postexposure samples (Suruda et al., 1993). Buccal, oral, and nasal epithelial cells present at the portal of entry may be directly exposed to formaldehyde and thus reports of clastogenic effects are consistent with direct interaction of formaldehyde at the POE. Mutagenicity in the form of *p*53 mutations has been observed in the SCCs of chronically-exposed rats, however, it is uncertain about the stage at which the mutations are induced (Recio et al., 1992). There is some supporting evidence for the mutagenicity of formaldehyde in human populations. Shaham et al. (2003) reported an increase in mutant *p*53 protein in the peripheral blood lymphocytes of individuals with mean formaldehyde exposure duration of 16 years. Additionally there was is a significant association between mutant *p*53 protein and DPC in this study suggesting a relationship between the formaldehyde's genotoxic effects. More recently, Zhang et al. (2010a) have reported aneuploidy in circulating hematopoietic stem cells in formaldehyde exposed workers with increases in both monosomy7 and trisomy 8.

In summary, there are several lines of evidence supporting mutagenic effects of formaldehyde exposure:

1) Formaldehyde directly interacts with DNA generating DPC, DNA adducts, and DDC
2) DPC in tissues at the POE exhibit a dose-response relationship to formaldehyde exposure,
3) Formaldehyde-induced DPC are associated with formaldehyde-induced MN and CAs,
4) Mutations induced by formaldehyde due to small deletions and rearrangements in DNA in various experimental systems are consistent with formaldehyde's observed clastogenic effects (MN and CAs),
5) Formaldehyde-induced mutations and clastogenic effects occur at levels below where significant cytotoxicity is detected, and

6) Formaldehyde exposure has been correlated to similar increased MN and CAs in human buccal and oral cells corresponding to sites where formaldehyde-induced tumors arise.

4.5.3.1.2. *Inhibition of DNA repair.*

Studies indicate that formaldehyde exposure may inhibit DNA repair mechanisms directly (see Section 4.3.1.5). Grafström (1985) first documented formaldehyde effects on DNA repair mechanisms, reporting that formaldehyde treatment of human bronchial fibroblasts in vitro inhibited repair of O^6-methyl-guuanine adducts induced by *N*-methyl-Nitrosurea (NMU). Inhibition of DNA repair in human keratinocytes and fibroblasts cultured at 10 µM formaldehyde affected repair of DNA single strand breaks from ultraviolet light but was specific to UVB and UVC, not impacting repair of single strand breaks from UVA (Emri et al., 2004).

To determine if formaldehyde may have similar effects in exposed humans, Hayes et al., (1997) assessed the activity O^6-alkylguanine-DNA alkyltransferase (AGT) an enzyme critical in repairing DNA damage induced by alkylating agents in formaldehyde-exposed mortuary students previously shown to have increased micronuclei in both buccal cells and peripheral lymphocytes (Suruda et al., 1993). AGT activity was lower in mortuary students with prior embalming exposures versus students with no prior exposure ($p = 0.08$). Seventeen of 23 students had lower AGT activity after the 9 week course ($p < 0.05$) with a larger proportion of naïve students demonstrating decreased activity (7 of 8) versus previously exposed students (10 of 15). Although detailed exposure measurements were taken for each student, the changes in AGT activity were not correlated to cumulative exposure (ppm-hrs).

4.5.3.1.3. *Protein to protein cross-links.*

Formaldehyde is a reactive molecule that is likely to interact with both low molecular weight cellular components (e.g., reduced glutathione [GSH]) as well as high molecular weight cellular components. Unlike nuclear DNA, which has additional membrane barriers to exposure (i.e., nucleus), extracellular and intracellular proteins, are obvious primary targets for interacting with formaldehyde. Formaldehyde is a well-known cross-linking agent that is used in the fixation of tissues, inactivation of toxins and viruses (e.g., preparation of vaccines), and study of protein-protein interactions (Metz et al., 2006). Using several identical synthetic polypeptides differing on one amino acid, Metz et al. (2004) have shown that formaldehyde initially reacts with the primary amino and thiol groups of amino acids forming unstable methylol adducts, which later are partially dehydrated forming labile Schiff bases that are capable of forming crosslinks with other amino acid residues, such as arginine, asparagine, glutamine, histidine, tryptophan, and tyrosine through methylene bridges, but not between two primary amino groups. The same group (Metz et al., 2006) has also shown that formaldehyde forms seven

intramolecular crosslinks in proteins with defined structure, such as insulin, involving arginine, tyrosine and lysine and the *N*-terminus of insulin was converted to a imidazolidinone adducts similar to that observed with the synthetic peptide (Metz et al., 2004). (Figure 3-1 provides a general reaction scheme for formaldehyde-mediated modifications of amino acids.)

4.5.3.1.4. *Break-down of the mucociliary apparatus.*

The mucociliary apparatus of the upper respiratory tract is the first line of defense against airborne toxicants. Comprised of a thick mucus layer (epiphase), hydrophase and ciliated epithelium, the mucociliary apparatus may entrain, neutralize and remove particulates and airborne chemicals from inspired air (see Figure 4-7). Formaldehyde reacts with the components of the mucous layer (proteins, glycoprotein, and lipids), crosslinking proteins. Formaldehyde exposure induces slowing of the mucous flow, stiffing and breaking up of the mucous layer and eventual mucostasis where gaps have been observed exposing the underlying hydrophase and epithelium. Although ciliary beat first increases in response to formaldehyde exposure, perhaps to compensate for reduced flow of the epiphase, ciliastasis ensues with both higher levels of exposure and increased duration of exposure. Altered ciliary beat has been noted in as little as 15 minutes of exposure (1.25 ppm) with functional deficits in the mucociliary apparatus at 30 minutes. Altered ciliary beat has been reported at the lowest concentration tested (0.5ppm) for a single 6 hour exposure. Severity of effects increase with both duration and level of exposure (see Section 4.2.1.2.1).

4.5.3.1.5. *Induced cell proliferation.*

There are several reports apparently demonstrating formaldehyde-induced proliferation in cells below cytotoxic levels of exposure. This phenomenon has been reported from studies involving both in vitro and in vivo exposures. Tyihak et al. (2001) demonstrated significantly increased cell proliferation in both HT-29 human colon carcinoma and human umbilical vein endothelial cell (HUVEC) lines treated with 0.1mM (the lowest dose) formaldehyde compared to untreated controls ($p < 0.0001$). This effect was quantified as both an increase in cell number over time (see Figure 4-35), and an increase in the percentage of cells undergoing mitosis at each time point. The authors also report a significant ($p < 0.01$) inhibition of apoptosis in formaldehyde-treated cells as compared to untreated cells (data not shown here). In a novel system using xenotransplanted human tracheobronchial epithelial cells, formaldehyde was shown to induce increased cell proliferation at doses below those required for a “massive toxic effect” (Ura et al., 1989).

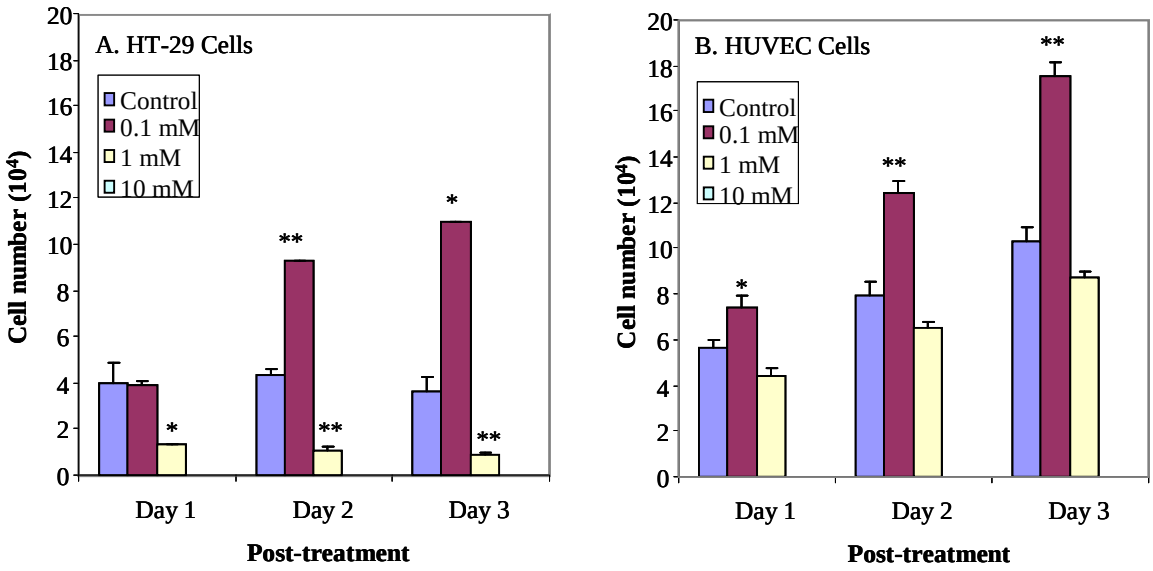

Figure 4-35. Effect of various doses of formaldehyde on cell number in (A) HT-29 human colon carcinoma cells and in (B) human umbilical vein epithelial cells (HUVEC).

Values are average of three samples ± SD; * $p < 0.01$ and ** $p < 0.0001$ compared to corresponding controls.

Source: Tyihak et al. (2001).

Some animal studies have demonstrated increased cell proliferation after formaldehyde exposures by both inhalation and ingestion (see Section 4.2.1). However, whether sustained increases in cell proliferation over baseline rates are observed upon exposure to subcytotoxic doses of formaldehyde remains unclear. Several of the rodent inhalation studies demonstrate increased cell proliferation in the nasal epithelium at formaldehyde exposures levels that were subcytotoxic—i.e., in the absence of significant cell death. Acute formaldehyde exposures (1 to 3 days) induced increased cell proliferation at discrete locations in the nasal mucosa, where cell proliferation was measured as a labeling index (percentage of cells pulse-labeled with tritiated-thymidine). Reuzel et al. (1990) reported increased cell proliferation in the nasal passages including the nasoturbinates, maxilloturbinates septum, and lateral wall in male Wistar rats exposed at 3 ppm, but not at 0.3 or 1 ppm, formaldehyde for 22 hours/day for 3 days.

Zwart et al. (1988) reported increased cell proliferation after exposure to 1 or 3 ppm formaldehyde, 6 hours/day for 3 days or 13 weeks in male and female albino Wistar rats. These increases were transient at level 3 but sustained at level 2 of the nose and were not correlated with cytotoxicity (see Table 4-17). In contrast, Wilmer et al. (1989), from the same group of

investigators using asimilar exposure regimen, reported no increase in cell proliferation after repeated 8−hour exposures at 1 or 2 ppm formaldehyde for 3 days or 4 weeks. Swenberg et al. (1986) demonstrated a transient increase in cell proliferation after a single 8-hour exposure to 0.5 or 2 ppm formaldehyde in male F344 rats but no increases after 3 days or repeated 8-hour exposures. The authors suggest that adaptive responses of the nasal mucosa contribute to the transient nature of formaldehyde-induced cell proliferation. After a series of acute studies at various formaldehyde concentrations, Swenberg and coworkers concluded that, in addition to cell proliferation being concentration-, dose- and time-dependent, the response varies by species and by location of exposure in the nose (Swenberg et al., 1983, Swenberg et al., 1986).

Other methods of quantifying cell proliferation in the nasal mucosa have demonstrated formaldehyde-induced cell proliferation at similar low exposure concentrations. For example, Roemer et al. (1992) measured cell proliferation by flow-cytometry in epithelial cells harvested from the nose and trachea of male Sprague-Dawley rats exposed to 2 ppm formaldehyde for 6 hours/day for 1 or 3 days and found increased cell proliferation after the 1-day exposure. These increases were transient and were not evident after 3 days of exposure. Cassee and Feron (1994) identified proliferating cells by staining for the presence of proliferating cell nuclear antigen (PCNA). Formaldehyde exposure at 3.6 ppm for 6 consecutive periods of 12 hours (8-hour exposures followed by 4-hour-periods of nonexposure) over three days, qualitatively increased the expression of PCNA in respiratory epithelium at levels 2 and 3 of the nose in albino male Wistar rats (Cassee and Feron, 1994). Hyperplasia, squamous metaplasia and frank necrosis were also reported for these tissues.

Monticello et al. (1990, 1991, 1996) conducted in vivo cell proliferation studies in which they exposed F344 rats for short durations (1, 4, 9 and 42 days) as well as much longer durations (13, 26, 52 and 78 weeks) to exposure concentrations of 0, 0.7, 2.0, 6.0, 10.0, and 15.0 ppm. These data are unique in that they also included low exposure concentrations. The authors reported statistically significant increases in cell proliferation only at 6.0 ppm and higher exposure concentrations in the short duration study and only at 10.0 ppm and higher concentrations in the longer duration study. These data have undergone considerable statistical analysis in several papers as well as in this document. Conolly et al. (2002, 2003) and Gaylor and Conolly (2004) interpreted these data, when combined, as indicating a nonmonotonic behavior at low dose. In other words, formaldehyde was judged to result in a reduction in cell proliferation at low dose in comparison to baseline rates, with increased proliferation effect kicking in only at exposures that were cytotoxic. However, as shown in Appendix C and in Subramaniam et al. (2008), Crump et al. (2008), analysis of the individual animal data shows considerable uncertainty and variability, both quantitative and qualitative, in the interpretation of

these cell proliferation data. (For example, even the control data vary over an order of magnitude in some cases. See Figures 5-22 and 5-23 in Appendix C.) These analyses (which were based on the replicate animal data used in the above studies) considered regional formaldehyde dose to the tissue (flux), nasal site and duration of exposure, as well as the number of cells at a given site. The overall conclusion in Section 5.3.3 (and detailed in Appendix C) is that the cell proliferation dose-response at low dose could be reasonably described by both monotonic (with and without a threshold) and nonmonotonic curves.

Only one study, by Monticello et al. (1989), quantified cell proliferation in primates after formaldehyde exposure; this study, reported an 18-fold increase in cell proliferation in the nasal epithelium (respiratory and transitional), larynx, trachea and carina of male Rhesus monkeys exposed to 6 ppm formaldehyde compared to controls (see Section 4.2.1 for detailed study description). The authors also noted that increased cell proliferation was seen in locations with minimal histological changes, indicating proliferation may be a more sensitive predictor of adverse health effects of formaldehyde exposure.

4.5.3.1.6. *Cytolethality and resulting regenerative cell proliferation.*

The toxic and cytolethal effects of formaldehyde exposure at the POE are well documented after both inhalation and oral exposures (see Section 4.2.1). The nature and progression of tissue injury has been best documented in rodent inhalation assays. Early effects on the nasal mucosa include altered ciliary beat and mucus flow, hyperplasia and metaplasia of nasal epithelium (Morgan et al., 1986a, b; Monteiro-Riviere and Popp, 1986; Maronpot et al., 1986; Rusch et al., 1983; Monticello et al., 1986). These first changes may be considered adaptive responses. Squamous epithelium may thicken and transitional epithelium may change to squamous epithelium as evidenced by squamous hyperplasia, squamous metaplasia and thickening of the epithelium in these anterior portions of the nose. Tissue damage may be transient at lower formaldehyde exposures as these changes serve to protect tissue from formaldehyde's reactivity. However, higher formaldehyde concentrations can overwhelm these adaptive responses and result in gross tissue damage. Frank necrosis and focal erosions have been reported in time- and concentration-dependent manner in rodent bioassays.

Both adaptive changes and cytolethality are associated with cell proliferation. However, where adaptive changes are successful, e.g., prevent continued toxic insult to the tissue, cell proliferation is transient. Exposure regimens where the adaptive changes are not adequate to protect the tissue, would result in continued cytoxicity and cell death. Sustained damage to the epithelium would result in sustained cell proliferation to compensate for cell death. A series of rodent bioassays present convincing evidence that chronic inhalation exposures 6 hours a day,

5 days a week at 6, 10, and 15 ppm formaldehyde doses do result in sustained damage to the nasal epithelium, sustained cell proliferation and tumor development (Kerns et al., 1983, Morgan et al., 1986; Monticello et al., 1990, 1991, 1996). Work by Monticello and coworkers demonstrate that chronic repeated inhalation exposures at 6, 10, or 15 ppm formaldehyde result in sustained cell proliferation at the lateral meatus, mid-septum and maxilloturbinates of rat nasal passages (Monticello et al., 1991, 1996).

4.5.3.1.7. ***Evidence for promotion.***

There is some evidence, although mixed, that formaldehyde may promote tumor development by other carcinogens, and known initiating agents by various routes of exposure. Formaldehyde exposure in drinking water (0.5% formalin) increased glandular stomach adenocarcinomas in male Wistar rats after initiation with 100 mg/L, *N*-methyl-*N*'-nitrosoguanidine (MNNG), compared to MNNG-only-treated rats (Takahashi et al., 1986). In white noninbred rats, inhalation exposures (3, 30, or 300 $\mu g/m^3$ formaldehyde 7hr/day, 5 days/week for 1 year) increased tumor multiplicity per animal and decreased latency of benzo[a]pyrene induced tumors in white noninbred rats (Yanysheva et al., 1998.) Similarly, formaldehyde skin application decreased tumor latency, in 7,12-dimethylbenz(a)anthracene (DMBA) initiated hairless Oslo mice (Iversen, 1986). Although formaldehyde exposure also increased the tumor multiplicity in Syrian golden hamsters where diethylnitrosamine (DEN) (0.25mg I.P.) was the tumor initiator, positive results were only reported for the exposure regimen where hamsters were exposed to formaldehyde via inhalation 48 hours prior to DEN injection, and then one a week thereafter for life. However, formaldehyde did not increase the number of tumors per tumor bearing animals when only administered beginning one week after all DEN injections. In contrast, bladder cancer was not enhanced by intravesical instillation of 0.5ml of 0.3% formalin, one week after instillation of *N*-methyl-*N*-nitrosourea (MNU) in male Fisher rats (Homma et al., 1986).

The observed promotion activity of formaldehyde has been tested in several systems, by different routes of exposure. By several routes of exposure, formaldehyde enhanced tumor development at a site where formaldehyde did not induce tumors alone, without the initiating agent (Takahashi et al., 1986, Yanysheva et al., 1998; Iversen, 1986). Promotion activity in these studies was evidenced by increased in tumor bearing animals (oral route), increase in tumors per animal (inhalation routes) and decreased tumor latency compared to those animals only exposed to the initiating agent (inhalation route) (Takahashi et al., 1986, Yanysheva et al., 1998; Iversen, 1986). Although these experiments do not indicate how formaldehyde acts as a

promoter in these systems, it is possible formaldehyde-induced mutation, increased cell proliferation or other toxic action could enhance tumor development from another agent.

4.5.3.1.8. *Localized immunosuppression*.

Formaldehyde exposure has induced localized immune suppression in experimental animals (Dean et al., 1984) and in exposed workers (Lyapina et al., 2004). Repeated inhalation exposures in rodents depopulated the URT and pulmonary tissues of resident macrophages, resulting in a transient decrease in POE host defenses (Admas et al., 1987). After cessation of exposure, the mononuclear phagocyte (MP) populations were replenished and there was a subsequent increase in host defense representing both increased MP numbers and increased bacteriocidal activity of the MPs. These data suggest that peak exposures of formaldehyde may present localized immunosuppression for components of the mononuclear phagocyte system (MPS) in tissues at the site of first contact.

A number of studies have evaluated the ability of formaldehyde to induce systemic immunotoxic effects in humans (Ohtani et al., 2004a, b; Erdei et al., 2003; Thrasher et al., 1990, 1987; Pross et al., 1987). Some studies have reported altered innate immune responses associated with formaldehyde exposure (Erdei et al., 2003), while others have noted adaptive immune response suppression associated with formaldehyde exposure (Thrasher et al., 1990, 1987) and changes associated with alterations to a predominant T—lymphocyte helper 2 (Th2) pattern (Ohtani et al., 2004a, b). In contrast, Pross et al. (1987) did not observe formaldehyde-associated changes in systemic immune function.

Numerous studies have reported increased respiratory tract infections in formaldehyde exposed individuals both in occupational and residential environments (Lyapina et al., 2004; Krzyzanowski et al., 1990; Holness and Nethercott, 1989). Incidences of physician-diagnosed chronic bronchitis were more prevalent in children (under age 15) living in homes with higher formaldehyde (>60 ppb) readings in the kitchen ($p < 0.001$) but this effect was more pronounced ($p < 0.001$) in children simultaneously exposed to environmental tobacco smoke (Kryzanowski et al., 1990). The prevalence of chronic cough was also increased in adults living in homes with measurable levels of formaldehyde, but data were not shown. Holness and Nethercott (1989) assessed chronic bronchitis in 87 funeral workers, where the average formaldehyde exposure was reported at 0.38 ± 0.19 ppm. Chronic bronchitis was observed in 20 funeral workers ($n = 87$) exposed to formaldehyde compared with 3 cases of chronic bronchitis in nonexposed referent controls ($n = 38$). A statistically significant association of self-reported chronic bronchitis and decreased resistance to URT infection was reported in formaldehyde exposed workers compared with controls ($p = 0.02$) (Lyapina et al., 2004). Of the workers, 41% had a history of chronic

respiratory infection and frequent long-lasting infectious inflammatory relapses (Group 1a). Another group (group 1b) consisted of 17 exposed workers, 12 of whom had no history of recurrent viral infections of the URT. There was a statistically significant association of frequency and duration of inflammatory relapses between groups 1a and 1b.

Lyapina et al. (2004) also reported effects of formaldehyde exposure on neutrophil respiratory burst activity (NRBA), the capacity of polymorphonuclear leukocytes to produce reactive oxygen radicals in response to chemical or microbial stimuli using flow cytometry. A suite of hematological tests and flow cytometric analysis for respiratory burst activity were performed. Although no significant difference was observed in the spontaneous and stimulated NRBA (median percentage of oxidizing cells) between the 29 exposed workers with URT inflammation and the healthy controls (0.83 vs. 1.35, respectively), a separate comparison of the NRBA of 12 workers with chronic, repeating URT infections and 17 workers with short, infrequent episodes of URT inflammations was significant (0.45 vs. 1.00, $p = 0.037$). When the NRBA of the group with chronic URT infections ($n = 12$) was separately compared with that of the healthy controls ($n = 21$), the results were also significant (0.45 vs. 1.35, $p = 0.012$). Individuals with chronic URT infections have reduced NRBA that could be due to formaldehyde exposure. Neutrophils respond to tissue damage or local invasion of microorganisms and act to phagocytize foreign cells. If neutrophilic activity is hampered or altered by formaldehyde exposure, then the ability to fight infection will be diminished, leading to prolonged infection. However, no dose-response pattern of formaldehyde exposure could be determined from this study.

4.5.3.1.8. *Potential for systemic transport of formaldehyde.*

In aqueous solution formaldehyde exists in equilibrium with its hydrated form methanediol (CH_2OH_2) ($K_d = 5.5x10\text{-}^4$). The equilibrium favors methanediol at physiological temperature and pH (>99.9%) and is readily reversible. In biological systems, as free formaldehyde is removed from aqueous solution through binding with serum proteins and cellular components, the equilibrium is reestablished by dehydration of methanediol to free formaldehyde. The reversible nature of this hydration reaction describes how a pool of free formaldehyde may be sustained in biological systems.

There is strong and consistent evidence in biological testing systems in vitro that treating cells with formaldehyde in an aqueous media results in significant cytoxicity, cell proliferation, clastogenic effects and clear evidence of mutational events (see Section 4.3). Similarly, animal bioassays where formaldehyde is administered in drinking water report portal of entry toxicity including hyperplasia, increased cell proliferation, focal lesions, and tumors (see Section 4.2.1).

It should be noted that URT tissues are covered by an aqueous mucous layer, through which formaldehyde must pass to react the cellular components of the URT. It has been postulated that formaldehyde transports through this mucous layer and the underlying tissues as methanediol (Georgieva et al., 2003).

The dynamic equilibrium between the hydrated and unhydrated forms of formaldehyde in biological systems is well understood. Since the hydration reaction favors methanediol, it is expected that exogenous formaldehyde which reaches the blood will primarily exist as methanediol and is subject to physiological elimination. As free, unhydrated formaldehyde continues to react with serum proteins and cellular components, the blood levels of methanediol are expected to reduce as it is dehydrated to maintain equilibrium. Although some attempts to measure significant changes in free formaldehyde levels in blood after inhalation exposure have not been successful, the half-life in blood has been measured after i.v. injection at approximately 2 minutes (McMartin et al., 1979). Additionally, the detection of antibodies to formaldehyde-hemoglobin adducts and formaldehyde-albumin adducts in exposures workers, smokers and laboratory animals exposed via inhalation provides direct evidence that formaldehyde is able to react with serum albumin and hemoglobin in biological systems (Thrasher et al., 1990, Grammer et al., 1990, 1993; Dykewicz et al., 1991; Varro et al., 1997; Li et al., 2007). These data support the hypothesis that exogenous formaldehyde may reach and transport through the blood. If so, formaldehyde (or methanediol) may reach sites distal to the portal of entry.

4.5.3.2. *Mode of Action Evaluation for Upper Respiratory Tract Cancer (Nasopharyngeal Cancer, Sino-nasal)*

From the above discussion, it can be seen that numerous mechanisms of action for formaldehyde-induced cancer can be reasonably supported based on various known biological actions of formaldehyde (e.g., mutation, cell proliferation, cytotoxicity, and regenerative cell proliferation). Additionally, alternative actions, such as immunosuppression or viral reactivation, are possible, although less data exist to evaluate these MOAs. Rather than a single MOA, it is plausible that a combination of these factors contribute to cancer incidence in an exposed population. Considering multiple factors may help to better understand the biological and mechanistic basis for the increases in cancer incidence observed in exposed human populations. Unlike animal bioassays, human epidemiological studies may reflect not only the effects of the agent of concern but also numerous other risk factors (e.g., viral status, diet, smoking, etc.). Additionally, human studies may be impacted by biological human variability across individuals, cancer biology (subtypes), wide variability in exposure regimens in human populations, etc. Therefore, if the purposes of exploring the carcinogenic MOA of an agent are to better understand the relevance of a given carcinogen to human populations and to inform the

exposure-response analysis, then discussions of MOAs which recognize the interaction of an agent with human variability and various risk factors is an appropriate analysis. For each of the below postulated MOAs, the detailed supporting evidence for the referenced key events are provided above in Section 4.5.3.1 (e.g., mutation, cell proliferation, cytotoxicity, and regenerative cell proliferation), and is not repeated here in detail each time a key event is proposed as part of a postulated MOA.

a) **Direct mutagenicity of formaldehyde in cells at the site of first contact:** Mutations, the permanent heritable changes in the genome of the cell, are a primary mechanism for the activation of oncogenes or the inactivation of tumor suppressor genes. Mutagenicity is the most widely recognized determinant of chemical-induced carcinogenicity, and it is difficult to set aside the relevance of direct formaldehyde-induced mutations from its demonstrated carcinogenicity. Formaldehyde-induced mutation in mucosal cells of the URT, throat and buccal cavity may serve to initiate cells, or provide subsequent mutagenic events to already initiated cells. Since the mucosal cells have proliferative capacity, and cell proliferation is a normal tissue function, mutations may be fixed and passed to daughter cells due to baseline cell proliferation of the tissue.

   Relevance to humans: This MOA is relevant to humans. The well-documented DNA reactivity (e.g., DPC and DNA adducts) and clastogenicity of formaldehyde in the URT of laboratory animals is a direct effect of formaldehyde on tissues of first contact. As this is a direct acting agent—no distribution or metabolism is required for the genotoxic action—there is little expected species variability. As discussed in Chapter 3, there are species differences in flux of formaldehyde into the respiratory mucosal tissues, but this introduces species differences in dosimetry—not mechanism. Finally, the clastogenic effects in nasal and buccal epithelial cells in formaldehyde- exposed workers confirms the direct genotoxic effects of formaldehyde at the first site of contact in humans.

b) **Decrease in DNA repair function within cells at the site of first contact:** A decrease in DNA repair capacity in these tissues by formaldehyde may increase total mutations over time due to either endogenous or exogenous sources of mutation. Although there are only a few studies which have explored the potential for formaldehyde to reduce DNA repair capacity, the evidence is positive, both in vitro testing systems, and in one study of occupationally exposed humans (Grafstrom, 1985; Hayes et al., 1997).

   Relevance to humans: This MOA is considered relevant to humans. The general population is exposed to various carcinogens, many with mutagenic potential, at sites of first contact including; air pollution, tobacco products, nitrosamines and viruses. Additionally, there are endogenous sources of DNA damage and mutagenicity in humans (e.g., lipid peroxidation, oxidative stress). The demonstration of reduced DNA repair activity (O6-alkylguanine-DNA alkyltransferase activity) in formaldehyde-exposed mortuary students suggests this toxic action of formaldehyde is possible in humans.

c) **Formaldehyde-induced cell proliferation:** Formaldehyde-induced cell proliferation in the oral and respiratory mucosa may be considered a key event in conjunction with the genotoxic effects, and induced mutational events observed with formaldehyde exposure.

This MOA is intended to describe events which occur below exposure levels which induce cell death and mucosal lesions. Therefore this MOA is comprised of two key events:

a. Formaldehyde-induced genotoxicity or mutation

b. Formaldehyde-induced cell proliferation

DNA replication during cell proliferation may serve to translate DNA damage or a formaldehyde-related DNA lesion into a permanent change in the sequence of nucleic acids during replication of the DNA—e.g. 'fix' a mutation from DNA damage. Additionally formaldehyde-induced cell proliferation may provide an opportunity for initiated cells to proliferate, increasing the potential for additional mutation events and transformation. The increased cell proliferation observed in the mucosal tissues in direct contact with formaldehyde during inhalation exposures may serve to amplify the risk of cell transformation from mutation alone. Researchers have noted that increased cell proliferation may be transient in some locations as adaptive responses compensate (Swenberg, 1983). However, evidence in both monkeys and rodents indicate that increased cell proliferation in repeated exposures across time do result in sustained cell proliferation. Data in Rhesus monkeys indicates increased cell proliferation is observed beyond the nasal cavities to the larynx, trachea and carina (first tracheal branching) (Monticello et al., 1989). Additionally, the authors note that cell proliferation is a more sensitive indicator of effects on the epithelium, observed even when minimal histological changes were present.

Human Relevance: Both formaldehyde-induced mutation and cell proliferation are direct effects on the oral and nasal mucosa, well documented in rodent models with supporting evidence in human epidemiological studies. Therefore both key events are relevant to humans. As noted above, there are species differences in localized flux of formaldehyde into the tissues of the oral and respiratory tract based on structural differences in the airways, as well as breathing patterns. Although these differences may effects the dosimetry of the formaldehyde absorption into the tissues, this only influences the magnitude of response at any given location. Data from exposed Rhesus monkeys which documents formaldehyde-induced cell proliferation in tissues beyond the nasal cavity, and tissues with minimal histological changes supports a role for cell-proliferation in the observed cancers in humans, which occur beyond the nasal cavities, and in tissues without formaldehyde-related focal lesions.

d) Cytotoxicity-induced cell proliferation (CICP): Cell death followed by compensatory cell proliferation is a reasonable MOA for agent-induced cancer. It should be noted that the exposure conditions which result in CICP in rodents is known to result in significant DNA reactivity and genotoxicity. Therefore, formaldehyde-induced mutations cannot be excluded from this MOA. The animal bioassays support the carcinogenic potential of formaldehyde in this context (Kerns et al., 1983; Selkemur et al., 1983; Monticello et al., 1986). The majority of squamous cell carcinomas (SCCs) seen in formaldehyde-exposed rats have been localized to the lateral meatus and mid-septum in the nasal passages (Morgan et al., 1986; Monticello et al., 1996), while polyploid adenomas have

predominantly been reported at the maxilloturbinates (Morgan et al., 1986; Monticello et al., 1996). Morgan et al. (1986) speculated that the maxilloturbinate was less susceptible to SCC due to metabolic differences. However, Monticello et al. (1996) later suggested that the smaller population of cells available at the maxilloturbinate accounted for fewer SCCs observed at that site. Regardless, for those locations where SCCs do arise in rats chronically exposed to formaldehyde, a clear temporal relationship can be demonstrated for dose regimens capable of producing sustained epithelial damage and sustained cell proliferation to eventual tumor development. Conversely, tumors are not observed in these rodent models at those sites in the nasal passages without sustained cell proliferation.

Relevance to humans: Human exposure to formaldehyde would most likely involve chronic exposures to indoor levels of formaldehyde, and episodic exposures in the environment or from an occupational exposure (see review in Chapter 2). An exposure scenario parallel to that used in chronic rodent bioassays is unlikely (e.g. 2-15 ppm 6−8 hours/day, 5 days/week for 10-30 months). Exposure conditions are difficult to assess especially in retrospective studies. However, only the most extreme industrial work conditions would result in human exposures similar to those that produce sustained compensatory cell proliferation in animal studies (i.e. 6-15 ppm 6 hours/day, 5 days per week). Gross tissue lesions as reported in rodents from repeated chronic exposures at 6 and 10 ppm formaldehyde have not been reported from workplace exposure, and only minor histopathological changes have been noted (Boysen et al., 1990; Holmström and Wilhelmsson et al., 1989). It is possible that workers were episodically exposed to formaldehyde levels which resulted in cell death and focal or gross lesions requiring cell proliferation for tissue remodeling or repair. However, it is unexpected that these conditions would be relevant to human environmental exposures. Therefore, although regenerative cell proliferation is retained as a reasonable MOA for formaldehyde carcinogenicity in experimental animals, it is unclear whether it is relevant to the extrapolation of health risks to formaldehyde exposures in the general environment.

e) Promotion: Several animal studies indicate that formaldehyde exposure may promote tumor formation due to other carcinogenic or initiating agents. There are positive data by several routes of exposure (oral, dermal and inhalation) and promotion has been reported as an increase in tumor bearing animals, an increase in tumors multiplicity or a decrease in tumor latency with formaldehyde exposure in conjunction with the initiating agent compared to tumors from the initiating agent along, or formaldehyde alone. The specific key events which may explain this promotion effect are unknown but may include several of the mechanisms discussed as potential MOAs for formaldehyde: mutagenicity, mitogenesis, cocarcinogenicity, immunosuppression. Promotion is considered here as a separate MOA, since these activities are noted for experimental conditions and tumor sites where formaldehyde did not induce tumors in the absence of the initiating agent.

Relevance to humans: Although the human epidemiologic literature doesn't address issues of tumor promotion, the nature of the cancers of concern indicate that chemical promotion may be relevant to cancer incidence for these sites. Many of the risk factors for nasopharyngeal cancer and other mouth and oral and URT cancers include direct mutagens (e.g., smoking, dietary nitrosamines) where a promoting agent would be expected to increase cancer incidence with these other risk factors. Additionally, the well

known viral risk factors for cancers of the mouth and URT also suggest a role for promoting agents to human cancer incidence. Although only tangential evidence, this does suggest that the promoting activity of a chemical agent, would be relevant to the agent's carcinogenicity at these sites. Therefore, the potential for formaldehyde to act as a promoter with other initiators—is considered relevant to formaldehyde's carcinogenic MOA.

f) **Increased URT infections/viral reactivation:** Inhalation exposure to formaldehyde has been shown to decrease the defenses of the body against infection through two mechanisms: (1) damage to the protective mucous barrier and function of the mucociliary apparatus; and (2) localized immunosuppression. These effects have been demonstrated in both exposed humans and controlled animal experiments. Additionally, increased respiratory tract infections are associated with formaldehyde exposure in several populations. Common viral agents (e.g., Epstein barr virus) are known risk factors for nasopharyngeal cancer, sinonasal cancers, and other URT cancers. Although direct evidence does support increased URT infections due to formaldehyde exposure, and URT infections are considered risk factors for URT cancers, direct evidence for formaldehyde-related infections leading to cancer is lacking. There is however one epidemiological study which finds the association between formaldehyde and nasopharyngeal cancer is strengthened in Epstein barr virus sero-positive cases versus sero-negative cases. These data suggest a possible role for formaldehyde in infection, viral reactivation, or cocarcinogenicity with a viral agent.

Relevance to humans: The potential role of increased URT infections and immunosuppression at the portal of entry is considered to relevant to humans. Data in humans are available to support both key events in this MOA. Additionally, epidemiological studies are conducted in human populations where individuals may be exposed to various viral agents across the study period. Therefore, toxic actions by formaldehyde which may increase URT infections, or viral-reactivation at the site of first contact, could be acting in conjunction with viral agents to contribute, in part, to observed associations between formaldehyde exposure and increased URT cancer.

Summary and integration of key events

Each of the hypothesized MOAs discussed above to better understand the carcinogenic potential of formaldehyde is supported by formaldehyde-specific evidence, either in animal studies, human studies, or both. For those key events studied in animal models such as cell proliferation, genotoxicity, degradation of the mucociliary apparatus and CICP, supporting evidence is available in more than one species, multiple strains (e.g., rats) and has been reported by multiple researchers. Therefore the overall database supporting these key events in laboratory studies, and their corresponding MOAs is fairly large. In contrast, some key events relevant to humans, but less studied in animal models may have a small supporting database (e.g., increased respiratory tract infections). These alternative MOAs are retained as potentially relevant to the carcinogenic action of formaldehyde as the intent of this discussion is to identify modes of action

will may contribute to the observation of increased upper respiratory tract cancers in exposed human populations. It is noted that additional study is needed to better understand the range of effects formaldehyde may have at sites of first contact in humans.

The MOAs which include genotoxicity, mutation, decreased DNA repair, increased cell proliferation and CICP are interrelated. Conditions which provide both a source of cell proliferation and increased mutation would be expected to increase neoplastic transformation. Formaldehyde acts on the target tissue, the respiratory epithelium, to induce each of these events. However, these key events operate across different exposure ranges and present different exposure response relationships. For example, formaldehyde-induced mutations would be expected across the exposure range, where any incremental increase in genotoxicity and formaldehyde-related mutation would contribute to background levels, with the potential to increase cancer risk incrementally. In contrast, focal and gross lesions to the respiratory mucosa due to cytolethality are not observed unless exposure concentrations are sufficient to provide localized tissue doses (flux) required to result in cell death and related compensatory cell proliferation. Since tissue dose (flux) is dependent on not only exposure concentration but also duration of exposure and location in the respiratory tract (see Section 3.4), and varies by species, correlation of exposure concentrations to tissue responses directly are complex. Exposure response relationships for the key events (cell proliferation, genotoxicity, degradation of the mucociliary apparatus and CICP) are reported by exposure concentration, not tissue flux, which would be a more biologically relevant measure.

Although the tissue dose-response relationships for formaldehyde induced mutation, mitogenesis and cytolethality are different, the effects at the tissue level cannot be easily disaggregated. At any given exposure concentration, target cells in the respiratory tract will experience different effective tissue concentrations of formaldehyde. Measurement of cell proliferation, DNA protein crosslinks, or genotoxicity may require examining a population of cells which would have been subject to different flux rates of formaldehyde (see Chapter 3). Similarly, when evaluating the tumor dose response, cells within the target tissue will represent a range of target tissue formaldehyde concentrations. Therefore, an integrated MOA scheme is hypothesized where key events may influence the observed tumor response differentially across the exposure response range (see Figure 4-36). This schematic illustrates the potential for genotoxicity and formaldehyde-induced mutation to occur where tissue dose (flux of formaldehyde into the tissue is minimal). Where tissue dose is increased, formaldehyde-induced cell proliferation is observed in addition to genotoxicity. As tissue dose increases and formaldehyde effects on the respiratory mucosa are more severe, gross pathology including focal and gross lesions due to cell death are noted. Therefore, several of the MOAs presented above

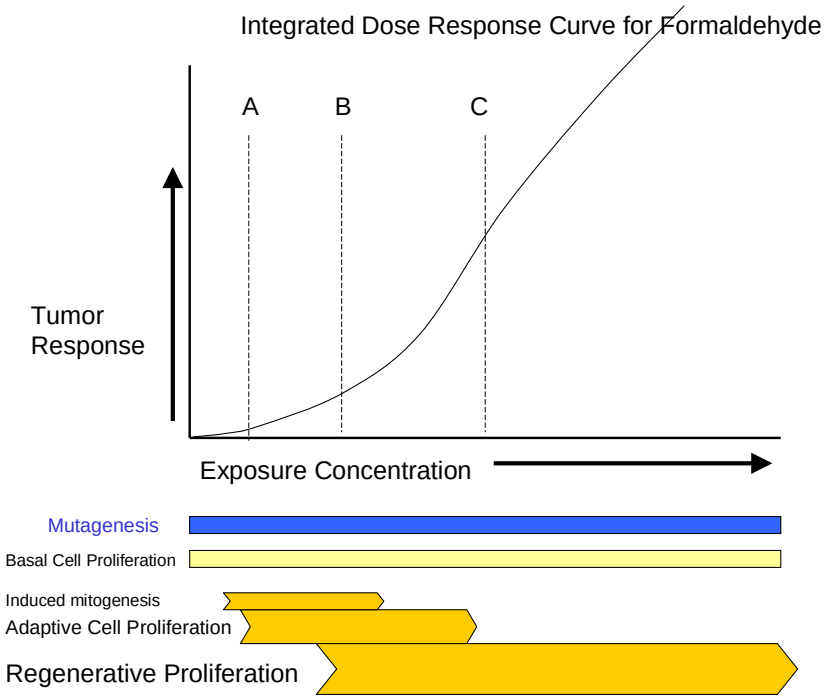

Figure 4-36. Integrated MOA scheme for respiratory tract tumors.

may be operative and relevant to human exposures at exposure levels resulting in minimal tissue flux—(a) direct formaldehyde genotoxicity and resulting mutation, (b) inhibition of DNA repair and c) formaldehyde induced cell proliferation in conjunction with mutation. CICP, which involves localized and gross tissue lesions, would be operative at higher exposure levels. There is little data to inform the dose range over which the remaining hypothesized MOAs may operate (promotion and increased respiratory tract infections/viral action).

4.5.3.3. *Mode(s) of Action for Lymphohematopoietic Malignancies*

4.5.3.3.1. *MOA evaluation for Leukemia.*

Leukemia may arise from stem cells or progenitor cells in the bone marrow (e.g., acute and chronic myeloid leukemia) or from mature lymphocytes (e.g., chronic lymphatic leukemia, hairy cell leukemia) (see Figure 4-32, Section 4.5.2). Although there is a consistent association between formaldehyde exposure and forms of leukemia when considered as group of diseases (see Table 4-91, Section 4.5.2), the strongest and most consistent associations are seen specifically with myeloid leukemia. Little evidence supports an association between formaldehyde exposure and other specific leukemia subtypes, although two studies support a strong association between formaldehyde and "other leukemia and unspecified leukemia (ICD-9

code 207). Therefore, this MOA evaluation will focus on mechanisms which may impact all forms of leukemia (e.g., bone marrow toxicity) or those specific to myeloid leukemia. The mechanistic data supporting the key events in this analysis are presented in Section 4.5.3.1. For each of the below postulated MOAs, the detailed supporting evidence for the referenced key events are provided above in Section 4.5.3.1 (e.g., mutation, cell proliferation, cytotoxicity, and regenerative cell proliferation), and is not repeated here in detail each time a key event is proposed as part of a postulated MOA.

a) **Direct effects of formaldehyde on a circulating stem cell or progenitor cell present at the portal of entry:** Hematopoietic stem cells do circulate throughout the body and can be harvested from peripheral blood. Formaldehyde exhibits a range of toxic effects at the site of first contact including genotoxic effects believed to be mediated by direct DNA reactivity (see Section 4.3). Formaldehyde is known to directly react with blood components in formaldehyde exposed humans and animals resulting in both hemoglobin and albumin adducts (Thrasher et al., 1990, Grammer et al., 1990; Grammer et al., 1993; Dykewicz et al., 1991; Varro et al., 1997; Li et al., 2007). Therefore, it has been hypothesized that formaldehyde could react with DNA in circulating hematopoietic stem cells (Zhang et al., 2009) resulting in heritable mutations which may contribute to leukemia incidence. Recently, Zhang et al. (2010a) have tested the hypothesis that exogenous formaldehyde may damage circulating stem cells. Clastogenic effects were found in circulating hematopoietic stem cells cultured from formaldehyde exposed workers. The reported aneuploidy was demonstrated as significant increases in both monosomy 7 and trisomy 8. These specific chromosomal changes are consistent with those reported for agent-induced myeloid leukemia (Zhang et al., 2010a).

   Relevance to Humans: This hypothesized MOA is considered relevant to humans. Supporting evidence is found in humans for formaldehyde direct reactivity with blood proteins (e.g., albumin and hemoglobin) as well as clastogenic effects in circulating hematopoietic stem cells in formaldehyde exposed workers.

b) **Bone marrow toxicity:** Direct bone marrow toxicity is the most studied leukemogenic action for an endogenous agent (e.g., benzene, ionizing radiation). It is believed that an agent which exerts its toxicity on the bone marrow, resulting in translocations and heritable mutations in hematopoietic stem cells may cause leukemia. It has been hypothesized that formaldehyde may transport to the bone marrow in its hydrated form (methandiol) and react with cellular proteins, and DNA causing direct effects on components of the bone marrow. A study by Ward et al. (1983) has shown that mice given a single dose of formaldehyde, or methanol by gavage were able to develop cytogenetic changes (SCEs, CAs and aneuploidy) in bone marrow cells suggesting that formaldehyde can reach to bone marrow and induce toxicity. Pancytopenia (a reduction in blood borne cells formed in the bone marrow) is a symptom of direct bone marrow toxicity and is observed with other leukemogenic agents (e.g., benzene, ionizing radiation). A recent review of 8 published studies of formaldehyde exposed workers in China by Tang et al. (2009) indicates 7 of the studies provide evidence of reduced white blood cell counts, platelet levels and hemoglobin levels associated with formaldehyde

exposure. A study of occupationally exposed nurses provided a correlation between decreased white blood cell counts and formaldehyde exposure (Kuo et al., 1997). A recent study by Zhang et al. (2010a) provides the best evidence for bone marrow toxicity, where they report not only a reduction in white blood cell counts, but reductions in cell counts of all the blood cells, as well as increased mean cell volume. Although these reductions did not meet the clinical definition of pancytopenia (when averaged across the study population), reduction of all blood borne cells formed in the bone marrow is consistent with the bone marrow toxicity associated with pancytopenia seen with other leukemogens (Zhang et al., 2010b).

Relevance to Humans: This hypothesized MOA is considered relevant to humans. Supporting evidence is found in humans for bone marrow toxicity in formaldehyde exposed workers.

4.5.3.3.2. *MOA evaluation for Lymphomas (e.g., Hodgkin's lymphoma, Multiple myeloma).*

The general MOA for formaldehyde is based on direct chemical reactivity and toxic effects at the portal of entry (POE). Formaldehyde is directly and indirectly genotoxic, and reacts with cellular proteins and DNA in cells which it comes into contact. Additionally, immunosuppression, viral reactivation and promotion effects are relevant to lymphoma and related malignancies. Therefore, the key events for the adult cell lymphoid cancers would include these actions. For each of the below postulated MOAs, the detailed supporting evidence for the referenced key events are provided above in Section 4.5.3.1 (e.g., mutation, cell proliferation, cytotoxicity, and regenerative cell proliferation), and is not repeated here in detail each time a key event is proposed as part of a postulated MOA.

Lymphoid tumors (e.g., lymphocytic leukemia, B-cell lymphoma, mantle cell lymphoma [a rare form on non-Hodgkin's lymphoma] and myeloma) may arise from cells present at the portal of entry (POE) (see Figure 4-32). The location and function of mature lymphocytes contribute to their vulnerability to transformation by agents at the POE. Therefore, a brief summary of the immuno-biology of these cells is provided in order to provide context for the MOA evaluation:

***Location*:** Lymphocytes are present in the oral and respiratory tract epithelium, as well as in cell aggregates and tertiary immune structures (e.g., germinal centers) in the mucosal tissues (Zuercher and Cebra, 2002; Zuercher et al., 2002; Wu et al., 1997; Kupper et al., 1990). These mucosa-associated lymphoid tissues (MALT) provide the opportunity for formaldehyde to directly interact with components of the immune system present at the POE (Wu et al., 1997, Claeys et al., 1996, Park et al., 2003; Fujimura, 2000). Intraepithelial lymphocytes are present in the pseudostratified epithelium of the nasopharyngeal passages and there are aggregates of immune cells and germinal cells present in these tissues. Crypts containing mature lymphocytes exist at the surface of the nasal epithelium (Fujimura, 2000). Microfold cells or M-cells form the crypts, where the

lymphocytes are covered by a thin membrane (see Figure 4-37). Functionally, these lymphocytes identify and process foreign antigens at the POE (Fujimura, 2000). Therefore the mature lymphocytes within these crypts, exposed to exogenous agents, are involved in active immune responses to foreign antigens.

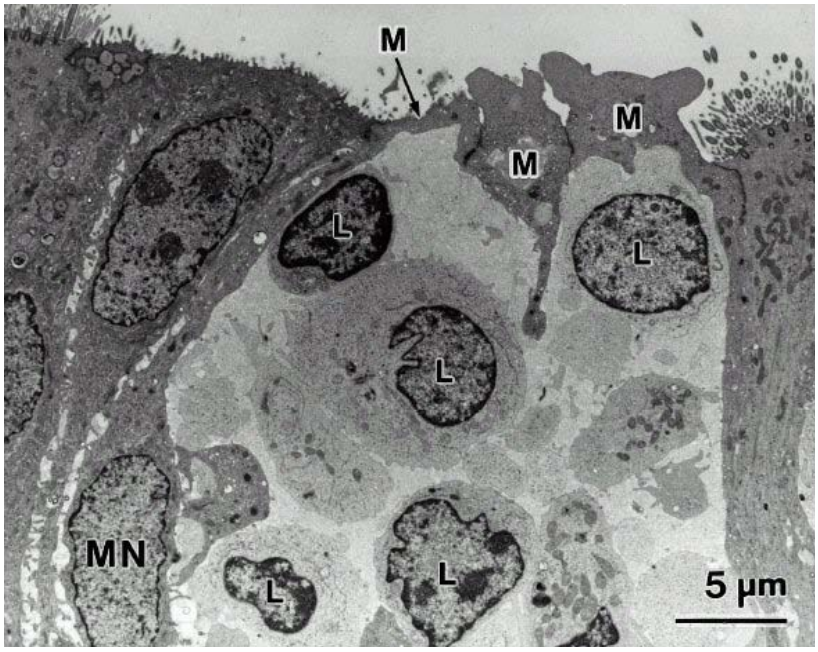

Figure 4-37. Location of intraepithelial lymphocytes along side epithelial cells in the human adenoid.

Source Fujimura et al., 2000

***Clonal expansion*:** Mature lymphocytes (both B and T-cells) clonally expand their populations in response to an exogenous antigen stimulation when a humoral immune response is stimulated. Therefore cell proliferation is a normal function of these mature lymphocytes and occurs every time there is an infection. Cell proliferation of mature B and T-cells, responsive to a particular antigen, occurs in active germinal centers (including those within the respiratory tract). Cells may be exposed to exogenous agents during the immune response, or cells responding in the germinal center may have previously been in the epithelium or M-cell crypt.

***Somatic hypermutation*:** Normal immune function includes the process of somatic hypermutation where B-cells undergo DNA rearrangement of the variable region genes to produce novel antibodies specific to a given antigen. This process is key to adaptive immunity and demonstrated by the basic principles of immuno-biology which underlie vaccination theory. Gene sequencing of adult B-cell lymphomas and leukemias indicate that the chromosomal regions involved in somatic hypermutation correspond to known oncogenes in these cancers. The vulnerability of these processes is evidenced by the

observation that approximately 90-95% of adult lymphomas and leukemias are of B-cell origin (Gordon et al., 2003). Formaldehyde-induced protein-protein crosslinking could disrupt cellular processes including somatic hypermutation and cell mitosis, resulting in agent-induced translations similar to those found in spontaneous B-cell malignancies.

Postulated MOAs for lymphomas (e.g., Hodgkin's lymphoma, multiple myeloma): For each of the below postulated MOAs, the detailed supporting evidence for the referenced key events are provided above in Section 4.5.3.1 (e.g., mutation, cell proliferation, cytotoxicity, and regenerative cell proliferation), and is not repeated here in detail each time a key event is proposed as part of a postulated MOA.

a) Direct or indirect formaldehyde-induced mutations in cells at the site of first contact: Immune cells including intraepithelial lymphocytes, and cells in MALT are collocated with the epithelial cells from which URT cancers arise (see Figure 4-37). Therefore direct and indirect mutagenic potential of formaldehyde is equally applicable to components of the immune system present in these tissues. Mutations, the permanent heritable changes in the genome of the cell, are a primary mechanism for the activation of oncogenes or the inactivation of tumor suppressor genes. Mutagenicity is the most widely recognized determinant of chemical-induced carcinogenicity, and it is difficult to set aside the relevance of direct formaldehyde-induced mutations from its demonstrated carcinogenicity. Formaldehyde-induced mutation in immune cells present at the site of first contact, may provide initiated cells or subsequent mutagenic events to already initiated cells. The competence of the immune system relies on the proliferation of peripheral blood lymphocytes in response to immune challenge (e.g. infection). Additionally, heritable changes to the variable gene regions in B-cells generated during somatic hypermutation are essential to adaptive immunity (e.g., immunization) demonstrating that permanent heritable changes in the DNA of peripheral B-cells are passed to daughter cells and retained in the body for decades. Any agent-induced mutations would be similarly propagated and retained with the potential to contribute to the transformation of mature lymphocytes.

Relevance to humans: This MOA is relevant to humans. The well-documented DNA reactivity (e.g., DPC and DNA adducts) and clastogenicity of formaldehyde at the POE in laboratory animals is a direct effect of formaldehyde on tissues of first contact, and these mechanisms are considered relevant to humans. As with epithelial cells, clastogenic effects in peripheral lymphocytes are documented in formaldehyde-exposed students and workers, confirming the genotoxic effects of formaldehyde in immune cells, from which lymphomas and related hematopoietic diseases may arise (see Section 4.5.2, Figure 4-32).

b) Formaldehyde-induced protein-protein crosslinks may disrupt somatic-hypermutation: Although not as well studied as DNA-protein crosslinks, formaldehyde also forms crosslinks between amino acids on proteins (see Section 4.5.3.1.3 for details). Specific oncogenes for malignancies which arise from mature B-cells are linked to errors in the process of somatic hyper-mutation (Greaves et al., 2004). If formaldehyde creates protein crosslinks in competent B-cells which affects the process of DNA rearrangement, formaldehyde may generate translocations and related oncogenes similar to those observed in spontaneous B-cell malignancies.

Relevance to humans: This hypothesis has not been tested in either exposed human or animal test systems. However, the link between somatic-hypermutation and B-cell oncogenes is well established and perturbation of this process by an exogenous agent is a reasonable extension of the current understanding of the etiology of B-cell malignancies.

c) **Increased URT infections/viral reactivation:** Inhalation exposure to formaldehyde has been shown to decrease the defenses of the body against infection through two mechanisms: (1) damage to the protective mucous barrier and function of the mucociliary apparatus in the nasal passages; and (2) localized immunosuppression (see Section 4.5.3.1). These effects have been demonstrated in both exposed humans and controlled animal experiments. Additionally, increased respiratory tract infections are associated with formaldehyde exposure in several populations. Common viral agents (e.g., Epstein barr virus) are known risk factors for malignancies which arise from mature lymphocytes. Thus, increased URT infections or viral reactivation due to formaldehyde exposure may influence the incidence of these cancers.

Relevance to humans: The potential role of increased URT infections and immunosuppression at the portal of entry is considered to be relevant to humans. Data in humans are available to support both key events in this MOA. Additionally, coexposure to infectious agents (including viruses) would be expected in participants in an epidemiological study, suggesting an MOA which acted in conjunction with infectious agents may be relevant to agent-induced cancer. Therefore, immunotoxic actions by formaldehyde which may increase URT infections, or viral-reactivation at the site of first contact, could be acting in conjunction with viral agents to contribute, in part, to observed associations between formaldehyde exposure and increased lymphoma and related diseases.

4.5.3.3.3. *Summary and evaluation of hypothesized MOA(s) for Lymphohematopoietic Malignancies.*

The well-documented direct toxic action of formaldehyde on cells at the site of first contact is a general effect based on the reactivity of formaldehyde with cellular components (e.g., proteins and DNA) (see Section 4.5.3.1). As a general effect, it is reasonable that these toxic effects would be relevant to all cells which come into contact with formaldehyde. The current debate regarding the biological plausibility of formaldehyde-induced lymphohematopoietic malignancies centers around a perspective that the diseases within this general grouping are systemic cancers arising only out of bone marrow toxicity (Heck et al., 2006, Pyatt et al., 2008) and that it is implausible for formaldehyde to induce bone marrow toxicity. The above MOA evaluation expands the current debate by considering the impact of POE toxicity on elements of the immune system and cancers might arise from these cells (see Section 4.5.3.3.2) and by presenting data which support the observation that formaldehyde is associated with bone marrow toxicity and damage to circulating stem cells in exposed humans (see Section 4.5.3.3.1).

As significant increases in free formaldehyde in peripheral blood from exogenous exposure has not been detected (Heck et al., 1987), it has been hypothesized that formaldehyde does not transport and therefore cannot exert toxic effects outside of the tissues at the site of first contact (Heck et al., 2006, Pyatt et al., 2008). In contrast to this hypothesis, at least one study by Ward et al. (1983) in rats has shown that orally administered formaldehyde was able to induce bone marrow genotoxicity, and also effects are seen in formaldehyde-exposed humans, which indicate systemic effects on the hematopoietic system including reduced white blood cell counts, clastogenic effects in peripheral blood lymphocytes and aneuploidy in circulating stem cells (Tang et al., 2009, Zhang et al., 2010a; Section 4.5.3.1). These observed effects in humans are consistent with agent-induced bone marrow toxicity and are observed with other well-studied exogenous leukemogens (e.g., benzene and ionizing radiation). It is unknown if formaldehyde is distributed systemically to exert its effects directly on cells in the bone marrow or if damage to circulating stem cells or progenitor cells would be sufficient to result in the observed effects in humans (Zhang et al., 2010b). Additional research is needed to better determine the potential for systemic transport of formaldehyde considering both detection of its hydrated form (methylene glycol) as well as formaldehyde protein adducts (e.g., formaldehyde-GSH, formaldehyde-Hb and formaldehyde-albumin). Similarly the results of Zhang et al. (2010a) need to be extended (analysis for additional chromosomal aberrations) and repeated. Although further evidence is needed to better understand the hypothesized mechanisms for formaldehyde-induced effects on hematopoietic stem cells, the observed hematologic effects in humans cannot be set aside. Therefore, however unlikely, the current data support the biological plausibility of formaldehyde effects on the hematopoietic system.

4.5.4. Hazard Characterization for Formaldehyde Carcinogenicity

Formaldehyde is Carcinogenic to Humans by the Inhalation Route of Exposure

Human epidemiological evidence is sufficient to conclude a causal association between formaldehyde exposure and nasopharyngeal cancer, nasal and paranasal cancer, all leukemias, myeloid leukemia and lymphohematopoietic cancers as a group. Epidemiological evidence is also strongly supportive of, but in itself not sufficient for, a conclusion of causal association for other upper-respiratory tract cancers, Hodgkins lymphoma, or multiple myeloma. Animal bioassays consistently demonstrate formaldehyde-induced nasal cancers in rodents which provide strong support for the observed upper respiratory tract cancers in humans. Limited evidence from animal bioassays is available to support the conclusion from human epidemiologic data that formaldehyde causes some types of lymphohematopoietic cancers.

The epidemiologic evidence is sufficient to characterize the association between formaldehyde exposure and nasopharyngeal cancer as causal in humans, based on the results from a large and well-followed longitudinal cohort study of industrial workers and several case-control studies (Hauptmann et al., 2004; Vaughan et al., 2000; West et al., 1993; Vaughan et al., 1986b). The epidemiologic evidence of association between formaldehyde exposure and other upper respiratory tract cancers (see Section 4.1.2.1.3) is consistent with, and supportive of, a causal association but insufficient on its own to reach a causal conclusion. Case-control studies have demonstrated associations between formaldehyde exposure and rare cancers of the URT. Luce et al. (2002) evaluated pooled data from 12 case-control studies and demonstrated a statistically significant increased risk between formaldehyde exposure and sinonasal cancer. Hypopharyngeal cancer was linked with formaldehyde exposure with an OR of 3.78 (95% CI: 1.50−9.49) in another case-control study (Laforest et al., 2000). Hauptmann and colleagues (2004) concluded that in spite of the small numbers of deaths from cancers of the URT, the positive associations with average intensity and peak exposure were consistent with the carcinogenicity of formaldehyde at these sites of first contact. The finding that formaldehyde inhalation causes nasal squamous cell carcinoma in animals (see Section 4.2.1.2) further supports a causal association of formaldehyde exposure and increased risk of upper respiratory tract cancer in humans. Both humans and animals developed tumors within the upper respiratory tract, the POE site expected to receive direct exposure to formaldehyde.

Also, overall, there is a consistent association between formaldehyde exposure and various forms of lymphohematopoietic (LHP) cancers, with all leukemias, myeloid leukemia specifically, Hodgkin's lymphoma and multiple myeloma demonstrating the greatest strength and consistency of results. Where exposure-response data exist, exposure-response trends have been seen for all LHP malignancies, all leukemia, myeloid leukemia and Hodgkin's lymphoma (Pinkerton et al., 2004; Beane-Freeman et al., 2009). Taken together, the data demonstrate a consistent association, across various worker populations, with the expected temporal association to exposure and defined exposure−response relationships in two different worker cohorts. The strongest associations tend to be with myeloid leukemia and Hodgkin's lymphoma. The criterion of reasonable biological plausibility is easily met for the majority of the diseases which contribute to an observation of all LHP cancers, specifically the cancers derived from mature lymphocytes. Although it is largely unknown how inhalation exposure to formaldehyde would influence bone-marrow derived malignancies, new evidence supports formaldehyde-induced bone marrow toxicity as well as damage to circulating stem cells which may be of importance to formaldehyde's leukemic potential (Zhang et al., 2010, Tang et al., 2009). Limited support for the potential for formaldehyde-induced LHP cancer is found when considering animal bioassays,

where formaldehyde exposure influenced leukemia and lymphoma incidence in female animals of two species (rats and mice) in a long-term bioassay (Battelle Laboratories, 1981).

4.6. SUSCEPTIBLE POPULATIONS

"Susceptible subpopulations" is used here to refer to factors, such as life stage, genetics, health status, etc., that may predispose individuals to greater response to an exposure. This greater response could be achieved either through differences in exposure to the chemical or differences in underlying toxicokinetic (TK) and toxicodynamic (TD) differences between the susceptible and other populations. For example, life stages may include the developing individual before and after birth up to maturity (e.g., preconception, embryo, fetus, young child, adolescent), adults, or aging individuals. Another susceptibility factor is genetics. Specifically, susceptible subpopulations may also include people with specific genetic polymorphisms that render them more vulnerable to a specific agent or people with specific diseases or pre-existing conditions (e.g., asthmatics). The term may also refer to gender differences, lifestyle choices, or nutritional state (U.S. EPA, 2002, Section 4.3.2.3).

A discussion of a comprehensive list of all possible susceptibility factors affecting exposure and response to formaldehyde, or any chemical, is not possible. Therefore, the discussion of susceptibility factors focuses on (1) factors hypothesized to be of importance to formaldehyde; and (2) factors for which there are available formaldehyde data. A partial list of these factors includes gender, genetic polymorphisms, preexisting disease status, nutritional status, diet, and previous or concurrent exposures to other chemicals. Qualitatively, the presence of multiple susceptibility factors will increase the variability that is seen in a population response to formaldehyde toxicity.

4.6.1. Life Stages

Individuals at different life stages are physiologically, anatomically, and biochemically different. Examples include physiological changes that occur through the lifespan (Selevan et al., 2000). They may also have distinctive exposure pathways (i.e., transplacental, breast milk ingestion), and exhibit differences in behavior (U.S. EPA, 2006b; NRC, 1993). Early life stages (i.e., during development, prior to mature adulthood) and the later life stages (i.e., aging) differ greatly from mature adulthood in body composition, organ function, and many other physiological parameters that can impact the TK and/or TD of chemicals and their metabolites (ILSI, 2003). This section presents and evaluates the pertinent published literature available to assess whether and how individuals of differing life stages may respond differently to formaldehyde.

4.6.1.1. *Early Life Stages*

4.6.1.1.1. *Factors influencing exposure and dosimetry.*

For all life stages, the primary exposure routes for formaldehyde include inhalation and, in some cases, ingestion (see Chapter 5). Some exposure scenarios may be child specific. For example, to the extent that the presence of baby furniture produced with formaldehyde in a child's house contributes to greater concentrations in a child's room, exposures for very young children in those circumstances may be increased (Environment California, 2008). As with all chemicals, placental transfer and breast milk ingestion are exposure pathways that are unique to early life stages. Studies assessing early life stage exposure pathways to formaldehyde have not been performed. Presumably, unmetabolized formaldehyde reacts too quickly to be effectively transported from mother to fetus by placental transfer; in addition, formaldehyde is not lipophilic and is therefore unlikely to accumulate in breast milk. However, the relevant dose metric for formaldehyde-related effects may vary depending on the specific target of concern (e.g., direct toxicity at the portal of entry versus systemic effects); insufficient information is currently available to determine whether individuals in different life stages are at higher risk for exposure to specific target tissues.

There are some calculations however which shed light on lifestage differences in the inhaled tissue dose at the portal of entry. Using respiratory tract surface areas and ventilation rates reported in the literature and the scheme in EPA (1994), Ginsberg et al. (2005) calculated that overall extrathoracic absorption of highly reactive and soluble gases is similar in adults and children. These results are in agreement with that of Garcia et al. (2009) who used computational fluid dynamics to study differences in the nasal dosimetry of reactive, water-soluble gases between 5 adults and 2 children, aged 7 and 8 years old. Overall uptake efficiency, average flux (rate of gas absorbed per unit surface area of the nasal lining) and maximum flux levels over the entire nasal lining did not vary substantially between adults and children (1.6-fold difference in average flux and much less in maximum flux). On the other hand, the local flux of formaldehyde varies between the two children by a factor of 2 to 4 at various distances along the septal axis of the nose. The results in Garcia et al. (2009) have been further described and evaluated in Appendix C.1. Under normal resting breathing conditions, it is expected that very little formaldehyde is delivered to the lung. However, under higher activity as well as mouth

breathing scenarios, both of which appear likely to happen more regularly in children, formaldehyde dose to the lung will be substantial.[7]

The toxicokinetic characteristics of formaldehyde are described in Chapter 3, with absorption and distribution studies discussed in Sections 3.2 and 3.3. Studies to assess differential absorption or distribution of formaldehyde in early life stages have not been performed and represent a significant data gap. The metabolism of formaldehyde is described in Section 3.4. Expression of the enzymes that metabolize formaldehyde (ALDH2 and FALDH, and specifically ADH3) is known to be developmentally regulated and thus may alter the TK of formaldehyde in early life stages. ADH3 is ubiquitously expressed and is present in the human fetus, neonate, and 1- to 10-year-old children (Hines and McCarver, 2002; Estonius et al., 1996). During early development in rodents, when neurulation first begins and forms collections of somites along the neural tube, ADH3 activities are significantly lower (at 8−10 and 11−13 somite stages) and suggest a decreased ability to detoxify formaldehyde in the early embryo (Harris et al., 2003). ADH mRNA expression levels appear to be age related, with decreased expression of ADH common in premature neonates and infants up to 5 months old. Thereafter, ADH expression increases and is dependent on body weight (Ginsberg et al., 2004). Benedetti et al. (2007) reported that decreased ADH expression persisted until age 2 to 5 years. Westerlund et al. (2005) tracked the ontogeny of ADH3 specifically and reported that ADH3 expression was ubiquitous in mouse and rat embryos and was the only ADH enzyme to be consistently localized to brain tissue, suggesting a housekeeping function. Thus, neonates and very young children may have a decreased ability to metabolize formaldehyde due to differential expression of ADH3 in development compared that of with adults; however, activity levels of this enzyme and alternate pathways specific to children are not available in the literature.

4.6.1.1.2. *Life-stage exposure and adverse health outcomes.*

In general, exposure to toxic agents during early development (i.e, preconception, prenatal stages, or postnatal development) may affect organ development and may also lead to increased disease susceptibility later in life. Following early life stage exposure to formaldehyde, a number of adverse health outcomes have been observed, including alterations in the respiratory, reproductive, and neurological systems. For example, the developing respiratory tract may be more vulnerable to insult compared with an adult respiratory tract, and thus, increase the severity of response. The potential for reproductive and developmental toxicity of formaldehyde is discussed in detail in Sections 4.1.1.7 (human studies) and 4.2.7 (animal

[7] For example, in the case of ozone concentrations of 0.1 ppm, a moderately reactive gas, Ginsberg et al. (2008) predict a 5-fold variation in the dose to the deep lung between quiet and heavy breathing conditions for an 8-year old child.

studies), while effects on the nervous system are discussed in Sections 4.1.1.6 and 4.2.6 (human and animal studies, respectively). The specific case of formaldehyde exposure and pulmonary effects is discussed in detail in Sections 4.1.1.1 to 4.1.1.4 and 4.2.1.1 to 4.2.1.4. A brief summary of identified effects of formaldehyde that may indicate susceptibility during particular life stages is provided below.

4.6.1.1.2.1. ***Preconception.***

Exposure prior to conception may damage reproductive organs and/or germ cells that could affect reproduction and/or damage the genetic makeup of the offspring. Effects on reproduction are discussed in Sections 4.1.1.7 and 4.2.7. In summary, an epidemiological study (Taskinen et al., 1999) reported significantly delayed conception among female workers exposed to formaldehyde at average daily ambient formaldehyde levels; these effects could be consistent with adverse effects on either preconceptional and/or gestational exposure. One animal study (Maronpot et al., 1986) reported endometrial hypoplasia and lack of ovarian luteal tissue in female mice exposed for 13 weeks to 40 ppm formaldehyde via inhalation, suggesting the potential for treatment-related alterations to the female reproductive system. Since the exposure was to the adult, the findings suggest that preconceptional formaldehyde exposure caused female reproductive system effects that in turn could affect pregnancy. In the rodent study of Kitaev et al. (1984), a three-fold increase in embryo degeneration on gestational days 2−3 was observed after formaldehyde exposure to the dams during premating. Since the exposure was to the adult in these three studies, the findings suggest that preconceptional formaldehyde exposure caused female reproductive system effects and/or affected the gametes.

4.6.1.1.2.2. ***Prenatal.***

A population-based study (Gražulevičiene et al., 1998) found an association between atmospheric formaldehyde exposure and low birth weight, yielding an adjusted OR of 1.37 (95% CI: 0.90−2.09). Three studies (Dulskiene and Gražulevičiene, 2005; Taskinen et al., 1994; Hemminki et al., 1985) that examined the effect of occupational exposures on the incidence of congenital malformation produced mixed results.

Results from Taskinen et al. (1999) support associations between formaldehyde exposure, subfertility, and spontaneous abortion. Subfertility and spontaneous abortion are biologically linked (subclinical pregnancy losses are increased among women with fertility problems) (Gray and Wu, 2000; Hakim et al., 1995), and both subfertility and spontaneous abortion may be related to sensitivity to environmental agents (Correa et al., 1996).

Two experimental animal studies (Martin, 1990; Saillenfait et al., 1989) evaluated a standard battery of developmental endpoints following inhalation exposure on GDs 6−10, but effects were minimal. Similarly, Chernoff and Kavlock (1982), Marks et al. (1980), and Hurni and Ohder (1973) reported minimal reproductive or developmental effects in rodents in studies in which dams were exposed orally during early gestation. When formaldehyde was administered via inhalation throughout gestation in female rats, some developmental effects, including increased pup weight and decreases in lung and liver weight in newborns, were reported at 0.01 and 0.4 ppm (Senichenkova and Chebotar, 1996; Senichenkova, 1991; Kitaev et al., 1984; Gofmekler and Bonashevskaya, 1969; Gofmekler, 1968; Pushkina et al., 1968). Two studies also reported changes in motor activity in offspring of dams exposed via inhalation to 0.4 ppm formaldehyde during gestation (Senichenkova, 1991; Sheveleva, 1971).

4.6.1.1.2.3. *Postnatal.*

Following early life stage exposure to formaldehyde, a number of adverse postnatal outcomes are possible, including effects on the developing and adult respiratory, reproductive, and neurological systems. The potential for increased risk of childhood cancer is also discussed below.

4.6.1.1.2.3.1. *Respiratory toxicity*. Formaldehyde is known to induce changes in pulmonary function and cause pulmonary irritation in human studies (Rumchev et al., 2002; Garrett et al., 1999; Krzyzanowski et al., 1990; Holmström et al., 1989; Holmström and Wilhelmsson, 1988; Ritchie and Lehnen, 1987) and animal studies (Ohtsuka et al., 2003, 1997; Riedel et al., 1996; Swiecichowski et al., 1993; Lee et al., 1984). Exposure to formaldehyde in early life can cause damage to the lungs and permanently influence airway function, resulting in increased vulnerability to toxicants later in life. Thus, young children may demonstrate increased susceptibility to formaldehyde-related health effects. Krzyzanowski et al. (1990) reported an association between physician-diagnosed asthma and chronic bronchitis in children who lived in homes with formaldehyde levels that were higher than 60 ppb, after controlling for socioeconomic status and ethnicity. Rumchev et al. (2002) reported a statistically significant increased risk of asthma with increased residential concentrations of formaldehyde. Garrett et al. (1999) found an increased association between bedroom concentration of formaldehyde and increased risk of atopy in children. These studies suggest that formaldehyde exposure may exacerbate responses in sensitive airways, particularly in children. Exacerbation of response has also been noted in asthmatic adults and will be discussed below.

Another child-specific concern is that respiratory irritation may have greater impact on lung function in children due to their smaller lung size; this is true even if the lung development is normal. Irritation is commonly accompanied by inflammation, which can have a greater impact on children's airways because they are narrower than adult airways. Thus, less inflammation is required for significant airway obstruction in children than in adults.

4.6.1.1.2.3.2. ***Developmental neurotoxicity*****.** In neonatal exposure paradigms, changes in brain structure (Sarsilmaz et al., 2007; Aslan et al., 2006), and brain chemistry (Songur et al., 2008) were seen in young rats following inhalation exposures (6,000 or 12,000 ppb, 5 days per week from postnatal day 0-30). In addition, Weiler and Apfelbach (1992) found juvenile animals to be more sensitive to formaldehyde-induced changes in olfactory thresholds when compared with adult animals (shifts in olfactory thresholds were greater when exposure [at 250 ppb] was initiated at PND 30 than at adult ages). These studies are consistent with the hypothesis that early life exposure to formaldehyde can lead to long-lasting neurological effects. Exposure levels in these studies (250−6,000 ppb) were in the same range as those producing the behavioral effects in adults (as low as 100 ppb), but provide limited information regarding relative sensitivity as no NOAELs were identified, and (with the exception of Weiler and Apfelbach), similar parameters were not measured in adult animals using the same exposure paradigms.

4.6.1.2. *Later Life Stages*

In general, older adults may be at risk for increased susceptibility to exposure to environmental chemicals by virtue of their slower metabolism and increased incidence of altered health status (Benedetti et al., 2007; Ginsberg et al., 2005; U.S. EPA, 2005a). Additionally, adverse effects of earlier exposure to some toxicants may be observed in older adults as a result of latency in expression of the effect (Olsen et al., 1997; Sweeney et al., 1986). No studies have examined the differential effects of formaldehyde exposure for elderly adults (>65 years old) as compared to other age groups.

4.6.1.3. *Conclusions on Life-Stage Susceptibility*

In summary, timing both of the exposure and of the assessment of health outcomes may be important for understanding the relative risk of adverse effects from formaldehyde exposure during different life stages. There are known developmental differences in kinetics across life stages, including differences in enzymes involved in formaldehyde metabolism, but the contribution of these differences to formaldehyde-related health effects is unknown. Similarly,

information regarding life-stage differences in respiratory physiology raises possible concern regarding increased exposure to children, but studies for formaldehyde are not available. Available data do support an increased risk for adverse effects on lung function in children. The overall body of evidence shows some support, although minimal, for susceptibility in reproductive or developmental endpoints associated with exposure to formaldehyde. Some studies observed altered development of the nervous system following formaldehyde exposure during early life. Older adults may be at risk for increased susceptibility to formaldehyde because of slower metabolism and clearance rates. Elderly adults have an increased probability of having both altered health status and altered metabolism, which could impact their ability to process and recover from an adverse effect. The available data are consistent with some life-stage susceptibility differences for formaldehyde at the level of TD or TK differences, the results are nonetheless inconclusive due to the number of data gaps.

4.6.2. Health/Disease Status

The factor for which we have the greatest evidence is pre-existing disease, and specifically asthma. Numerous studies have assessed the potential for increased susceptibility to formaldehyde in asthmatics. Formaldehyde does not induce airway hyperreactivity directly (Sheppard et al., 1984) and has not been shown to increase airway hyperreactivity in either asthmatics or nonasthmatics (Pazdrak et al., 1993; Harving et al., 1991; Kulle et al., 1987). Significantly decreased forced expiratory volume (FEV_1) measurements were reported among asthmatics in two studies (Casset et al., 2006; Green et al., 1987), while others did not find any significant change in FEV_1 following formaldehyde exposure (Ezratty et al., 2007; Frigas et al., 1984).

A few available case reports of bronchial asthma do suggest direct respiratory tract sensitization to formaldehyde gas (Lemiere et al., 1995; Burge et al., 1985; Hendrick et al., 1982; Hendrick and Lane, 1977, 1975). All cases displayed marked changes in FEV_1 or pulmonary airflow rate in response to acute challenges with formaldehyde gas at exposure levels <3 ppm. Formaldehyde-induced IgE production has been reported in some studies (Vandenplas et al., 2004; Wantke et al., 1996a).

There is a large quantity of human data providing evidence of an association between formaldehyde exposure and increased incidence of asthma or exacerbation of asthmatic responses in compromised individuals. For example, Krzyzanowski et al. (1990) reported an association between physician-diagnosed asthma and chronic bronchitis in children who lived in homes with formaldehyde levels that were higher than 60 ppb, after controlling for socioeconomic status and ethnicity. Rumchev et al. (2002) reported a statistically significant

increased risk of asthma with increased residential concentrations of formaldehyde. Garrett et al. (1999) found an increased association between bedroom concentration of formaldehyde and risk of atopy in children. These studies suggest that formaldehyde exposure may exacerbate responses in sensitive airways, particularly in children. Exacerbation of response has also been noted in adults. Kriebel et al. (1993) reported a greater decrease in peak expiratory flow (PEF) in asthmatic medical students (7.3% decrement) compared with nonasthmatic medical students (2% decrement) after 2 weeks exposure to formaldehyde (average concentration 0.73 ppm) in an anatomy lab. This effect does not appear to be immunogenic in nature (Fujimaki et al., 2004a; Lee et al., 1984).

Several animal studies document increased airway resistance and bronchial constriction following inhalation exposure to formaldehyde (Nielson et al., 1999; Swiecichowski et al., 1993; Biagini et al., 1989; Amdur et al., 1960). Sadakane et al. (2002) demonstrated that formaldehyde exposure exacerbated sensitization and challenge with a common dust mite allergen (Der f) as measured by increased eosinophil infiltration into the interstitium around the bronchi and bronchioles as well as goblet cell proliferation in the bronchial epithelium; they suggested that formaldehyde exposure may aggravate eosinophilic infiltration and goblet cell proliferation that accompanies allergic responses. The MOA underlying this response is unknown. These decrements may occur indirectly in response to formaldehyde and may be mediated via neurogenic potentiation (Sadakane et al., 2002; Riedel et al., 1996; Tarkowski and Gorski, 1995). In particular, Tarkowski and Gorski (1995) suggest that formaldehyde may increase permeability of respiratory epithelium and destruction of immunologic barriers. Thus, the respiratory tract may become vulnerable to inhaled allergens after formaldehyde exposure (Tarkowski and Gorski, 1995).

In summary, the data indicate that formaldehyde exposure can aggravate a type I hypersensitivity response and that this hypersensitivity may in turn increase the susceptibility to formaldehyde exposure in these individuals. Formaldehyde exposure may predetermine an asthmatic phenotype or may induce new incidences of asthma via indirect mechanisms, though definitive evidence and a proposed mechanism remain to be determined. Individuals that exhibit chemically induced sensitivity and are exposed acutely or chronically to formaldehyde in residential and occupational settings might exhibit adverse responses at lower concentrations of formaldehyde than the average healthy person.

4.6.3. Nutritional Status

Limited available data indicate that certain types of malnutrition may increase susceptibility to formaldehyde exposure. Senichenkova and Chebotar (1996) reported increased

fetal anomalies in fetuses from iron-deficient pregnant mice after formaldehyde exposure compared with anemic mice that had not been exposed to formaldehyde. Forced iron reduction (induced by addition of bipyridyl treatment in pregnant mice) *in utero* increased the overall incidence of fetal anomalies when paired with formaldehyde exposure (Senichenkova and Chebotar, 1996). The findings are difficult to evaluate due to poor reporting and have not been substantiated by other laboratories.

4.6.4. Gender Differences

Males and females can differ greatly in body composition, organ function, and many other physiological parameters that may influence the toxicokinetics of chemicals and their metabolites in the body (Gochfeld, 2007; Gandhi et al., 2004).

The human epidemiology data set does not support any specific gender susceptibilities for noncancer effects due to formaldehyde exposure. In general, data suggest that nonpregnant women, on a per kg body weight basis, may have slightly lower air intake than men, which would suggest that women may be less susceptible to inhaled pollutants like formaldehyde than men, but this has not been investigated in the available formaldehyde literature.

A few isolated reports have investigated potential gender differences in development of nasal pharyngeal carcinomas following exposure to formaldehyde. One case-control study identified a higher OR for sinonasal adenocarcinomas in women (OR = 6.2 [95% CI: 2.2−19.7]) compared with the OR observed in men (OR = 3.0 [95% CI: 1.5−5.7]) following exposure to formaldehyde (Luce et al., 2002). However, the overall body of evidence remains scant.

There are a few reports concerning differential formaldehyde-induced effects on the male and female reproductive systems. Özen et al. (2002), Sarsilmaz et al. (1999), and Woutersen et al. (1987) reported reduced Leydig cell numbers in adult male rats exposed by inhalation. In female mice, inhalation exposure to formaldehyde resulted in endometrial hypoplasia and lack of ovarian luteal tissue (Maronpot et al., 1986). The clinical significance of these effects in humans is unknown, and due to limited data it is unclear whether the female or male reproductive system is more susceptible to perturbation by formaldehyde.

4.6.5. Genetic Differences

There are some data for polymorphisms in humans that affect formaldehyde TK. As discussed in Section 3.4, the primary metabolizing enzymes of formaldehyde are ALDH2 and ADH3, with the latter enzyme considered more relevant to low exposures. Polymorphisms in ALDH2 have been shown to have implications in human risk assessment, specifically in regard to acetaldehyde metabolism (Ginsberg et al., 2002). Teng et al. (2001) demonstrated the

importance of ALDH2 for formaldehyde metabolism in rat hepatocytes at fairly high formaldehyde concentrations (2.5 mM and greater). Cheng et al. (2008) investigated the relationship between occupational exposure to formaldehyde and genetic polymorphisms of ALDH2 and CYP2E1. There was a positive relationship between the concentration of formic acid in the urine and ALDH2 genotypes ($\chi^2 = 9.241$, $p < 0.05$). Urinary formic acid concentration may be affected by formaldehyde exposure concentration and ALDH2 genotype (Cheng et al., 2008) for individuals that have high exposure levels. Thus, although ALDH2 may not be involved in formaldehyde metabolism if exposure levels are low, polymorphisms of this enzyme may lead to differences in response at higher exposure levels.

Wu et al. (2007) looked for and identified two SNPs in ADH3 among a population of Mexican asthmatic children 4 to 17 years of age. Carrying one or two copies of the minor allele for one SNP resulted in a decreased RR of asthma (RR = 0.66−0.77). For the second SNP, homozygotes for the minor allele had an RR of 1.6 for asthma. The functional characteristics of these SNPs are unknown. Studies evaluating whether any of the polymorphisms affect the expression, regulation, stability, or activity of the enzyme *in vivo* are lacking; therefore, the relative susceptibility of individuals with different polymorphisms cannot be characterized at this time.

One study (Hedberg et al., 2001) identified three polymorphisms in human ADH3 involving four base-pair substitutions in the promoter region of which one (C→T) showed reduced activity (~50−70% of control). Hedberg et al. (2001) reported differences in allele frequencies among Chinese, Spanish, and Swedish groups, consisting of Asian-Caucasian differences and ethnic subgroups among Caucasians. Their results suggest that a small percentage of Caucasians may have decreased ADH3 expression and thus, be more susceptible to formaldehyde exposure. Additional studies to validate these findings have not been performed.

The relative activity level of these enzymes may also impact the metabolism of formaldehyde. In pharmacokinetic studies, deletion of ADH3 increased the sensitivity of mice to formaldehyde (Deltour et al., 1999) and was deleterious to yeast (Achkor et al., 2003). These results suggest that deficiencies in ADH3 may confer an increased susceptibility to formaldehyde toxicity (Teng et al., 2001). The importance of properly functioning enzymes also suggests that genetic differences in ADH3 or ALDH2 may affect the response to formaldehyde exposure. However, comparable human data are not available.

Race/ethnicity may be a surrogate for genetic differences but racial or ethnic groups may also reflect socioeconomic, and/or cultural factors that are distinct from genetics. Possible ethnic differences may be related to genetic polymorphisms of enzymes ALDH2 and ADH3, relevant for formaldehyde metabolism. ALDH2 variants, present primarily in East Asians, are known to

have protective effects against alcoholism but were not found in the people of Indo-Trinidadian descent (Moore et al., 2007) or in American Indians or Alaska natives (Ehlers, 2007). However, there is no direct evidence to associate these variants to differential susceptibility to formaldehyde exposure, nor is there direct evidence to associate these ethnic groups specifically with differential susceptibility to formaldehyde. Further, no studies have specifically assessed ethnic variability in responses to formaldehyde.

There are complex pathways through which genetic polymorphisms in ADH3 can potentially affect differential susceptibility to formaldehyde. Firstly, ADH3 is central to the metabolism of formaldehyde. However, ADH3 itself may indirectly contribute to the adverse effects of formaldehyde on pulmonary physiology (Thompson et al., 2009; Staab et al., 2008a, b; Thompson and Grafström, 2008). Exposure to formaldehyde is itself thought to alter the activity of ADH3 resulting in the perturbation of critical metabolic pathways. ADH3 participates in the oxidation of retinol and long-chain primary alcohols, as well as the reduction of S-nitrosoglutathione (GSNO). The activity of ADH3 toward some of these substrates has been shown to be significantly increased in the presence of formaldehyde. ADH3 has recently also been shown to contribute to NO signaling through its dual role in metabolizing GSNO, an endogenous bronchodilator and reservoir of NO (Staab et al., 2008a; Hess et al., 2005; Jensen et al. 1998). Through its regulatory function on GSNO, ADH3 may thus play a central role in regulating bronchial tone allergen-induced hyperresponsiveness (Gerard, 2005; Que et al., 2005). As concluded by California Environmental Protection Agency (CalEPA) (2008), "the dysregulation of NO by formaldehyde [in this manner] helps to explain the variety and variability in the toxic manifestations following formaldehyde inhalation."

4.6.6. Coexposures

4.6.6.1. *Cumulative Risk*

When considering health risks, it is important to consider the impact of coexposures to other agents that may interact with the chemical under evaluation. Coexposure to other pollutants, particularly those that produce some of the same metabolites and similar health effects as formaldehyde, is likely to occur in both occupational and nonoccupational settings.

Due to effects on metabolic enzymes (inducing and/or inhibition) as well as direct effects on organ system function, coexposures may alter the way in which formaldehyde is metabolized and cleared from the body. Inhibition or induction of the enzymes responsible for metabolism of chemicals may alter susceptibility to toxicity (Lash and Parker, 2001; IARC, 1995; U.S. EPA, 1985a). Smokers may be at increased risk for effects of formaldehyde exposure, because formaldehyde is one of the components of cigarette smoke and is likely to heighten the point-of-

entry effect when combined with occupational or residential exposures to inhaled formaldehyde. However, no evidence is available to evaluate the potential aggregate effects.

4.6.6.2. *Aggregate Exposure*

In addition, multiple routes of exposure to a single agent may increase the cumulative risk by increasing the overall body burden of the chemical. A human aggregate exposure model developed by McKone and Daniels (1991) incorporated likely exposures from air, water, and soil media through inhalation, ingestion, and dermal contact. The authors hypothesized that the aggregate exposure could be age-dependent but did not present any data for persons of differing life stages. The role of multiple exposures on different genders, genetic susceptibility, or altered health and nutrition status has not been investigated. The available database regarding the potential for multiple routes of exposure (or aggregate exposure) formaldehyde is limited.

Guseva (1972) specifically assessed the reproductive and developmental effects caused by coexposure to formaldehyde via both inhalation (0.25 mg/m^3) and ingestion (0.01 mg/L) routes in male rats. The authors reported reduced nucleic acid levels in testes to 88 and 92% of controls, which suggests a possible toxic gonadotropic effect. The ability of male rats (receiving combined exposure to formaldehyde at a low concentration level for a long period of time) to reproduce was preserved since all the cohabited females were impregnated. The number and weight of the fetuses and newborn rat pups in the experimental coexposure groups did not differ substantially from those figures observed in the control group. No developmental defects or anomalies were observed in the offspring for up to 1 month postnatally. Thus, at low exposures, the reproductive effects due to combined ingestion and inhalation exposure are unknown.

4.6.7. Uncertainties of Database

There is a need to better characterize the implications of formaldehyde exposures to susceptible populations. A number of areas where the database is currently insufficient are identified below.

4.6.7.1. *Uncertainties of Exposure*

Although information exists on early life exposure to formaldehyde, a number of uncertainties regarding children's susceptibility remain. First, inhalation is believed to be of most concern for formaldehyde, since formaldehyde vapors are released from insulation or from ambient sources of formaldehyde, including secondary production from other pollutants involved in photo-oxidant reactions. Any additional pathways of exposure for children have not been characterized. Since formaldehyde is nearly ubiquitous in the environment, it is difficult to

quantify the total exposure. Second, children have different respiratory, metabolic, and activity rates compared with healthy adults, potentially influencing ADME and target tissue exposure to formaldehyde. However, studies to identify the specific changes in absorption of formaldehyde and its metabolites across developmental stages and across organs have not been performed. In addition, exposure prenatally may be altered based on whether formaldehyde or its metabolites pass through the placenta, but placental transfer data are not available. Third, no quantitative models have been developed to characterize these differences for formaldehyde. Formaldehyde-specific PBPK models and their validation will aid in understanding the uncertainties associated with formaldehyde exposure in children.

Given the large proportion of time that most individuals in the U.S. spend indoors, exposure scenarios where indoor concentrations to formaldehyde are high (e.g., in homes or in trailers; see Section 2.3.1) may play a significant role and may be of particular concern to the elderly or health-impaired individuals who spend relatively more time at home. Further evaluation of the effects of coexposures and pathways of exposure and aggregate risk is needed. An estimate of the multiple exposure pathways is needed to know where along the dose-response curve to place an incremental exposure to formaldehyde.

4.6.7.2. *Uncertainties of Effect*

Studies specifically designed to evaluate effects after early and later life stage exposure are needed in order to more fully characterize potential life-stage-related differences in formaldehyde toxicity, including the defining of critical windows during development. For example, life-stage-specific neurotoxic and pulmonary effects, particularly in the developing fetus, need further evaluation. The preconceptional period may be a critical window for formaldehyde exposure and reproductive and developmental effects, based on rodent studies of reproductive, embryonic and gamete effects. Data specific to the carcinogenic effects of formaldehyde exposure during early life stages do not exist. The reduction in fertility seen in some studies (Gray and Wu, 2000; Taskinen et al., 1999; Hakim et al., 1995) is not adequately described and a well-established MOA has not been identified, but some have been hypothesized including altered sperm quality (Özen et al., 2002; Sarsilmaz et al., 1999; Woutersen et al., 1987). Further, spontaneous abortion/fetal loss occurring early in gestation, prior to maternal knowledge of the pregnancy, can lead to misclassification of the effect as infertility (see Sections 4.1.1.7 and 4.2.7).

More research is needed to clarify the role of genetic polymorphisms in formaldehyde metabolism. Similarly, data gaps pertaining to gender differences remain. A potential impact of nutritional status and iron deficiency on formaldehyde toxicity needs further investigation.

A fair body of evidence suggests that asthmatics are more susceptible to formaldehyde exposure than the general population, however, the mechanism of action for this increased susceptibility is unknown.

In the studies discussed above, there are a number of examples of studies that assessed multiple susceptibility factors that are worth noting. For example, the Krzyzanowski et al. (1990) study reported asthma and chronic bronchitis cases for two interacting potential susceptible groups, in children and those with high exposure (due to living in homes with formaldehyde levels that were higher than 60 ppb). Similarly, the Garrett et al. (1999) study assessed the same two interacting potential susceptible groups.

The study of Senichenkova and Chebotar (1996) assessed developmental effects in mouse fetuses after *in utero* iron-deficiency and formaldehyde exposure. Thus, the study findings must be considered in light of possible interactions between life stage exposure differences and nutritional status differences.

Studies to understand the nature of the interactions between the various susceptibility factors for formaldehyde have not been performed.

4.6.8. Summary of Potential Susceptibility

There is some evidence to demonstrate susceptibility for various populations exposed to formaldehyde. Available data are summarized in Table 4-95 where formaldehyde susceptibility factors are presented by those with data for increased formaldehyde susceptibility and those with data for differences but with an unknown impact on formaldehyde susceptibility.

Exposure to formaldehyde during early developmental and later life stages may be of concern. However, human exposure to the developing fetus is unknown since it is not known whether formaldehyde or one of its metabolites crosses the placenta. However, there is very limited life-stage-specific information regarding the TK of formaldehyde. Life-stage-specific TK has not been characterized, and, thus, no PBPK models exist to effectively evaluate the risk to early life stages. Children may be more susceptible to noncancer health effects as a result of inhalation exposure to formaldehyde due to increased respiratory rates. There are no studies to evaluate whether formaldehyde exposure in early life (e.g., pregnancy) is associated with an increased risk of childhood cancer.

The weight of evidence supports a plausible association between formaldehyde exposure and aggravated asthmatic responses in humans and this association is corroborated by limited evidence from animal studies. Formaldehyde does not appear to directly induce airway hyperreactivity but may sensitize airways to subsequent exposures. One issue in interpreting the available studies that assessed the relationship between asthma and formaldehyde could not

Table 4-95. Available evidence for susceptibility factors of concern for formaldehyde exposure

Factor	Evidence that factors increase susceptibility to formaldehyde	Evidence that factors show differences but unknown impact on susceptibility
Life stage	Developmental effects reported suggesting that critical windows of exposure may be relevant:	
▪ Preconception	▪ Reproductive outcomes (Taskinen et al., 1999; Maronpot et al., 1986) ▪ Embryo effects (Kitaev et al., 1984)	
▪ Prenatal	▪ Structural- and functional developmental outcomes (Martin, 1990; Saillenfait et al., 1989; Sheveleva, 1971; Seninchenkova, 1991)	▪ Possible life stage level differences in some enzymes involved in formaldehyde metabolism (Harris et al., 2003; Ginsberg et al., 2004; Westerlund et al., 2005; Benedetti et al., 2007) ▪ Mixed reports of associations between prenatal exposure and developmental outcomes in human studies (positive association: Gražulevičiene et al., 1998)
▪ Postnatal	▪ Lung function outcome (Krzyzanowski et al., 1990; Rumchev et al., 2002; Garrett et al., 1999) ▪ Developmental neurotoxicity (Weiler and Apfelbach, 1992)	▪ Possible life stage level differences in some enzymes involved in formaldehyde metabolism (e.g., ↓ADH expression over first 5 months; Ginsberg et al., 2004) ▪ Developmental neurotoxicity (Sarsilmaz et al., 2007; Aslan et al., 2006; Songur et al., 2008)
Disease status	▪ Bronchial asthma (Lemiere et al., 1995; Burge et al., 1985; Hendrick et al., 1982; Hendrick and Lane, 1977, 1975) ▪ Increased airway resistance and bronchial constriction (Nielson et al., 1999; Swiecichowski et al., 1993; Biagini et al., 1989; Amdur et al., 1960)	▪ Mixed results for forced expiratory volume (FEV1) measures affected by formaldehyde exposure in asthmatics (Casset et al., 2006; Green et al., 1987; Ezratty et al., 2007; Frigas et al., 1984)
Nutritional status/diet	▪ Iron-deficiency *in utero* (Senichenkova and Chebotar, 1996).	
Genetics ▪ Polymorphisms	▪ For high formaldehyde exposure: Urinary formic acid levels affected by ALDH2 genotype (Cheng et al., 2008) ▪ In mice, ADH3 increased sensitivity to formaldehyde (Achkor et al., 2003)	▪ Differences among ADH3 alleles and asthma outcome (Wu et al., 2007) ▪ Differences among ethnic groups in ADH3 alleles (Hedberg et al., 2001)
Gender		▪ Gender differences in incidence of nasopharyngeal carcinoma following formaldehyde exposure (Luce et al., 2002)

distinguish between the cases of asthma that were due to earlier formaldehyde exposure vs. those without a direct link to formaldehyde exposure.

No direct link exists between formaldehyde exposure and differential susceptibility in different ethnic groups, although genetic polymorphisms in the enzymes involved with formaldehyde metabolism, ADH3 and ALDH2, provide some support for differential susceptibility to alcoholism in a number of ethnic groups. The evidence for differential gender responses to formaldehyde exposure is equivocal. Coexposures may result in altered metabolism and clearance, but there is no evidence that coexposures are a critical part of formaldehyde-mediated differential susceptibility.

Thus, given the available data, increased susceptibility to adverse effects of formaldehyde is most strongly supported for three populations: (1) Preconception and perinatal exposure based on reproductive and developmental effects; (2) children, whose exposure may be higher by virtue of their increased activity level and respiratory rate; and (3) asthmatics who may exhibit exacerbation of response to formaldehyde.

- End of Volume II -

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TOXICOLOGICAL REVIEW OF FORMALDEHYDE - INHALATION ASSESSMENT

(CAS No. 50-00-0)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

VOLUME III of IV

Quantitative Assessment, Major Conclusions in the Characterization of Hazard and Dose Response, and References

June 2, 2010

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5. QUANTITATIVE ASSESSMENT: INHALATION EXPOSURE

This chapter presents the quantitative assessments conducted by EPA for both cancer and noncancer health effects associated with formaldehyde exposure. The quantitative assessment is focused on the inhalation route of exposure. The current IRIS reference dose (RfD) is not reevaluated in this assessment. Formaldehyde's carcinogenicity via the oral route of exposure is not evaluated herein nor is an oral slope factor considered at this time. Therefore, the following sections address derivation of a reference concentration (RfC) and cancer unit risk estimate for inhalation exposures.

For noncancer effects, the RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a NOAEL, LOAEL, or benchmark concentration, with uncertainty factors generally applied to reflect limitations of the data used. Data from the previous chapters are evaluated to determine the health effects associated with formaldehyde exposure and which studies may best inform the exposure response relationship for RfC derivation. Section 5.1 summarizes the observed noncancer health effects, selecting key studies and critical effects for consideration. Candidate RfCs are derived for each identified key study. Several alternatives are considered for uncertainty factors addressing human variability for key studies and alternatives presented (see Section 5.1.2.3). Options for addressing the overall database uncertainty factor are provided which may modify the final RfC (see Section 5.1.3).

The derivation of the cancer inhalation unit risk estimate considered data regarding both respiratory tract cancers and lymphohematopoietic malignancies. Exposure-response modeling from epidemiologic studies was used to derive a combined unit risk estimate for nasopharyngeal cancer and lymphohematopoietic cancers (see Section 5.2). This unit risk estimate is supported by an analysis of exposure-response modeling of respiratory tract cancer risk using data from experimental animal studies (see Section 5.3). Analysis of the animal bioassays includes an evaluation of a published biologically based dose-response model as well as an appraisal of published dose-response modeling of genomics data and a presentation of benchmark dose modeling approaches. Finally, Section 5.4 provides a summary and conclusions from the cancer exposure-response modeling, presenting the final unit risk estimate based on the combined risk of nasopharyngeal cancer and lymphohematopoietic cancers observed in the epidemiology studies.

5.1. INHALATION REFERENCE CONCENTRATION (RFC)

Prior to the current assessment, the EPA IRIS file for formaldehyde did not provide an inhalation RfC. As presented in the hazard identification in Chapter 4, a number of noncancer health effects are associated with formaldehyde exposure. Section 5.1.1 describes each of the health effect categories considered for RfC derivation and the specific endpoints considered for each category. The identified effect categories are: sensory irritation (eye, nose, and throat); upper respiratory tract (URT) pathology; pulmonary function; increased asthma and atopic sensitization; altered immune function; neurotoxicity and reproductive and developmental toxicity. For each health effect category, studies that may adequately inform the exposure-response relationship for specific critical effects are identified for consideration in RfC derivation.

EPA employed a screening process across the different health effect categories to select key studies that would best support the derivation of an inhalation RfC (as described in Section 5.1.2.1). The following factors were considered in this evaluation: characteristics of the study population, exposure regimen, quality of exposure assessment, quality of exposure-response assessment, exposure levels at which effects were seen and statistical power of the study. Based on this analysis, seven studies were considered for RfC derivation. Candidate RfC derivation from a key study includes the following steps: 1) define the critical effect(s); 2) determine appropriate point(s) of departure (PODs) on the basis of inhaled concentration; 3) adjust each POD by endpoint/study-specific uncertainty factors (UFs), to account for uncertainties in the extrapolation of study results to conditions of human environmental exposure. All of the identified key studies were observational epidemiology studies of people and several studies included potentially susceptible individuals (e.g., children, asthmatics). The uncertainty factor for human variability has sometimes been reduced for studies of susceptible populations or lifestages. However, for five of the seven key studies it was unclear if an uncertainty factor of 3 or 1 for human variability was most appropriate. Therefore, alternatives are presented for consideration. Candidate RfCs (cRfCs) are derived for sensory irritation, decreased pulmonary function in children, increased asthma incidence in children, increased allergic sensitization to common allergens in children, and decreased fecundability density ratio (FDR) in women (increased time to pregnancy) (see Table 5-7). All of these cRfCs are derived from endpoints identified in residential studies, with the exception of decreased FDR (observed in an occupational study of women in the woodworking industry).

The overall literature database of both human and laboratory animal studies examining the health effects from formaldehyde exposure is large; however, the available studies for some types of effects are limited. Limitations in the existing database are discussed in Section 5.1.3,

specifically regarding understanding the reproductive and developmental effects and the exposure-response relationship for the observed neurological and behavioral effects from formaldehyde exposure. EPA considers 3 options for addressing these database uncertainties in the final RfC: (1) providing an RfC derived from studies of respiratory and allergenic responses and protective of sensory irritation effects, without further adjustment for uncertainties in the database (noting the need for further research to elucidate reproductive, developmental and neurotoxic effects); (2) providing an RfC with a database uncertainty factor incorporated to reflect the potential that reproductive, developmental, or neurotoxic effects might occur at lower doses; or (3) provide a range for the RfC which encompasses the above two options for the database uncertainty factor.

5.1.1. Candidate Critical Effects by Health Effect Category

The following subsections describe the best available studies and endpoints for quantitative RfC derivation within each health effect category. These studies are considered representative of the health effects attributed to formaldehyde exposure. For more details on specific studies discussed here, see Sections 4.1.1 and 4.2.1. The identified health effect categories are: sensory irritation (eye, nose, and throat); upper respiratory tract (URT) pathology; pulmonary function; increased asthma and allergic sensitization; altered immune function; neurotoxicity and reproductive and developmental toxicity. Discussions in each subsection below describe the various health effects observed in human and animal studies for each category.

For each health effect category, specific studies that may adequately inform the exposure-response relationship for critical effects are identified for consideration in RfC derivation. In general, studies are included where study quality and ability to define exposures are considered adequate for RfC derivation. Whenever possible, greater consideration is typically given to human data from observational epidemiology studies for derivation of an RfC.

5.1.1.1. *Sensory Irritation of the Eyes, Nose, and Throat*

Eye, nose, and throat irritation are common effects of chemically induced sensory irritation; specific effects include lacrimation, burning of the eyes and nose, rhinitis, burning of the throat, and cough (Feron et al., 2001). Chemical irritants such as formaldehyde bind to protein receptors of the trigeminal nerve, triggering a burning and painful sensation. This process is distinct from taste and smell (Cometto-Muniz and Cain, 1992; Nielsen, 1991). The trigeminal nerve has three branches (ophthalmic, maxillary, and mandibular) and not only acts as an afferent nerve relaying these sensations to the central nervous system but has efferent nerve

activity as well (Meggs, 1993). Stimulation of the trigeminal nerve may result in reflex responses, including lacrimation, coughing, and sneezing. In this assessment, both the reflex responses and the sensations (such as burning, pain, and itching of the eyes, nose, and throat) are considered adverse effects (see Section 4.1 for a full discussion of available human data).

There are studies noting irritant effects in rodents (Sarsilmaz et al., 1999; Holmström et al., 1989; Dubreuil et al., 1976) and monkeys (Monticello et al., 1989; Rusch et al., 1983). These animal studies are supportive of the health effects reported in humans. However, given the uncertainties in extrapolation from responses in laboratory animals to expected responses in humans, the available human studies are preferred.

In human studies, the endpoints for assessing irritation include subjective self reporting of symptoms (e.g., pain, burning, itching, increased cough) via questionnaires or objective measures of irritation that can be assessed during controlled acute exposures (e.g., eye-blink counts, lacrimation). Several acute chamber studies support development of a concentration-response relationship for sensory irritation, identifying an effect level for various exposure durations (Kulle, 1993; Andersen and Mølhave, 1983; Bender et al., 1983; Weber-Tschopp et al., 1977). Arts et al. (2006b) reviewed several studies and performed BMD analyses, reporting 10% extra risk BMCL values for reported eye discomfort of 560 and 240 ppb for 3 and 5 hour exposures, respectively. LOAELs of 1,000 ppb and 1,700 ppb were reported for 1−2 minute exposures (Bender et al., 1983; Weber-Tschopp et al., 1977). These acute studies support a role for both concentration and duration in the effect level for eye irritation. Although exposure concentrations are well-defined in these chamber studies, the chamber studies are not appropriate for RfC derivation because they are of acute duration and the exposure levels used are much higher than those reported for chronic exposure scenarios, both occupational and residential.

A study of industrial workers assessed sensory irritation and provided an average exposure derived from in-plant exposure measurements and the work history of each study participant (Holmström and Wilhelmsson, 1988). Although average daily exposures were estimated for each employee, these data were not used to explore an exposure-response relationship within the worker cohort. The symptom prevalence for sensory irritation (e.g., nasal discomfort, eye discomfort, and airway discomfort) relative to the referent group was reported for the cohort as a whole, where worker exposure ranged from 0.05 to 0.5 mg/m^3 formaldehyde 8-hour time-weighted average (TWA), with a mean of 0.26 mg/m^3 (210 ppb). The daily TWA does not reflect the peak exposures experienced during specific work tasks. Although this study demonstrated marked increases in symptoms of sensory irritation in the workplace due to formaldehyde exposure, it provided little data to inform the exposure-response relationship, especially in the range of environmental exposures.

There are three studies that report sensory irritation in humans from chronic exposures in a residential environment and provide sufficient exposure data to support quantitative assessment (Liu et al., 1991; Ritchie and Lehnen, 1987; Hanrahan et al., 1984). Each study reports site-specific exposure measurements and presents some metric of individual exposure. These residential studies employ in-home measurements for each study participant, either as average exposure level (Ritchie and Lehnen, 1987; Hanrahan et al., 1984) or as calculated cumulative exposure based on the time in the home (Liu et al., 1991). Eye irritation is reported at similar levels of residential formaldehyde exposure in the three studies (see Section 5.1.2.2.4). Each study provides an exposure-response relationship for prevalence of sensory irritation in relation to in-home formaldehyde exposure based on individual-level data. The detailed exposure information and chronic nature of the exposures support the selection of these studies as potential principal studies for RfC derivation. Each of these studies is further evaluated and a cRfC developed for consideration (see Section 5.1.2).

5.1.1.2. *Upper Respiratory Tract Pathology*

Formaldehyde-induced respiratory tract pathology includes inflammation, rhinitis, goblet cell hyperplasia, metaplastic changes, squamous cell hyperplasia, and impaired mucociliary transport. A series of laboratory animal studies assessing formaldehyde-induced changes in the nasal mucosa suggests that these changes may be a protective or adaptive response and that increased mucus flow and metaplastic changes will progress in relation to the concentration and duration of exposure to protect the underlying tissue (Swenberg et al., 1983). The degree of inflammation, hyperplasia, and metaplastic change that is due to sensory irritation-induced inflammatory responses versus inflammation and tissue remodeling from formaldehyde-induced direct cell damage cannot be distinguished. These changes have been noted as sensitive indicators of formaldehyde-induced effects, occurring before gross cellular damage and focal lesions (Monticello et al., 1989). These responses are considered for RfC derivation, especially for exposure concentrations where gross damage of the underlying tissue is not expected. Although well-documented studies demonstrating formaldehyde-induced upper respiratory tract (URT) pathology have been performed in laboratory animals, including the rat (Zwart et al., 1988; Woutersen et al., 1987; Morgan et al., 1986a, b, 1983; Swenberg et al., 1986, 1983) and monkey (Rusch et al., 1983), robust human data from epidemiologic studies are available, and these human data are preferred for RfC derivation.

Six epidemiology studies examined the effects of formaldehyde exposure on URT pathology (Pazdrak et al., 1993; Boysen et al., 1990; Holmström et al., 1989; Edling et al., 1988; Holmström and Wilhelmsson, 1988; Andersen and Mølhave, 1983). Of these studies,

Holmström and Wilhelmsson (1988) and Holmström et al. (1989) were identified as the most robust and sensitive and are included as candidate studies for RfC derivation. Both studies address the same cohort and, thus, were considered together. The Holmström and Wilhelmsson (1988) study is discussed above under sensory irritation effects. In this study of 70 factory workers exposed to a TWA formaldehyde concentration of 210 ppb, impaired mucociliary clearance was reported in 20% of the exposed workers and 3% of the 36 nonexposed workers. Using rhinomanometry, Holmström and Wilhelmsson (1988) also found an increase in nasal resistance due to mucosal swelling, though this increase was not statistically significant. In Holmström et al. (1989), nasal biopsy samples were collected from 62 of the 70 formaldehyde-exposed factory workers (these 62 had been exposed to a TWA formaldehyde concentration of 240 ppb) and also from 32 of the nonexposed workers. A pathologist scored each sample by using a scale of 0 (normal respiratory epithelium) to 8 (carcinoma). Biopsy scores for both the exposed and control groups ranged from 0 (normal respiratory epithelium) to 4 (stratified squamous epithelium with marked horny layer). Although the mean biopsy scores for the two groups were similar—2.16 for the formaldehyde-exposed workers and 1.56 for the unexposed workers—the difference was statistically significant and the authors reported that the loss of cilia, goblet cell hyperplasia, and the incidence of cuboidal and squamous cell metaplasia replacing the columnar epithelium were more frequent in the group exposed to formaldehyde. There was no correlation between the duration of exposure and histologic changes or between smoking habits and biopsy scores. The URT effects, taken together (decreased mucous flow, increased inflammation, decreased nasal flow, and degradation of the respiratory epithelium), demonstrate a range of formaldehyde-induced URT pathology consistent with effects observed in controlled animal studies.

5.1.1.3. *Pulmonary Function Effects*

A synthesis of the literature evaluating formaldehyde exposure and pulmonary function is provided in Section 4.4.2. The potential effects of formaldehyde exposure on pulmonary function in humans can be examined on several time-scales of interest. There are reports examining effects from acute exposures among naively exposed anatomy graduate students (Kriebel et al., 1993; 2001), anatomy graduate students with several weeks of episodic exposure (Kriebel et al., 1993), as well as postshift versus preshift differences in pulmonary function in

workers with regular occupational exposure (Malaka and Kodama, 1990; Herbert et al., 1994; Alexandersson et al., 1982; Alexandersson and Hedenstierna, 1989). Depending on whether the exposures are naïve, the epidemiologic studies that assessed the pulmonary effects of acute exposures to formaldehyde may be assessing different biological responses, namely, the acute effect alone or the acute effect(s) in people who may have already been sensitized to formaldehyde effects.

Pulmonary effects of acute formaldehyde exposure have been studied in both healthy volunteers and sensitive populations under controlled conditions (e.g., acute chamber studies). Although acute chamber studies have the advantage of measured controlled exposures, other factors can limit the usefulness of the studies for RfC derivation including: acute duration, small study populations and lack of statistical power to assess the measured parameters. The acute chamber studies are more fully evaluated in Section 4.1.1 and will not be further considered here for RfC derivation.

The observed effects in the previously unexposed anatomy students provide additional information on acute exposures in two naïve populations (Kriebel et al., 1993; 2001), as well as insight into the intermediate stages of possible sensitization (Kriebel et al., 1993). Kriebel and colleagues (1993) examined the prelaboratory and postlaboratory peak expiratory flow (PEF) in students attending anatomy classes once per week. They found the strongest pulmonary response when examining the average cross-laboratory decrement in peak expiratory flow in the first 2 weeks of the study when formaldehyde concentrations collected in the breathing zones had a geometric average concentration of 0.73 ppm. Overall, the students exhibited a 2% decrement in PEF, while the students with any history of asthma showed a 7.3% decrement in PEF. These findings of acute decreases in PEF following students' initial anatomy sessions were corroborated by the Kriebel et al. (2001) study, which used a similar study design applied to another class of anatomy students.

The first Kriebel et al. (1993) study also shows how the acute effects of formaldehyde exposure were altered following several weeks of episodic exposure. By the 5^{th} week of class, the pre- and post-laboratory measurements of PEF were no longer reflecting a clearly demonstrated acute effect but following the 7^{th} week of episodic exposure, both pre-and post-laboratory PEF continued to drop steadily until the class adjourned after 10 weeks time. While the acute effects of formaldehyde exposure appeared to diminish after several weeks of exposure, the intermediate effect across 10 weeks was a 27 liter/minute drop in PEF that was statistically significant ($p<0.01$) after statistical control for random person effects, asthma, an interaction between time and asthma and eye and nose symptoms of irritation. The Kriebel et al. (1993) study is considered of sufficient quality to support an acute RfC but the quantitative

details on the initial acute effects among the naively exposed students are not adequately provided. The findings of the Kriebel et al. (2001) study may have been influenced by decreased class attendance, which dropped from 37 in the first week to 20 in week 6 and to just 10 students by week 10. While the Kriebel et al. (2001) study could be useful as a supportive study for naively exposed students, the longitudinal component is not strong enough to support RfC development.

Several studies of workers assess both cross-shift and chronic effects of formaldehyde exposure (Malaka and Kodama, 1990; Herbert et al., 1994; Alexandersson et al., 1982; Alexandersson and Hedenstierna, 1989). Since formaldehyde exposure may have cumulative effects over chronic exposures, occupational studies generally showed clinically small but statistically significant decrements in pulmonary function across shifts. In general these studies did not identify, have information on or have appropriate statistical control of, potential confounding coexposures. While these occupational studies provide evidence that is clearly and consistently supportive of an acute effect on pulmonary function, they do not directly support RfC development of an acute effect divorced of the concomitant chronic effects.

Several studies allowed for the examination of potential chronic effects of formaldehyde exposure. These included an occupational study (Malaka and Kodama, 1990) that reported preshift pulmonary function as a percentage of expected among the formaldehyde exposed compared to comparable people not exposed to formaldehyde. Studies that did not report preshift pulmonary function as a percentage of expected function (Herbert et al., 1994; Alexandersson et al., 1982) contribute less to an assessment of potential chronic effects because, post hoc, it is difficult to calibrate for cross-study comparison the multiple pulmonary function data without knowledge of the age, gender, smoking status, height, year of birth, etc. that are important determinants of the pulmonary function metrics of concern. The single study (Malaka and Kodama, 1990) that did report functional measures in relation to expected value, found that an average 8-hour time weighted average formaldehyde exposure of 1.13 ppm from area samples was associated with statistically significant decrements in FEV_1, FEV_1/FVC and $FEF_{25–75}$ compared to a referent population. The strongest response was for $FEF_{25–75,}$ which showed a 12% drop in observed function compared to expected function in the unexposed, but it is unclear how to interpret the potential chronic health effect(s) with just the magnitude of the decrement and the length of the average occupational tenure at this plywood facility (6.5 years), which was not reported by exposure status.

One study reported on the longitudinal follow-up of workers exposed to formaldehyde (Alexandersson and Hedenstierna, 1989). This investigation not only examined the acute effects of exposure across shift, but was able to do so among some of the same workers that had been

studied five years earlier (Alexandersson et al., 1982). Statistically significant decreases in FEV_1/FVC and $FEF_{25–75}$ were noted over the intervening five years in nonsmokers after correction for normal aging. The decrease in FEF_{25-75} was 0.212 liters/s (SD = 0.066 liters/s) for each year of exposure and was highly significant ($p < 0.01$). For comparison with the 12% drop in the same pulmonary metric reported by Malaka and Kodama (1990) over an estimated 6.5 years, EPA computed the extrapolated percentage decrease in FEF_{25-75} for the Alexandersson and Hedenstierna (1989) using the reported yearly decrement applied to the preshift values at the time of the initial study period. EPA calculated that from the predicted value of 4.57 liters/s, a decrease of 0.168 liters/s could be estimated for each year of exposure regardless of smoking status. For 6.5 years of exposure, this would result in a 24% drop in FEF_{25-75}. Formaldehyde concentrations were estimated at 0.42 ppm in the first Alexandersson et al. (1982) study and at 0.50 ppm in the second study, but without better exposure measures, the results of the longitudinal follow-up cannot support quantitative RfC development.

Information is lacking in these studies such as length or tenure of employment associated with the preshift pulmonary function or how long the residents had lived in their homes. Likewise, knowledge of how occupational or residential exposure may have changed over time would have allowed for an examination of the progression of any decrement in function associated with long-term episodic exposure. Among these studies, the best designed and executed of the cross-sectional studies was that of Krzyzanowski and colleagues (1990). Municipal employees and their children (613 adults and 298 children) were randomly sampled and were considered to be representative of a diverse local population. Residential exposures to formaldehyde were based on repeated samples from each individual's kitchen, living area and bedroom. The average formaldehyde concentration was 26 ppb, with a maximum sample value of 140 ppb. The majority of subjects (83%) lived in homes with 2-week average concentrations below 40 ppb. Subjects' peak expiratory flow rates (PEFR) were determined 4 times daily in the morning, at noon, in the early evening and before bed for 2 weeks. A statistically significant linear relationship between increased formaldehyde exposure and decreased peak expiratory flow rate was reported in children but not adults. All statistical models controlled for socioeconomic status, tobacco smoking (current active or environmental tobacco smoking) and nitrogen dioxide concentrations. In children, formaldehyde concentrations of 60−140 ppb increased the prevalence of physician-diagnosed asthma and bronchitis. Among adults who smoked, there was a statistically significant nonlinear relationship with decreased morning PEFR for formaldehyde concentration > 40 ppb. This well-conducted study had only minor weaknesses such as non-differential measurement error. However, random measurement error tends to attenuate any true effect and is unlikely to have produced a spurious effect. It is

unlikely that these findings were the product of unmeasured or residual confounding as the analyses controlled for smoking as well as nitrogen dioxide levels and there is no evidence of alternative factors that were temporally correlated with formaldehyde concentrations and more strongly associated with decrements in pulmonary function. This study of a large and representative sample from a diverse study population with a well-quantified concentration-response function and is further considered for RfC derivation.

5.1.1.4. *Asthma and Allergic Sensitization (Atopy)*

Sensitization to inhalational chemical exposure may manifest as an allergic or asthmatic response that is characterized by bronchial constriction (BC) or bronchial hyperresponsiveness (BHR). This sensitization may be a result of immune involvement, as in the case of hypersensitivity, or a neurogenic sensitization, where a chemical may directly stimulate inflammation. Asthma is a specific manifestation of IgE-mediated hypersensitivity, characterized by BHR and airway inflammation, resulting in lower airway obstruction (Fireman, 2003; Kuby, 1991).

A variety of hypersensitivity reactions have been reported following exposure to formaldehyde. Rashes and skin reactions have been reported in some individuals after dermal exposures to formaldehyde. Increased expression of Th-2 cytokines in the lymph nodes of mice given dermal applications of formaldehyde indicates the involvement of an immune component to the observed sensitization (Dearman et al., 2005; Hilton et al., 1998; Arts et al., 1997). However, the response does not appear to be IgE mediated (Arts et al., 1997; Lee et al., 1984). Gorski et al. (1992) observed an increase in formaldehyde-mediated neutrophil burst in dermatitis patients exposed in a controlled chamber study and suggests a putative role of oxidative stress and reactive oxygen species (ROS).

5.1.1.4.1. ***Epidemiologic studies.***

A synthesis of the literature evaluating formaldehyde exposure and asthma and allergic sensitization is provided in Section 4.4.3. Inhalation exposure has been associated with increased asthmatic responses in asthmatics in occupational settings. While few available case reports of bronchial asthma suggest direct respiratory tract sensitization to formaldehyde gas (Lemiere et al., 1995; Burge et al., 1985; Hendrick et al., 1982; Hendrick and Lane, 1977, 1975), a greater body of epidemiological data provides evidence of an association between formaldehyde exposure and exacerbation of asthmatic responses in compromised individuals (Kriebel et al., 1993) and particularly in children (Rumchev et al., 2002; Garrett et al., 1999a,b; Krzyzanowski et al., 1990). Asthma incidence in children increased with in-home exposure to

formaldehyde (Rumchev et al., 2002). Similarly, the frequency of respiratory symptoms associated with asthmatic responses and measures of allergic sensitization in children increased with in-home formaldehyde exposure (Garrett et al., 1999 a,b).

The association between formaldehyde and asthma has been studied by examining occupational exposures (Fransman et al., 2003; Malaka and Kodama, 1990), school-related exposures (Zhao et al., 2008; Smedje and Norback, 2001; Norback et al., 2000) and residential exposures (Matsunaga et al., 2008; Tavernier et al., 2006; Gee et al., 2005; Delfino et al., 2003; Rumchev et al., 2002; Garrett et al., 1999 a,b; Palczynski et al., 1999; Norback et al., 1995; Krzyzanowski et al., 1990). The two occupational studies examined the respiratory health of plywood workers (Fransman et al., 2003; Malaka and Kodama, 1990). The most recent of these was conducted in New Zealand by Fransman et al. (2003). Personal samples of formaldehyde exposure were taken. The mean level of exposure was 0.08 mg/m^3 (65 ppb) and the majority of samples were below the limit of detection which was reported to be 0.03 mg/m^3 (24 ppb). Compared with those with low levels of formaldehyde exposure, workers with high levels of exposure were more likely to report having asthma (OR = 4.3 [95% CI: 0.7–27.7]). The association was not seen when examining formaldehyde exposure and use of asthma medication. The second study of plywood workers was completed in Indonesia. Background levels of formaldehyde ranged from 0.003 to 0.07 ppm. The highest concentration of formaldehyde detected in an air sample was in the particleboard unit (range 1.16 to 3.48 ppm). The occurrence of asthma was found to be positively associated with formaldehyde exposure, where asthma was defined as, "Have you ever had an attack of wheezing that made you feel short of breath?", (Malaka and Kodama, 1990).

Studies of exposure to formaldehyde at schools have been performed in China (Zhao et al., 2008) and in Sweden (Smedje and Norback, 2001). In the study from China (Zhao et al., 2008), mean levels of formaldehyde were reported to be 2.3 µg/m^3 (range 1.0–5.0 µg/m^3) indoors and 5.8 µg/m^3 (range 5.0–7.0 µg/m^3) outdoors. Cumulative asthma (i.e., physician-diagnosed asthma since birth) and daytime attacks of breathlessness were found to be associated with outdoor formaldehyde levels. Neither of these outcomes was associated with indoor concentrations of formaldehyde; however, indoor levels were found to be associated with nocturnal attacks of breathlessness. In Sweden (Smedje and Norback, 2001), the levels of formaldehyde measured indoors were higher (arithmetic mean 8 µg/m^3, geometric mean 4 µg/m^3, range <5.0–72 µg/m^3). One difference between the Swedish study and the study conducted in China is that the Swedish study examined the incidence of asthma over a 4-year period and did not report an association between formaldehyde exposure and the incidence of asthma (OR 1.2 [95% CI: 0.8–1.7]) among the whole study population. However, when the

investigators stratified based on history of atopy, they reported that among children without a history of atopy, a new diagnosis of asthma was significantly more likely at higher concentrations of formaldehyde (OR 1.7 per 10 μg/m^3 [95% CI: 1.1–2.6]) and at higher total concentrations of mold (OR = 4.7 per 10-fold increased in total molds [95% CI: 1.2–18.4] in the classroom air. The finding in increase health effects due to formaldehyde and mold exposures did not appear to control for the other exposure and no information on the potential correlation between the two exposures was provided. In order to evaluate the potential for confounding of the reported formaldehyde association by the reported mold association, the magnitude of effects must be compared on an appropriate scale since the magnitude of an odds ratio depends on the magnitude of the change in exposure level that is expected to produce increased risk. After standardizing the units to the reported geometric standard deviation (GSD), the results for formaldehyde is OR[1] = 1.13 per GSD increase in formaldehyde concentration and the results for mold is OR[2]=1.02 per GSD increase in mold exposure (based on a 10-fold increase from the mean mold exposure) or alternatively, OR[3] = 1.06 per GSD increase in mold exposure (based on a 10-fold increase from the minimum mold exposure). As it appears that the magnitude of the formaldehyde effect is stronger than that of the mold effect (following standardization of exposure increment), it can be concluded that the reported formaldehyde effect could not have been due to uncontrolled confounding by mold.

The results of studies measuring residential exposure to formaldehyde and asthma are varied, with some demonstrating an association and others finding no relationship. A recent study (Matsunaga et al., 2008) found no association between 24-hour formaldehyde and prevalence of asthma when pregnant women with an exposure to ≥47 ppb were compared to those with exposure to <18 ppb. However, they reported an increased risk of atopic eczema. This study did not assess the risk of incident asthma. A study utilizing self-reported asthma prevalence as an outcome also found no association with levels of formaldehyde (mean 25.9 μg/m^3, range 2.0–66.8 μg/m^3) (Palczynski et al., 1999), although they noted the incidence of allergic diseases was greatest in the highest formaldehyde exposure group but that the groups were too small for statistical evaluation.

A study performed by Tuthill (1984) measured formaldehyde exposure for children grades K through 6 by using a combination of proxy variables. Overall, there was no association, but some individual variables showed an increased risk. For example, the reported risk ratio for having new construction or remodeling performed in the house in the past 4 months

[1] OR per GSD of formaldehyde = xp[ln(OR per μg/m^3)/10 μg/m^3 * 2.3 μg/^{3}] = exp[ln(1.7)/10*2.3] = 1.13
[2] OR per GSD of mold = xp[ln(OR per 10-fold increase)/ (9*Geo. Mean)*1.6 μg/^{3}] = exp[ln(4.7)/162*1.6] = 1.02
[3] OR per GSD of mold = xp[ln(OR per 10-fold increase)/ (9*Minimum)*1.6 μg/^{3}] = exp[ln(4.7)/45*1.6] = 1.06

was 2.5 (95% CI: 1.7–3.9). The risk ratio for having new or upholstered furniture in the house (within the past 4 months) was 2.2 (95% CI: 1.2–3.9).

The study by Delfino et al. (2003) assessed whether the ambient formaldehyde concentration measured at a central monitoring site was associated with asthma symptoms. The study examined 22 10–15 year olds with at least 1 year of physician-diagnosed asthma and living in a nonsmoking household. The mean levels of formaldehyde were measured to be 7.21 ppb (range 4.27–14.02 ppb). There was a positive association between asthma symptom scores (comparing children who report symptoms interfering with their daily activities versus those with no symptoms or symptoms not great enough to affect their daily activities) and high current levels of formaldehyde (OR 1.90 [95% CI: 1.13–3.19]).

Three studies (Tavernier et al., 2006; Gee et al., 2005; Garrett et al., 1999 a,b) were performed by matching children with and without asthma and comparing the levels of formaldehyde in their homes. Gee et al. (2005) reported median formaldehyde levels of 0.03 ppm in living rooms and 0.04 ppm in bedrooms. Analyses were limited to univariate comparisons of formaldehyde levels for cases of existing asthma and controls without asthma. The concentrations did not differ in a statistically significant manner. The study by Gee et al (2005) was followed up with a more sophisticated analysis of the same children in the same home. Tavernier et al. (2006) reiterated the earlier finding by Gee et al. (2005) that formaldehyde was not found to be associated with existing asthma. Tavernier et al. (2006) did not report the measured levels of formaldehyde but gave the OR for the highest tertile of exposure compared with the lowest tertile of exposure as 0.99 (95% CI: 0.39–2.50).

Garrett et al. (1999 a,b) reported on the risk of allergy and asthma-like respiratory symptoms due to formaldehyde exposure in a cross-sectional survey of households with children with (n = 53) or without (n = 88) doctor-diagnosed asthma. Formaldehyde exposure was characterized by 4 seasonal in-home sampling events across the year for bedrooms and 4-day passive samples collected in living rooms, kitchens and outdoors. Statistically significant linear trends for increased risk of having asthma were seen with increasing formaldehyde levels ($p < 0.02$); however, the ORs for the association did not remain statistically significant after controlling for parental allergy and asthma (exact ORs and 95% CIs not given). Garrett et al (1999 a,b) also evaluated the prevalence and severity of allergic sensitization to 12 common allergens and reported increased prevalence with increasing formaldehyde concentration in the home. The respiratory symptom score was also increased and demonstrated a significant effect for formaldehyde in a multiple regression after adjusting for multiple risk factors and interactions. For the atopy and respiratory symptom endpoints, severity/prevalence was increased in the medium (20–50 $\mu g/m^3$) and high (>50 $\mu g/m^3$) exposure groups relative to the

low (<20 μg/m^3) exposure group, based on the highest of four seasonal 4-day formaldehyde measurements in the home. The associations between formaldehyde concentrations and severity of allergic sensitization are clearly shown and further substantiated with multivariate regression controlling for potential confounders which showed that the unadjusted effect estimate was not confounded. In logistic regressions, both the prevalence and severity of allergic sensitization to 12 common allergens increased with increasing formaldehyde concentration in the home. The crude association for atopy with an increase in formaldehyde concentration per 10 μg/m^3 was OR = 1.34 which increased only slightly when adjusted for parental asthma and gender to and odds ratio of 1.42 per 10 μg/m^3 (95% CI: 0.99–2.04). Passive smoking, the presence of pets, indoor nitrogen dioxide concentrations, airborne fungal spores and house-dust-mite allergens did not influence the effect estimates and were unlikely to be confounders. Additionally, a calculated respiratory symptom score was increased and demonstrated a significant relationship to increased formaldehyde concentration in a multiple linear regression after adjusting for multiple risk factors and interactions. For each of these endpoints, severity/prevalence was increased in the medium (20–50 μg/m^3) and high (>50 μg/m^3) exposure groups relative to the low (<20 μg/m^3) exposure group, based on the highest of four seasonal 4-day formaldehyde measurements in the home.

Residential formaldehyde exposure was associated with an increased risk of asthma in a population-based case-control study of 192 children aged 6 months to 3 years (Rumchev et al., 2002). The study, which comprises 88 cases of children discharged from the emergency department of a children's hospital in Perth, Australia, with a primary diagnosis of asthma and 104 controls, provides a positive exposure-response relationship. Seasonal in-home formaldehyde measurements taken in the living room and subject's bedroom were used to assess exposure (8-hour passive sampler). The odds ratios (ORs) for risk of asthma by formaldehyde exposure level category were adjusted for numerous risk factors both familial and environmental including, familial history of asthma, age, sex, smoking, presence of pets, and attributes of the home. Of these, age, allergic sensitization to common allergens, and family history of allergy were independent risk factors for asthma (ORs of 1.09, 2.57, and 2.66, respectively). Categorical analysis of the data indicates the ORs for asthma were increased in the two highest formaldehyde exposure groups, reaching statistical significance for household exposures >60 μg/m^3 (48 ppb) (OR of 1.39). Analysis of the data with formaldehyde as a continuous variable indicated there was a statistically significant increase in the risk of asthma (3 % increase in risk per every 10 ug/m3 increase in formaldehyde level. All analyses controlled for other indoor air pollutants, allergen levels, relative humidity, and indoor temperature as well as other risk factors.

A study of 202 households (mean formaldehyde level of 26 ppb) found that among children aged 6–15 years old and exposed to environmental tobacco smoke, the prevalence of asthma was 45.5% for those with measured levels of formaldehyde in the kitchen >60 ppb. The prevalence of asthma dropped to 15.1% for levels ≤40 ppb and 0% for 41–60 ppb. No trend in asthma prevalence was seen for children who were not exposed to environmental tobacco smoke (Krzyzanowski et al., 1990).

Finally, a study by Norback et al. (1995) reported mean levels of formaldehyde were 29 μg/m^3 (range <5–110 μg/m^3) in the bedrooms of individuals experiencing nocturnal breathlessness compared with formaldehyde levels of 17 μg/m^3 (<5–60 μg/m^3) among those without nocturnal breathlessness. The OR for this association was 12.5 (95% CI: 2.0–77.9) and the effect was substantially stronger in magnitude than the associations observed for toluene, terpenes and volatile organic compounds which makes confounding by those coexposures unlikely.

5.1.1.4.2. ***Supporting animal studies.***

Several animal studies report increased airway resistance and BC due to inhalation exposures to formaldehyde (Nielsen et al., 1999; Swiecichowski et al., 1993; Biagini et al., 1989; Amdur, 1960). Changes in pulmonary resistance were observed as early as 10 minutes after exposure (Biagini et al., 1989), and reported effect levels ranged from 0.3–13 ppm. Other pulmonary effects were reported in conjunction with BHR, such as increased tracheal reactivity and decreased pulmonary elasticity (Swiecichowski et al., 1993; Amdur, 1960). Although BHR is a common result of Type I hypersensitivity reaction to an allergen, the observation of BHR alone is not sufficient to demonstrate that an agent induces Type 1 hypersensitivity.

BHR may be directly induced both pharmacologically and neurogenically (Joos, 2003; Cain, 2001; Meggs, 1995). There is little evidence that formaldehyde itself is an allergen recognized by the immune system, especially via inhalation (Lee et al., 1984). Although formaldehyde exposure has been reported to alter cytokine levels and immunoglobulins in some experimental systems, these immunomodulatory effects do not support a type 1 hypersensitivity. IgE was unchanged (Fujimaki et al., 2004a; Lee et al., 1984), and cytokine profiles were not consistent with the Th-2 cytokines expected in IgE mediated hypersensitivity (Fujimaki et al., 2004a; Ohtsuka et al., 2003).

Formaldehyde-induced dermal sensitization show parallel results. The physical signs of irritation and sensitization are consistently shown (e.g., rashes, edema). Some involvement of the immune response has been demonstrated with positive LLNA assays, indicating proliferation of lymphocytes in lymph nodes draining the affected area (Hilton et al., 1998; Arts et al., 1997).

Increased expression of Th-2 cytokines in the lymph nodes of mice given dermal applications of formaldehyde does indicate an immune component to the observed sensitization. However, the response does not seem to be mediated by IgE (Arts et al., 1997; Lee et al., 1984).

Ito et al. (1996) reported that a tachykinin NK_1 receptor, but not the histamine H_1 or bradykinin B_2 receptors, is involved in formaldehyde-induced vascular permeability. Neuropeptides NGF and substance P were affected in BAL and stimulated splenocytes from formaldehyde-exposed mice, with greater effects seen in OVA-immunized mice. Tachykinins (e.g., substance P and neurokinin A) are produced by nerve cells and can directly stimulate bronchoconstriction (Van Schoor et al., 2000). Substance P is also a mediator of neurogenic inflammation. Therefore, although formaldehyde may induce some of the symptoms of type 1 hypersensitivity, these symptoms are more likely neurogenic than immunogenic in origin.

In contrast, formaldehyde enhances immunogenic hypersensitivity of known allergens (Sadakane et al., 2002; Riedel et al., 1996; Tarkowski and Gorski, 1995). This potentiation varied based on sensitization protocols (respiratory tract versus systemic, frequency and timing of immunization, allergen, etc.) and formaldehyde exposure regimens (concentration, continuous versus intermittent exposures). Taken as a whole, the results support the finding that formaldehyde exposure can aggravate a type 1 hypersensitivity response (see Table 4-53).

The mechanism underlying this response has not been elucidated. Formaldehyde-induced IgE production has been reported in some studies (Vandenplas et al., 2004; Wantke et al., 1996a). Other studies suggest that this effect does not appear to be immunogenic in nature (Fujimaki et al., 2004a; Lee et al., 1984). Although formaldehyde exposure has been reported to alter cytokine levels and immunoglobulins in some experimental systems (Fujimaki et al., 2004a; Ohtsuka et al., 2003), these immunomodulatory effects do not support immunogenically mediated type 1 hypersensitivity.

These decrements may be mediated via neurogenic potentiation (Sadakane et al., 2002; Riedel et al., 1996; Tarkowski and Gorski, 1995). Tarkowski and Gorski (1995) suggest that formaldehyde may increase permeability of respiratory epithelium and destruction of immunologic barriers. Tachykinin NK1 receptor and various neuropeptides (NGF and substance P) have been implicated in formaldehyde-induced sensitization and lend weight of evidence to a neurogenic MOA (Van Schoor et al., 2000; Ito et al. 1996).

5.1.1.5. *Immune Function*

Although there are some indications of formaldehyde-induced immunomodulation in laboratory animal studies (Jakab, 1992; Morgan et al., 1986a, b, c; Leach et al., 1983) and reports of increased upper respiratory tract infections in formaldehyde-exposed workers

(Lyapina et al., 2004; Krzyzanowski et al., 1990; Holness and Nethercott, 1989), the overall database for toxic effects on immune function and competence is very limited. A study of workers using carbamide-formaldehyde glue indicates decreased neutrophil respiratory burst activity (NRBA) (Lyapina et al., 2004). NRBA was reduced in workers with URT inflammation and long-lasting respiratory tract infections, compared with healthy controls, and in formaldehyde-exposed workers with slight or no respiratory infections. The authors hypothesized that the decreased NRBA in symptomatic workers may be an indication of formaldehyde effects in a susceptible population. Since the workers have increased respiratory tract infections as compared with controls, a formaldehyde-specific effect cannot be excluded. These indications of a functional deficit of the immune system are considered adverse and appropriate for consideration as a critical effect. Although this was a small study (n = 29), the exposed workers had increased chronic URT infections and decreased resistance to infections compared with a control population. Additionally, duration of employment was negatively correlated with both erythrocyte count and hematocrit. Measured formaldehyde concentrations for a work shift were 870 ± 390 μg/m^3 (722 ± 324 ppb). This average work-shift concentration is considered to be the LOAEL for increased respiratory tract inflammation and decreased resistance to infections in a worker population.

5.1.1.6. *Neurological and Behavioral Toxicity*

Studies evaluating the effects of formaldehyde on nervous system structure or function are described in detail in Sections 4.1.1.6 and 4.2.6 and summarized in Section 4.4.8 and Table 4-58. Taken together, the animal and human data support the conclusion that formaldehyde exposure results in neurological and behavioral toxicity. Observed health effects include impaired memory and learning, developmental effects seen as both structural changes in the brain and behavioral changes, and a potential for increased mortality from amyotrophic lateral sclerosis (ALS). Although studies appropriate for RfC derivation do not exist for each potential neurological and behavioral health effect, several studies are available that provide information that needs to be considered when selecting the formaldehyde RfC.

Seven of the available neurotoxicity studies were considered as candidates for RfC development (listed in Table 5-1). All seven studies provided reliable documentation of

Table 5-1. Candidate points of departure (PODs) including duration adjustments for nervous system toxicity in key human and animal studies

Reference	Species	POD[a]		Exposure scenario			POD duration adjustments[b]							Ratio[c]	Effect
		Type	ppb	Hours/day	Days/week	Duration	POD	×	Hours/day	×	Days/week	=	ppb		
Developmental neuropathology effects															
Sarsilmaz et al. (2007)	Rat	LOAEL	6,000	6	5	30 days	6,000	×	6/24	×	5/7	=	1,070	5.6	Volume and cell number change in brain regions following neonatal exposure
Aslan et al. (2006)	Rat	LOAEL	6,000	6	5	30 days	6,000	×	6/24	×	5/7	=	1,070	5.6	Volume and cell number change in brain regions following neonatal exposure
Human neurobehavioral outcomes															
Bach et al. (1990)[d]	Human	NOAEL	170	5.5	1	1 day	170[d]	×		×		=	170	1	Changes in short-term memory and ability to concentrate. Single 5.5-hour exposure
Psychomotor effects															
Senichenkova (1991)	Rat	LOAEL	400	4	7	GD 1–19	400	×	4/24	×	7/7	=	67	6	Changes in open field motor activity (exploratory activity and habituation in offspring following in utero exposure

Table 5-1. Points of departure (POD) for nervous system toxicity in key human and animal studies (continued)

Reference	Species	POD[a]		Exposure scenario			POD duration adjustments[b]							Ratio[c]	Effect
		Type	ppb	Hours/day	Days/week	Duration	POD	×	Hours/day	×	Days/week	=	ppb		
Cognitive effects															
Malek et al. (2003c)	Rat	LOAEL	130[e]	2	1	1 day	130	×	2/4[e]	×		=	65	2	Concentration-dependent decreases in activity by a variety of measures following a single exposure
Pitten et al. (2000)[f]	Rat	LOAEL	2,600	0.17	7	90 days	2,600[f]		--		--		--	--	Impaired memory in a spatial maze. Magnitude of effect increased with continued exposure through 12 weeks
Malek et al. (2003a)	Rat	LOAEL	100[e]	2	7	10 days	100	×	2/4[e]	×	7/7	=	50	2	Impaired learning in a water maze. Short-term (10 day) exposure with testing conducted 2 hours following daily exposure.

[a]1 mg/m^3 = 0.813 ppm. All identified PODs were based on statistically significant findings at the study LOAELs. Full study details are provided in Section 4.1.1.6 (Bach et al., 1990) or 4.2.1.6 and Table 4-57 (all other studies).
[b]Both actual levels of experimental exposures, and duration adjusted PODs are shown.
[c]POD unadjusted dose/duration-adjusted dose.
[d]Testing was conducted during or following exposure, duration was not adjusted.
[e]Testing was conducted 2 hours postexposure; duration was adjusted to 4 hours to include the entire period between start of exposure and testing.
[f]Due to the uncertainty in continuous exposure adjustments and the unusually short (10 minutes) exposure in this study, no adjustment to continuous exposure is presented.exposure, study design, and evaluation

procedures, and all demonstrated robust findings of changes in nervous system structure or function following formaldehyde exposure. All but one of the candidate studies present information at multiple exposure levels to provide an understanding of the exposure response relationship. One selected study (Senichenkova, 1991) provided less robust information, with evaluation at only a single exposure level, but was considered useful as supporting the findings of two other studies (Sarsilmaz et al., 2007; Aslan et al., 2006) regarding neurological sequelae of developmental exposure. All of the selected studies using experimental animals were conducted in rats, although several studies in mice demonstrated dose-related neurotoxic effects following formaldehyde exposure. These studies in mice were not considered for RfC development because of the possibility that results might be confounded by reflex bradypnea at the doses tested in each study; selected behavioral studies in rats were not similarly confounded by reflex bradypnea because the effect occurs in rats only at doses above those at which the effects of concern were seen (see Section 4.2.6 for details).

In order to improve transparency and facilitate comparison of health effect levels across study types and health effects, Table 5-1 summarizes the PODs and exposure scenarios for each selected study and describes the effects on which the selected POD is based. Dose conversions used to adjust from actual experimental exposure concentrations to continuous exposure concentrations are detailed. It should be noted that available studies providing dose-response information regarding the effects of formaldehyde exposure on the nervous system were all of short duration, and thus information regarding the relationship between formaldehyde toxicity and exposure duration (i.e., whether toxicity increases with longer exposures at a given exposure level, or is more related to the maximum exposure concentration) is limited. However, the rodent study by Pitten et al. (2000) and the epidemiology study by Weisskopf et al. (2009) provide strong support for an association between increasing neurotoxicity and increasing duration of exposure.

Although chronic human studies are preferred for RfC derivation, no adequate human study of chronic duration is available (see Section 4.1.1.6 for detailed discussion of available human studies). The available human studies were sufficiently strong to raise concern regarding formaldehyde effects on the nervous system; however, most did not provide sufficient exposure information to permit derivation of a POD for use in quantitative dose-response assessment. Available epidemiologic studies (most notably Weisskopf et al. [2009] and Kilburn et al. [1987, 1985]) provided limited exposure information. Weisskopf et al. (2009) reported a non-statistically significant increase in the rate ratio for ALS for ever being exposed to formaldehyde with RR=1.34 (95% CI: 0.93-1.92) among 987,229 people followed by an American Cancer Society study, but no information regarding exposure concentrations was available. However,

when the cohort was restricted to people without missing data on duration of exposure to formaldehyde, a statistically significant association was demonstrated as RR=2.47 (95% CI: 1.59-3.86, p<0.0001). Weisskopf et al. (2009) also demonstrated statistically significant increased exposure-response for risk of mortality from ALS associated with increased duration of formaldehyde exposure (p = 0.0004). Interpretation of the findings of Kilburn et al. (1987, 1985) is complicated by concomitant exposure of many subjects to other solvents. Although the chamber study by Lang et al. (2008) included a concentration-response assessment of changes in reaction time, as previously discussed, the effects detected were difficult to interpret and the study was not considered useful for RfC derivation.

One acute human study, Bach et al. (1990), which evaluated changes in cognitive function following a single formaldehyde exposure, was considered for evaluation of a cRfC as the chamber exposures were well defined and effects at multiple levels of exposure were reported. In that study, concentration-related changes in short-term memory and ability to concentrate were seen during a single 5.5-hour exposure at a range of levels (32, 170, 390, and 890 ppb). The study was designed as a comparison of effects of short-term formaldehyde exposure in previously occupationally exposed individuals with effects in controls without previous occupational exposure. Because occupational exposure levels were not assessed, exposure measurements from the previously exposed workers are not appropriate for use in RfC derivation. The authors reported a statistically significant exposure-response relationship for three related cognitive measures (number of additions completed, number of errors, and reaction time) in the 'addition test' assessment indicating a deficit in performance. Complete data were not presented, but graphical presentations in the article indicated that the effect was seen at all doses tested, with an apparent NOAEL of 170 ppb (see Figure 5-1).

No BMD modeling could be performed on these data because the graphical representation could not be accurately digitized. The statistical analysis indicated no interaction between formaldehyde effect and previous occupational exposure (i.e., the magnitude and direction of the effect were similar in previously exposed and previously unexposed subjects) and separate data were not presented for the two groups; thus, the LOAEL represents effects in the combined study groups. Overall, the published paper lacks detail and it is difficult to evaluate some aspects of the reported findings, in particular where magnitude and direction of effect are not provided. Finally, the authors noted that controls and the high-exposure group were not well matched on two key parameters (age and education level), adding uncertainty to the reported exposure-response relationship (at the high dose). Although this study was considered valuable in documenting neurological effects in humans following exposure to relatively low concentrations of formaldehyde, the above concerns limit its utility for

quantitative human health risk assessment. Therefore, this study is not considered of sufficient quality for RfC derivation.

In the absence of adequate human data, controlled studies in laboratory animals are considered (see Section 4.2.1.6 for detailed discussion of available animal studies). There are no

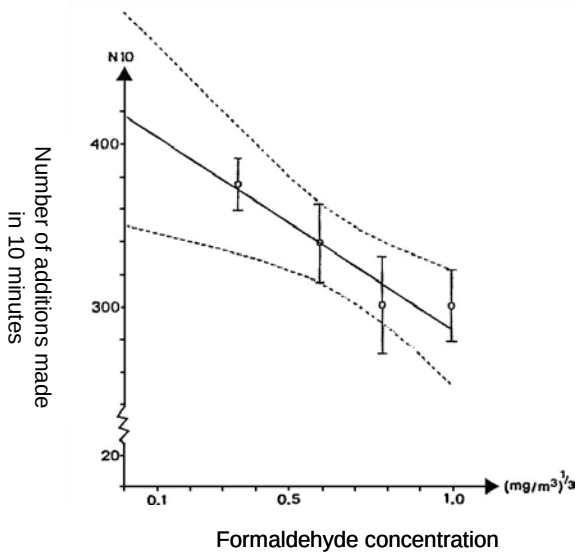


Figure 5-1. Change in number of additions made in 10 minutes following formaldehyde exposure at 0.04, 0.21, 0.48, or 1.1 mg/m^3 (32, 170, 390, or 890 ppb).

Note: Vertical bars are the standard errors of the means, dashed line shows the 95% CI.

Source: Bach et al. (1990).

chronic studies and only one subchronic animal study evaluating neurological and behavioral effects of formaldehyde exposure. Pitten et al. (2000) demonstrated impaired retention of a previously learned task in rats exposed at concentrations of 2,600 or 4,600 ppb, 10 minutes per day, 7 days/week, for 90 days (statistically significant, $p<0.05$). In this study, the magnitude of the impairment increased over time, even though testing was performed 22 hours after exposure, indicating that repeated formaldehyde exposure led to a worsening of effect. The study design, test methods, and reporting of the results are all of adequate quality for both hazard assessment and quantitative risk assessment. However, the short duration (10 minutes) of the repeated daily exposures is a severe limitation to establishing a chronic RfC based on this study, due to uncertainties in extrapolating from 10 minutes to a 24-hour exposure (see Table 5-1). Because

this study as designed indicates an accumulation of effect with repeated exposure, it is useful in documenting the existence of a duration component to the exposure-response relationship. It follows that concentration alone, without an adjustment for duration of exposure, would be inadequate as an exposure metric; however inadequate information is available to inform the appropriate magnitude of the duration effect. Therefore, although Pitten et al. (2000) is a well-conducted study, the data are of limited utility for RfC derivation.

Finally, there are several well-documented acute and subacute animal studies that provide exposure-response information for neurological and behavioral endpoints relevant for RfC derivation. Several laboratory animal studies that evaluate neurological effects following in utero or neonatal exposure address potentially susceptible life stages. Sarsilmaz et al. (2007) and Aslan et al. (2006) observed changes in brain structure (cell number and/or volume changes in specific brain regions) following 30 days of exposure to neonatal rats ($p<0.001$). A related finding by Senichenkova (1991) demonstrated changes in behavior (open field motor activity, including habituation) in young rats following in utero exposure ($p<0.05$). Effects of concern were seen at all doses in these studies, resulting in PODs of 67 ppb following in utero exposure and 1,070 ppb following early postnatal exposure, based on LOAEL values adjusted for continuous exposure (see Table 5-1). These studies support the possibility of neurodevelopmental effects attributable to in utero or early postnatal formaldehyde exposure, at levels similar to or below those causing other types of effects.

The other three studies in Table 5-1 evaluate behavioral changes in rats following exposure to formaldehyde. Malek et al. (2003c) found concentration-related changes in motor activity following a single 2-hour exposure at concentrations from 130–5,180 ppb (with testing 2 hours following cessation of exposure; $p<0.005$)). In a second study, Malek et al. (2003a) found concentrated-related changes in performance on a learning task at similar exposure levels (100–5,400 ppb) when 2-hour exposures were repeated for 10 consecutive days ($p<0.05$); performance was evaluated 2 hours after cessation of exposure, and concentration-related learning deficits were seen at all exposure levels (see Table 5-2 and Figure 5-2).

Although other studies evaluating neurobehavioral effects were available in the formaldehyde database (see Chapter 4), these studies by Malek et al. (2003a, c) were considered to be the most robust, documenting effects at relatively low exposure levels. Both studies also included evaluation at multiple concentrations and showed concentration-related increases in effect. In the Malek et al. (2003a) study with repeated exposures, it is unclear whether or not the measured effect primarily reflects the most recent exposure or cumulative exposure; therefore, the adjustment for continuous exposure was made over the final exposure period and the two hours following exposure (4 hours total), as was done for the single-exposure study (Malek

Table 5-2. Effects of formaldehyde exposure on completion of the labyrinth test by male and female LEW.1K rats

	Swimming time (sec)			Error rate (mean)		
Male rats	**Day 1**	**Day 6**	**Day 10**	**Day 1**	**Day 6**	**Day 10**
Control	105	12.2	6.33	7.4	0.5	0.0
0.1 ppm[a]	100	12.9	6.07	7.7	5.0[c]	3.2[c]
0.5 ppm	97	16.7[c]	7.60[b]	7.6	4.4[c]	1.8[c]
5.4 ppm	105	25.7[c]	10.9[c]	7.7	5.0[c]	2.8[c]
	Swimming time (sec)			**Error rate (mean)**		
Female rats	**Day 1**	**Day 6**	**Day 10**	**Day 1**	**Day 6**	**Day 10**
Control	103	12.5	6.47	7.9	0	0.0
0.1 ppm	96	12.3	7.53	7.1	5.2[c]	3.0[c]
0.5 ppm	97	14.6[c]	7.60[b]	8.0	4.6[c]	2.2[c]
5.4 ppm	98	23.5[c]	9.73[c]	7.9	5.2[c]	2.6[c]

[a]Rats were exposed to formaldehyde for 2 hours/day, for 10 consecutive days.
[b]Different from control, $p < 0.05$.
[c]Different from control, $p < 0.005$.

Source: Malek et al. (2003a).

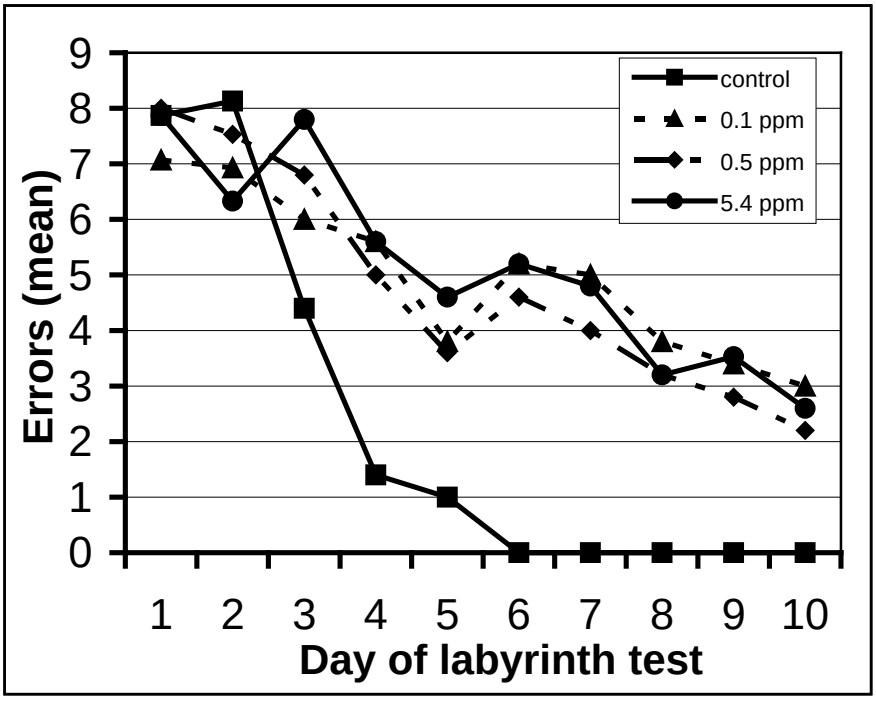


Figure 5-2. Effects of formaldehyde exposure on the error rate of female LEW.1K rats performing the water labyrinth learning test.

Source: Drawn from data reported in Malek et al. (2003a).

et al., 2003c). After appropriate duration adjustments, PODs for these studies range from 50 to 67 ppb (based on LOAELs), and the types of effects seen provide support for the Bach et al. (1990) study that detected cognitive impairments in humans following a single exposure (with a NOAEL of 170 ppb).

Summary of neurological and behavioral effects. In summary, the available studies for formaldehyde and nervous system outcomes have demonstrated that the nervous system is a sensitive target following inhalation of formaldehyde. In experimental animals, changes in nervous system function were seen following acute and subchronic exposures; studies evaluating neurological changes following chronic exposure were unavailable. Available human studies that evaluated nervous system effects following inhalation exposure were found to have many study-specific uncertainties and, thus, were not suitable to serve as the primary basis for a chronic RfC. The Weisskopf et al. (2009) study of ALS, in particular, suggests that humans may be at risk for severe neurological effects from formaldehyde exposure; however, this study

lacked the exposure concentration information necessary to derive an RfC. Neurological findings from the rodent inhalation (acute and subchronic) studies that were judged to be adequate for dose-response assessment identified unadjusted LOAELs ranging from 100 to 6,000 ppb, with LOAELs adjusted for continuous exposure in the range of 50 to 1,070 ppb. Use of these PODs in risk assessment would require addressing uncertainties regarding animal-to-human extrapolation, short study durations, and extrapolation from LOAELs.

Among the adequate studies, EPA considered Malek et al. (2003a) to be the most appropriate for calculation of a cRfC for neurological and behavioral toxicity, based on the exposure level at which effects were seen (100 ppb), the type of effect (impaired learning), which is relevant to humans, and the use of a repeated-exposure paradigm (2 hours/day over a period of 10 days), which addresses different exposure durations. This choice is supported by similar effects seen in other studies (Lu et al., 2008; Pitten et al., 2000; Bach et al., 1990) and by other neurologic effects seen at similar exposure levels (Malek et al., 2003c; Senichenkova, 1991; Sheveleva, 1971).

5.1.1.7. *Developmental and Reproductive Toxicity*

As described in Sections 4.1 and 4.2, both human epidemiologic data (see Section 4.1.1.7) and experimental animal studies (see Section 4.2.7 and Tables 4-70 and 4-73) demonstrate an association between formaldehyde inhalation exposure and adverse developmental and reproductive effects, where adversity is characterized as per EPA risk assessment guidelines (U.S. EPA, 1991a, available at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=23162; 1996, available at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=2838). Adverse outcomes were observed across the various manifestations of developmental toxicity, including fetal death, structural alterations (including congenital malformations), growth retardation, and functional development. Additionally, in spite of the lack of a comprehensive database of studies for the evaluation of the overall effects of formaldehyde on the reproductive system and its function, the available evidence demonstrates toxicity to the male reproductive system in multiple animal studies, as well as effects on the female reproductive system in both rodents and epidemiologic studies, where an association with impaired fertility and increased spontaneous abortions were noted.

Potential principal studies for specific adverse outcomes are presented and evaluated below including reproductive effects (male and female), fetal death, growth retardation, and structural alterations. The only available evidence for functional alterations is based on developmental neurotoxicity studies which are presented and evaluated in Section 5.1.1.6.

Among the animal studies with developmental and reproductive effects after inhalation exposure (presented in Tables 4-70 ad 4-73), 12 endpoints from nine studies were selected for candidate PODs (see Table 5-3). The criteria for inclusion are that the studies provided reliable documentation of exposure, study design, and positive findings of developmental or reproductive structural, functional or precursor effects. Six of the studies evaluated effects after multiple dose levels, providing dose-response information. The other three studies, with a control and a single FA dose, were included as candidate PODs because effects were observed for endpoints that were either not assessed or observed in the other six studies (e.g., cryptorchidism). Table 5-3 summarizes animal studies deemed suitable for deriving quantitative dose-response information for reproductive and developmental outcomes and their corresponding PODs, adjusted for continuous exposure. Calculations that were used in dose conversions and exposure duration adjustments for the POD values are included. In general, repeated daily exposures of laboratory animals are adjusted from a partial day to a 24-hour exposure and then weighted for the number of days per week the exposures occurred. No chronic animal studies evaluating these endpoints were available, so only subchronic and acute studies are considered.

The human epidemiologic data on developmental and reproductive outcomes are discussed in Section 5.1.1.7.1. below. Exposure duration adjustments to the only suitable human study (Taskinen et al., 1999) are more complex due to uncertainties in the exposure data and the potential for nonoccupational exposures. For this discussion the reported 8-hour TWA exposures will be used for the Taskinen et al. (1999) study. Further duration adjustments to this study are discussed in Section 5.1.2.2.5 for cRfC derivation.

5.1.1.7.1. ***Spontaneous abortion and fetal death.***

Increased risk of spontaneous abortion following maternal occupational formaldehyde exposure was reported in a number of epidemiologic studies (Taskinen et al., 1999, 1994; John et al., 1994; Seitz and Baron, 1990; Axelsson et al., 1984). The studies did not appear to be overtly influenced by common principle biases found in epidemiologic studies. Considered together, the studies are consistent with an adverse effect of formaldehyde exposure on pregnancy loss, where adversity is characterized as per EPA risk assessment guidelines (U.S. EPA, 1991a, 1996). Of these studies, Taskinen et al. (1999) had the higher quality quantitative exposure data reporting reduced fecundity and spontaneous abortion in the exposed workers. Taskinen et al. (1999) is an occupational study with a well-considered study design, including measurements of exposure and outcomes, and relatively high study power. The study population consisted of 602 female workers in Finland who had at least one successful childbirth and first employment in the

Table 5-3. Candidate PODs including duration adjustments for developmental and reproductive toxicity in key animal studies

Reference	Species	POD		Exposure scenario			POD duration adjustments						Ratio[b]	Effect; comments
		Type	ppb[a]	Hours/ day	Days/ week	Duration	POD (ppb)	×	Hours/ day	×	Days/ week	= Adjusted POD (ppb)		
Spontaneous abortion and fetal death														
Kitaev et al. (1984)	Rat	LOAEL	400	4	5	6 months premating	400	×	4/24	×	5/7	= 50	8	Increased (>threefold) embryo degeneration on gestational days 2–3 after 4 months maternal premating treatment
Sheveleva (1971)	Rat	LOAEL	400	4	7	GDs 1-19	400	×	4/24	×	7/7	= 70	5.7	Increased (50%) preimplantation loss[g]
Structural alterations[c]														
Senichenkova (1991)	Rat	LOAEL	400	4	7	GDs 1-19	400	×	4/24	×	7/7	= 70	5.7	Increased (13%) litter incidence of internal organ anomalies, including 20% increase in undescended testes; 9% decreased fetal incidence of hyoid ossification[g]
Senichenkova and Chetobar (1996)	Rat	LOAEL	400	4	7	GDs 1-19	400	×	4/24	×	7/7	= 70	5.7	Increased (21%) fetal and litter incidences of cryptorchidism and increased (6%) fetal incidences of total anomalies[g]
Growth retardation														
Saillenfait et al. (1989)	Rat	BMCL	1,300	6	7	GDs 6-20	1,300	×	6/24	×	5/7	= 325	4	Decreased male fetal body weights[g] (BMR = 5%)

Table 5-3. Candidate PODs including duration adjustments for developmental and reproductive toxicity in key animal studies (continued)

Reference	Species	POD		Exposure scenario			POD duration adjustments						Ratio[b]	Effect; comments
		Type	ppb[a]	Hours/ day	Days/ week	Duration	POD (ppb)	×	Hours/ day	×	Days/ week	= Adjusted POD (ppb)		
Functional development[d]														
Male reproductive toxicity														
Özen et al. (2002)	Rat	LOAEL	10,000	8	5	4 or 13 weeks	10,000	×	8/24	×	5/7	= 2,380	4.2	Decreased testis weight at 4 weeks (2%) and 13 weeks (8%)
Özen et al. (2005)	Rat	LOAEL	5,000	8	5	91 days	5,000	×	8/24	×	5/7	= 1,190	4.2	Decreased (40%) serum testosterone levels at 91 days
Sarsilmaz et al. (1999)	Rat	LOAEL	10,000	8	7	4 weeks	10,000	×	8/24	×	7/7	= 2,380	4.2	Decreased (5%) Leydig cell numbers at 4 weeks
Zhou et al. (2006)	Rat	LOAEL	8,050	12	7	2 weeks	8,050	×	12/24	×	7/7	= 4,025	2	Decreased (~25%) testis weight; alteration of epididymal sperm [decreased (38%) count, decreased (19%) motility, and increased (>3-fold) abnormal morphology] at 2 weeks
Female reproductive toxicity														
Kitaev et al. (1984)	Rat	NOAEL	400	4	5	4 months premating	400	×	4/24	×	5/7	= 50	8	Increased (~66%) follicle-stimulating hormone at 4 months

[a]1 mg/m^3 = 0.813 ppm. All identified PODs were based on statistically significant findings at the study LOAELs. The study details are provided in Section 4.2.1.7. and Tables 4-70 and 4-73. For Saillenfait et al. (1989), the BMCL was calculated (see "effect; comments" column above for details).

[b]POD unadjusted dose/duration-adjusted dose.

[c]Neuropathological alterations following exposures during postnatal development (from the studies by Aslan et al. [2006] and Sarsilmaz et al. [2007]) are addressed in the neurobehavioral toxicity Section 4.2.6 and Table 5-2.

[d]Functional developmental endpoints (from the study by Senichenkova [1991]) are addressed in the neurobehavioral toxicity Section 4.2.6 and Table 5-2.

GDs = Gestation days

wood-working industry beginning at least 6 months prior to the studied pregnancy. Mean daily formaldehyde inhalation exposures during the time-to-pregnancy period were estimated for each worker, based on task-level exposure measurements and work history.

Exposure was reported as a daily exposure index representing the average daily exposure for the time-to-pregnancy period, and three exposure classes were defined as low, medium and high with equivalent mean work-shift TWA exposure of 18, 76 and 219 ppb, respectively. Fecundity density ratio (FDR) was significantly reduced in the high exposure group compared to the referent group (FDR=0.64, 95% CI: 0.43-0.92, p = 0.02) indicating that it took longer for the highly exposed women to become pregnant compared to women who were unexposed. The investigators stratified the 39 women in the high exposure group by glove use. While stratification by glove use reduced the statistical power of each comparison, the magnitude of the effect in each strata was not markedly effected and the 95% confidence intervals of the unstratified and stratified results all overlapped. Figure 5-3 shows the study results stratified by glove use in women in the high-exposure group. While the adverse effect of high exposure to formaldehyde was somewhat more pronounced among the women who did not wear gloves, possibly suggesting that a component of dermal exposure might contribute to the effect, it is unclear what, if any, dermal exposure is expected based on the nature of the work. Regardless, there remains uncertainty as to whether effects are solely due to inhalation exposure. Taskinen et al. (1999) also reported the risk of spontaneous abortions was statistically significantly increased with reported ORs of 3.2 (95% CI: 1.2−8.3), 1.8 (95% CI: 0.8−4) and 2.4 (95% CI: 1.2−4.8) for the high, medium and low exposure groups, respectively. The finding of increased risk of spontaneous abortion is consistent with the finding of delayed conception as measured by the fecundity density ratio.

In some available rodent studies (Kitaev et al., 1984; Sheveleva, 1971), evidence of increased embryo degeneration in early gestation or of preimplantation loss (findings that are generally comparable to spontaneous abortion in humans) was observed. In the Kitaev et al. (1984) study, early implantation losses resulted following treatment of dams prior to mating. This may support a possible contribution of prepregnancy exposures to the spontaneous abortions observed in Taskinen et al. (1999). Quantification of the findings by Kitaev et al. (1984) and Sheveleva (1971) resulted in adjusted PODs of 50 and 70 ppb, respectively, based upon study LOAELs (see Table 5-3).

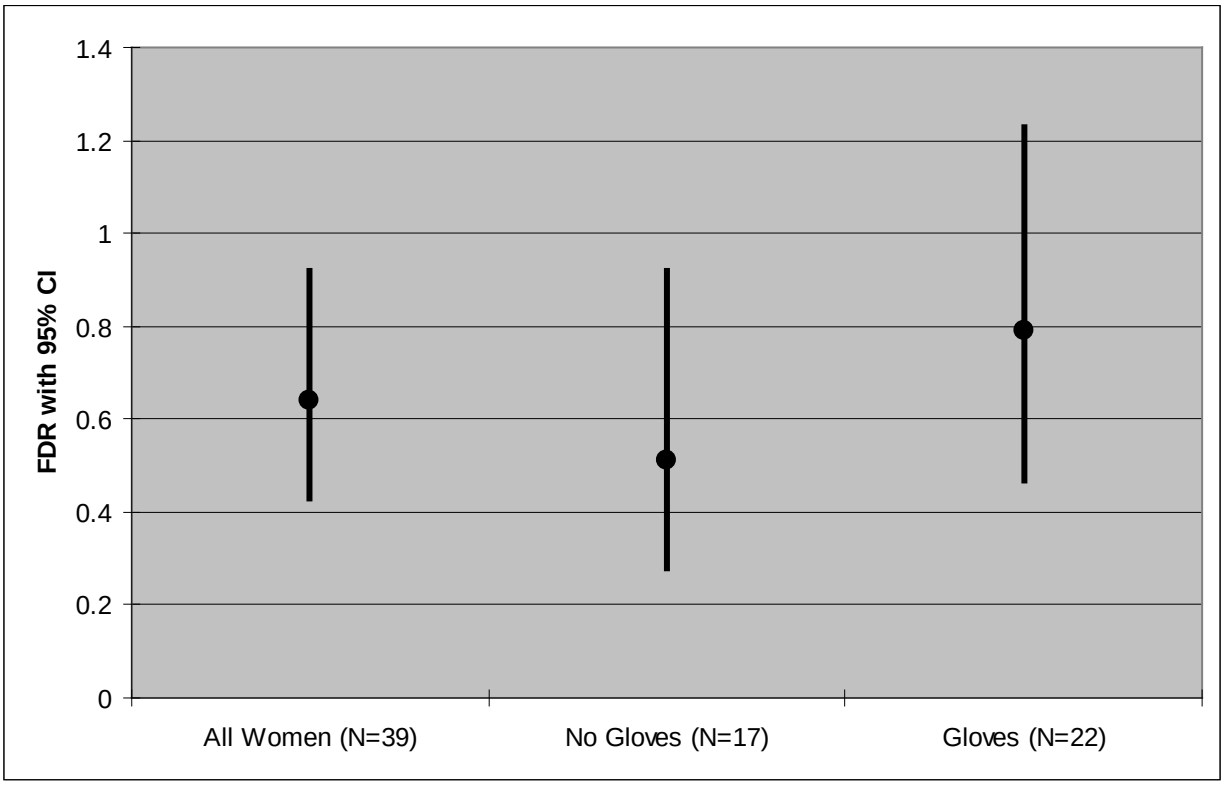


Figure 5-3. Fecundity density ratio among women exposed to formaldehyde in the high exposure index category with 8-hour time-weighted average formaldehyde exposure concentration of 219 ppb (Taskinen et al., 1999)

5.1.1.7.2. ***Structural alterations.***

Studies of occupational exposures to formaldehyde examined the incidence of congenital malformations, but exposure and outcome data were not fully characterized and therefore could not be carried forward to RfC development. Animal studies (Senichenkova and Chetobar, 1996; Senichenkova, 1991) reported increases in internal organ anomalies; the most frequently observed structural anomaly was a delay in fetal testis descent (at times characterized as cryptorchidism in the study reports). For both studies, which exposed rats to formaldehyde for 4 hours/day during gestation, adjusted PODs based upon LOAELs were 70 ppb (see Table 5-3). These studies included only one treatment level, precluding the ability to establish a dose-response relationship, and the observed outcomes were not noted in other developmental toxicity studies with similar exposure scenarios, thus limiting the strength of the studies for use in RfC derivation.

5.1.1.7.3. ***Growth retardation.***

Decreased fetal weight was observed in a number of animal studies that exposed pregnant rats to formaldehyde during gestation. Of these, based on adequacy of dose-response information, Saillenfait et al. (1989) was considered appropriate for consideration for RfC development. In this study, rats were administered formaldehyde 6 hours/day on gestational days (GDs) 6–20. Decreased male fetal body weight (BW) was modeled with a BMR of 5% mean change, a BMCL was established, and, as shown in Table 5-3, the resulting duration-adjusted POD of 325 ppb was derived. The relevance of this finding to human exposures was qualitatively supported by a population-based study by Grazuleviciene et al. (1998) that reported an association between atmospheric formaldehyde exposure and low birth weight; although a dose-response relationship could not be adequately quantified from the information provided.

5.1.1.7.4. ***Male reproductive toxicity.***

Evidence of adverse effects on male reproductive system endpoints following inhalation exposure to formaldehyde was observed in a number of animal studies, where adversity is characterized as per EPA risk assessment guidelines (U.S. EPA, 1991a, 1996). The effects include decreased testes weight, changes in Leydig cell quantity and quality, degeneration of seminiferous tubules, decreased testosterone levels, alterations in biomarkers of toxicity in the testes, and alterations in sperm count, morphology, and/or motility (Golalipour et al., 2007; Xing et al., 2007; Zhou et al., 2006; Özen et al., 2005, 2002; Sarsilmaz et al., 1999; Guseva, 1972). Several of these studies included inhalation exposure of rats to formaldehyde 8 hours/day, 5 days/week for 4 and/or 13 weeks (Özen et al., 2005, 2002; Sarsilmaz et al., 1999) and included exposure-response information that was considered adequate for RfC derivation. In a study by Özen et al. (2002), increased severity of statistically significant testes weight decreases was related to both dose and duration of treatment. Similarly, in the study by Golalipour et al. (2007), seminiferous tubular diameter and epithelial height were reduced in rats following 18 weeks of formaldehyde inhalation exposure, with the severity of outcome positively correlated to the number of hours/week that the animals were exposed. Sarsilmaz et al. (1999) noted dose dependent decreases in Leydig cell quantity after 4 weeks of treatment, while decreased testis weight and atrophy of seminiferous tubules were observed by Zhou et al. (2006) after only 2 weeks of treatment. The reported outcomes in these independent studies illustrate a biologically consistent toxicological profile of treatment-related male reproductive toxicity. PODs, adjusted for continuous exposure, ranged from 1,190 to 4,025 ppb, where the lowest POD was associated with the longest exposure period and vice verse (see Table 5-3).

5.1.1.7.5. ***Female reproductive toxicity.***

Evidence of decreased fecundability was observed in the study by Taskinen et al. (1999), which was described above for spontaneous abortions. Delays in the time to conception that characterized this outcome, as well as increases in the incidence of endometriosis, were statistically significantly associated with occupational exposures to formaldehyde. As these effects were observed in the high exposure group, the unadjusted NOAEL for each of these effects is 76 ppb (8 hour-TWA) based on the next lowest exposure group. Uncertainties included lack of information human variability, as well as on the extrapolation of data from studies of short duration to risk estimates for chronic exposures. As discussed above for spontaneous abortions, the use of these data for cRfC derivation could result in values that would likely be an underestimation of risk because they assume that all the risk was from inhalation exposure and ignore the apparent contribution of dermal exposure (i.e., the dermal-exposure-adjusted candidate inhalation RfCs might be higher). For decreased fecundability, a POD can also be identified based on the data from only the women who wore gloves. The fecundability density ratio (FDR) for the women in the highest exposure group was statistically significantly reduced at FDR=0.64 (95% confidence interval [CI] 0.43–0.92). Evidence of spontaneous abortions in the same study, as described above, may also be indicative of female reproductive toxicity.

In animal studies, assessment of the female reproductive system was quite limited. An increase in the mean follicle-stimulating hormone (FSH) levels in rats, observed at the highest exposure level tested in Kitaev et al. (1984) was found to be sufficient to derive a duration-adjusted POD of 50 ppb (see Table 5-3).

5.1.1.7.6. ***Summary of developmental and reproductive toxicity studies suitable for RfC development.***

A review of the developmental and reproductive toxicity studies in humans and animals that would be suitable for cRfC development demonstrated that the developing organism and the reproductive system are targets for toxicity following formaldehyde exposure by inhalation. In the animal studies, effects during early development were observed following maternal premating or gestational exposures at duration-adjusted PODs ranging from 50−325 ppb. The minimal data available on female reproductive toxicity demonstrated an adjusted POD of 50 ppb with subchronic (4-month) premating exposure, while more extensive evaluation of male reproductive outcomes identified adjusted PODs of 1,190−4,025 for testicular and sperm abnormalities after exposures of from 2 weeks to 3 months in duration. The animal studies demonstrate the broad range of adverse outcomes to the reproductive system and the developing organism following inhalation exposure to formaldehyde and highlight concerns regarding the

inadequacy of the database for the assessment of these outcomes (as described in Chapter 4). These data also support the human relevance of female reproductive and/or embryonic and fetal developmental effects, since some outcomes were similarly observed in both human and animal studies.

The animal study data were not selected for RfC derivation, since a high-quality epidemiology study (Taskinen et al., 1999) was available for the purpose of deriving a chronic RfC. This study, a well-designed population-based case-control study of women who were occupationally exposed to formaldehyde, included a well-defined study population which was adequately selected to allow for meaningful comparisons of health effects among individuals with different levels of exposure to formaldehyde. Potential confounding factors such a selection bias and inaccurate self-reporting were not considered to have had a significant influence on the study findings. The increased risk of spontaneous abortion observed in Taskinen et al. (1999), and perhaps the observed decrease in fecundity, is internally consistent and coherent with other reports of increased risk of pregnancy loss associated with exposure to formaldehyde (John et al., 1994; Taskinen et al., 1994; Seitz and Baron, 1990; Axelsson et al., 1984). It is also supported by similar adverse outcomes observed in the animal data (Kitaev et al., 1984; Sheveleva, 1971).

5.1.2. Summary of Critical Effects and Candidate RfCs

5.1.2.1. *Selection of Studies for Candidate RfC Derivation*

The above reviews of data from both human and animal studies identified health effects associated with formaldehyde exposure. Detailed information on these findings is given in Chapter 4 (see Sections 4.1 and 4.2), and a qualitative summary of the noncancer hazard identification is provided in Section 4.4 for each of the identified health effect categories: sensory irritation, upper respiratory tract pathology, respiratory effects, increased atopic response, immune function, reproductive and developmental toxicity, and neurobehavioral toxicity. In this chapter, results for each health effect category are reviewed and studies are identified which are adequate to inform the exposure-response relationship for health effects from inhalation exposure (see Section 5.1.1). Although the database of published studies that are currently available does not provide adequate quantitative data to derive cRfCs for all qualitatively identified endpoints, at least one adequate study was identified for each of the health effect categories discussed above. For all but one of the categories, at least one study was available that provided epidemiologic (human) data, based on occupational or residential exposures, which was judged adequate to provide a quantitative basis for a cRfC.

In order to select the principal study or studies most appropriate for use as the basis of the RfC for formaldehyde, the relative merits of these studies were evaluated with respect to study quality, characteristics of the study population, the quality and frequency of exposure measurements, and the exposure levels at which effects are observed. The ideal RfC would be derived from a reported exposure level without an appreciable risk of deleterious effects in humans, including sensitive populations, with little uncertainty. Additionally, where possible, the RfC should be derived with consideration of all of the identified health effects. The several factors that were collectively taken into consideration for these studies (in no particular order) included the following:

- Were studies of laboratory animals or humans?
  - Human studies were generally preferred over laboratory animal studies for similar health effects, when both were of good quality, given the uncertainties in interspecies extrapolation.
- What was the study size?
  - Larger studies were generally preferred over smaller studies because they can give more precise estimates of response levels associated with specific exposure levels.
- Among the epidemiologic (human) studies, were exposures from an occupational setting or from a residential setting?
  - Studies of health effects from residential exposures were generally preferred over studies of health effects from occupational exposures because residential exposures tend to have a smaller range of variability and are less prone to large intermittent exposure peaks.
  - Residential exposures are more representative of the exposures of the general population.
- Among the epidemiologic (human) studies, were children among the study population in which health effects were observed?
  - Studies of health effects that assessed the effect of formaldehyde on children's health, representing a potentially more susceptible life-stage for some effects, were given some preference because they provide formaldehyde-specific data relevant to the components of the RfC derivation that address potentially sensitive life-stages and populations.
- Relative to the other studies under consideration for RfC development, how accurately were formaldehyde concentrations measured?
  - Studies based on relatively more accurately measured formaldehyde concentrations were generally preferred over studies that estimated exposures.

- Studies that reported effects at relatively lower formaldehyde concentrations, potentially indicative of more sensitive endpoints, were generally preferred.

Taking all the factors into consideration collectively, the individual studies are presented in Table 5-4.

For sensory irritation, four studies are identified with adequate exposure information for RfC derivation, and all are observational studies of humans (see Table 5-4). Of these, 3 studies were conducted in residential populations, including children and the elderly (Liu et al.,1991; Ritchie and Lehnen, 1987; Hanrahan et al., 1984). Each of these studies includes in-home formaldehyde measurements for each participant. Liu et al. (1991) provide the best exposure measurements, with 7-day in-home passive air samples collected in two seasons. The occupational study by Holmström and Wilhelmsson (1988) provides evidence of sensory irritation in workers; however, only the mean and range of exposures for all workers is given. Furthermore, occupational exposures can include high peak exposures. The residential studies are preferred for development of candidate RfC. Although there are differences in study size and the quality of exposure measurements between the three residential studies, their results are mutually supportive, defining similar effect levels in similar populations, and the use of the three residential studies was considered to provide adequate consideration of the sensory irritation endpoint. Therefore, all 3 studies are selected (Liu et al.,1991; Ritchie and Lehnen, 1987; Hanrahan et al., 1984) and will be evaluated together in the following section.

Histological changes in the upper respiratory tract are well documented in animal studies and have been observed in several worker studies (see Section 4.4). Although the study of resin production workers (Holmström and Wilhelmsson, 1988; Holmström et al., 1989) provides the best documentation of effect level for this health category in humans, it is not carried through for development of a candidate RfC. As with the sensory irritation endpoint reported in these studies, exposure is described for the worker cohort by a simple mean, with a range of exposures given for all workers. Therefore, these data do not provide an exposure-response relationship and the POD would be the mean exposure level of all workers, regardless of effect. This is less exact than other available studies which provide exposure-response relationships. Additionally, animal studies provide a broad database which supports sensory irritation as a more sensitive endpoint than histological changes in the nasal mucosa.

Reduced pulmonary function is associated with formaldehyde exposure in several human studies (students and workers). The best single study demonstrating decreased pulmonary

Table 5-4. Summary of candidate studies for formaldehyde RfC development by health endpoint category

Health endpoint category	Study	Species	Setting	Children	Study size	Formaldehyde measurements	Specific endpoints	Observed effects[a] (ppb)	POD (ppb)
Sensory Irritation	Liu et al. (1991)	Human	Residential	Yes	1,394	Two locations at one time period (winter or summer); 7-day passive monitors	Eye irritation	95	LOAEL=95
	Ritchie and Lehnen (1987)	Human	Residential	Yes	2,007	Two locations at one time period; 30-minute sample	Eye, nose, and throat sensory irritation	200	NOAEL=50
	Hanrahan et al. (1984)	Human	Residential	Yes (teenagers)	61	Two locations at one time period; 60-minute sample	10% increased prevalence of burning eyes	130	$BMCL_{10}$=70
	Holmström and Wilhelmsson (1988)	Human	Occupational	No	106	Several measurements at factory workstations taken over 7 years	Eye irritation	210	NOAEL=70
Upper Respiratory Tract Pathology	Holmström and Wilhelmsson (1988); Holmström et al. (1989)	Human	Occupational	No	132 68 with pathology	Several measurements at factory workstations taken over 7 years	Loss of ciliated epithelium; goblet cell hyperplasia; squamous cell metaplasia	240	LOAEL=240
Sensitization: Asthma and atopy	Garrett et al. (1999 a,b)	Human	Residential	Yes	148	Four locations over up to four time periods; 4-day passive monitors	Increased allergy; increased asthma-like symptoms	28	LOAEL=28

Table 5-4. Summary of candidate studies for formaldehyde RfC development by health endpoint category (continued)

Health endpoint category	Study	Species	Setting	Children	Study size	Formaldehyde measurements	Specific endpoints	Observed effectsa (ppb)	POD (ppb)
Pulmonary Function	Krzyzanowski et al. (1990)	Human	Residential	Yes	208	Four locations over two time periods (opposite seasons); 7-day passive monitors	10% Reduction in PEFR	27	$BMCL_{10}$=17
Neurological	Malek et al. (2003a)	Rat	Laboratory	--	120	Intentional exposures at specific levels	Impaired learning	100	LOAEL=100
Reproductive and Developmental effects	Taskinen et al. (1999) (FDR)	Human	Occupational	No	602	Actual and surrogate measurements estimated by occupational hygienist	Decreased fecundity density ratio (FDR)	226[b]	NOAEL=86
	Taskinen et al. (1999) (SAB)	Human	Occupational	No	602	Actual and surrogate measurements estimated by occupational hygienist	Increased risk of spontaneous abortion (SAB)	26[b]	LOAEL=26
Immune Function	Lyapina et al. (2004)	Human	Occupational	No	29	Average shift concentrations based on measures 8-hour exposures	Increased respiratory tract infections, decreased neutrophil respiratory burst activity	722	LOAEL=722

[a]This is the lowest level of exposure at which adverse effects were observed, the LOAEL, in effect, or the cut-off point for adversity for BMCLs.
[b]See Section 5.1.2.6.2 for methods to adjust exposure levels from Taskinen et al. (1999).

function is the moderate residential study by Krzyzanowski et al. (1990). The study was specifically designed to include homes with children between the ages of 5−15. Results presented for children (n = 208) provide an exposure-response relationship for reduced PEFR. Data quality is considered high for this study, both in terms of the in-home exposure measurements (7-day passive monitors, two time periods) and the contemporaneous in-home measurement of pulmonary function. Sources of potential confounding or bias were considered by the study authors and adequately taken into account in the study. Therefore, this study is retained for derivation of a candidate RfC.

Several studies report increased asthma and/or allergic sensitization in children associated with increased formaldehyde exposure in school or homes (see Section 5.1.4). Of these, two studies are further evaluated here (Garrett et al., 1999 a,b; Rumchev et al., 2002). The study by Rumchev et al. (2002) is a case-control study of asthma incidence in children, and the study by Garrett et al. (1999 a,b) is designed to study several related health effects (asthma, sensitization and respiratory symptoms) in asthmatic and nonasthmatic children. Both studies measure in-home formaldehyde levels with multi-day passive samples. Survey data and health outcome data are considered of high quality in each study. Additionally, sources of potential confounding or bias were considered by the study authors and adequately taken into account in the study. Therefore, both studies are retained for derivation of a candidate RfCs. Although several studies of school children support these findings, the residential studies were considered more appropriate for RfC derivation because individual in-home formaldehyde levels were associated with the health outcome data.

Multiple lines of evidence support the occurrence of neurotoxicity following exposure to formaldehyde, however, none of the available studies in humans were considered to be of adequate quality for derivation of a point of departure for use in quantitative assessment. Of the available neurotoxicity studies, Malek et al. (2003a), in which impaired learning was seen in rats following exposure at 100 ppb, was selected as a potential candidate for RfC development (see Section 5.1.6). A NOAEL was not identified for this effect. In view of the other studies available in the formaldehyde database (including multiple human studies of potentially sensitive populations), and considering the uncertainty in extrapolating from the exposure conditions in the Malek et al. (2003a) study (two hour exposures, repeated on ten consecutive days) to a chronic exposure scenario, this study was not carried forward for derivation of a candidate RfC. It is important to note that the resulting RfC may therefore not fully consider the documented neurotoxic effects of formaldehyde.

Of the various reproductive and developmental effects associated with formaldehyde exposure, reduced fecundity and increased risk of spontaneous abortions are primarily studied in

humans (see Section 5.1.7). Of the available epidemiology studies, only one study provides individual exposure estimates of adequate quality to support RfC development (Taskinen et al., 1999). Exposure-response relationships for decreased fecundability density ratio and increased risk of spontaneous abortions are seen with increased categories of worker exposures. Several potential confounding exposures are evaluated in the study, and the association of decreased fecundability density ratio observed in the study is most convincingly associated with increased formaldehyde exposure (Taskinen et al., 1999). Potential sources of bias were also adequately addressed in the study. This is considered a high quality study and is retained for cRfC derivation.

Although Lyapina et al. (2004) have documented decreased neutrophil respiratory burst activity in exposed workers, the overall weight of evidence for deficit in immune function due to formaldehyde exposure is weak. There is a trend for increased respiratory tract infections in formaldehyde-exposed individuals, but it is a direct result of impaired immune function or, perhaps, increased infection due to direct effects on the protective barriers of the nasal mucosa. Animal studies do not support a finding of a deficit in immune function with formaldehyde exposure. The study by Lyapina et al. (2004) is a small study, and the findings of decreased neutrophil respiratory burst activity were in those individuals with more upper respiratory tract infections, so there is some question of causality. The data evaluation does not provide an exposure-response relationship, but, rather, exposure for the cohort is expressed as a mean exposure of 722 ppb. Although the potential for impairment of immune function is an important health effect, the overall evidence for this effect and this specific study are relatively weak compared to other data available to support RfC derivation for formaldehyde. Therefore, this study is not carried further in the quantitative analysis.

In summary, the best studies evaluated herein for the derivation of an RfC for formaldehyde exposure and the related health effects are: 1) Sensory irritation (Liu et al.,1991; Ritchie and Lehnen, 1987; Hanrahan et al., 1984); 2) reduced pulmonary function (Krzyzanowski et al., 1990); 3) sensitization (atopy and asthma) (Garrett et al., 1999 a,b and Rumchev et al., 2002); and 4) reduced fecundity and increased spontaneous abortion (Taskinen et al., 1999). It is recognized that not all identified health effects are represented in these studies.

5.1.2.2. *Derivation of Candidate RfCs from Key Studies*

5.1.2.2.1. *Candidate RfC derivation for Krzyzanowski et al. (1990) (Pulmonary function).*

The study by Krzyzanowski et al. (1990) is a high quality epidemiology (human) study of health effects in a random sample of residents and their families. The study was specifically designed to include only households that had children 5–15 years of age, a sensitive life-stage for

respiratory effects. The study was of moderate size, when the effects in children were analyzed separately from adults, with the final analysis based on 208 children—a cohort large enough to show statistically significant results. The formaldehyde monitors were prepared by the Lawrence Berkeley Laboratories and were considered to be precise and highly reliable. The 7-day passive formaldehyde monitors generally provide the lowest limit of formaldehyde detection. The investigators specifically tested an a priori hypothesis and conclusively demonstrated to a high level of statistical significance that increased residential formaldehyde exposures were associated with decreased pulmonary function as measured by peak expiratory flow rate (PEFR) in children. This effect was clearly shown at relatively low concentrations of formaldehyde as the mean concentration in the homes was 26 ppb with more than 83% of homes having measured concentration less than 40 ppb. This study also reported specific regression modeling results that allowed EPA to calculate the point of departure for RfC development using a BMCL as the point of departure.

The effects of formaldehyde exposure on pulmonary function represent a sensitive endpoint with a reported 10% reduction in PEFR at 27 ppb. Among children with physician-diagnosed asthma, the observed effects of increased formaldehyde exposure on decreased PEFR were more pronounced—a clear indication of variability in response. The American Thoracic Society (ATS, 2000) considers decreased pulmonary function an adverse health effect, even when it is transient and subclinical. "Assuming that the relationship between the risk factor and the disease is causal, the committee considered that such a shift in the risk factor distribution, and hence the risk profile of the exposed population, should be considered adverse, even in the absence of the immediate occurrence of frank illness" (ATS, 2000). The ATS (2000) stated that individuals in an exposed population experiencing a shift in the distribution of pulmonary function were at potential risk from another agent due to the reduction in their reserve capacity to address additional insults. In the study by Krzyzanowski et al. (1990), the investigators demonstrated statistically significant interaction between formaldehyde exposures, smoking, and chronic cough. That is, a formaldehyde concentration that caused decreased pulmonary function at residential levels also caused chronic cough in the presence of environmental tobacco exposures. Higher prevalence rates of physician-diagnosed asthma and chronic bronchitis were also shown at higher concentrations of formaldehyde (60–140 ppb), an effect that was exacerbated by environmental tobacco exposures.

Figure 5-4 illustrates the reductions in peak expiratory flow rate (PEFR) in children (<15 years of age) in relation to indoor residential formaldehyde concentrations estimated by a random effects model based on 3,021 observations in 208 subjects. Formaldehyde levels in the

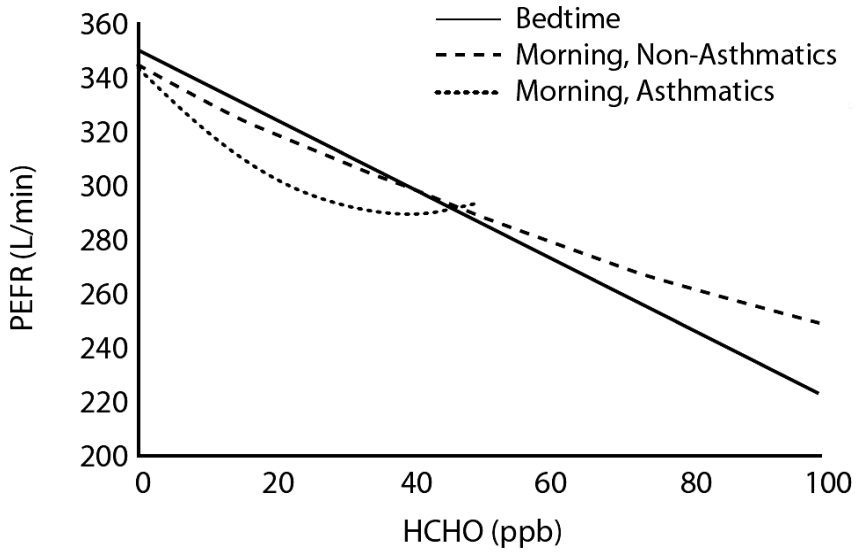


Figure 5-4. Estimated reduction in peak expiratory flow rate (PEFR) in children in relation to indoor residential formaldehyde concentrations.
Source: Krzyzanowski et al. (1990).

home were significantly related to reductions in PEFR in children both at bedtime and in the morning ($p < 0.05$). PEFR measurements in the morning versus at bedtime were significantly different ($p < 0.05$). Formaldehyde-related reductions in PEFR were greater in the morning in asthmatic children than in nonasthmatic children ($p < 0.05$).

Candidate RfC derivation based on Krzyzanowski et al. (1990):

Critical effect: Based on this study, which specifically included a susceptible population, the critical effect is reduction in PEFR in children. PEFR was the most sensitive measure of disease or impaired lung function reported in this population, with decreases in lung function reported in children who lived in homes with average measured formaldehyde concentrations as low as 30 ppb (Krzyzanowski et al. (1990). Children were more sensitive to formaldehyde-associated decreases in PEFR than adults, so the cRfC derived focused on the results in the 208 children.

Point of departure: A BMR of 10% reduction in PEFR was selected as a cut-off point for adversity, based on rationales articulated by the ATS (2000)[4]. Using this BMR and the model coefficient in Table 5 of Krzyzanowski et al. (1990), a $BMCL_{10}$ of 17 ppb (BMC_{10} = 27 ppb) was derived for all children.[5] Although the authors noted that asthmatic children were more sensitive, the necessary data were not provided in the report to calculate a BMCL for asthmatic children alone. Thus, 17 ppb, the BMCL based on all children in the study, was used as the POD.

Application of study-specific Uncertainty Factors (UFs):

Interspecies UF = 1: No interspecies adjustment is needed, as this is a human study.

LOAEL-to-NOAEL UF = 1: Because a BMCL was used for the POD and the BMR of 10% reduction in PEFR was considered to be a cut point for adversity, no LOAEL-to-NOAEL UF was needed (UF_L = 1).

Subchronic-to-chronic UF = 1: The study addresses ongoing residential exposure to formaldehyde. Although information on the duration of exposure for each participant is not provided, the residential nature of the study suggests a longer term exposure than the duration of the study. It was judged that a population-based study of residential exposures is sufficient to derive a chronic RfC without adjusting for a subchronic observation period — at least for adults and older children, and the children in this study were mostly older children (e.g., older than 7 years).

[4] The ATS (2000) recommended that "a small, transient loss of lung function, by itself, should not automatically be designated as adverse" and cited EPA's 1989 review of ozone, which offered a graded classification of lung function changes in persons with asthma as "mild," "moderate," or "severe" for reductions of less than 10, 10–20, and more than 20%, respectively (U.S. EPA, 1989). ATS (2000) concluded that, in evaluating the adverse health effects of air pollution at the level of population health (compared to individual risk), "[a]ssuming that the relationship between the risk factor and the disease is causal, the committee considered that such a shift in the risk factor distribution, and hence the risk profile of the exposed population, should be considered adverse." This was specifically considered by ATS (2000) even when "[e]xposure to air pollution could shift the distribution towards lower levels without bringing any individual child to a level that is associated with clinically relevant consequences." A moderate adverse effect at functional decrements of 10–20% was considered the best indicator of adverse effects in the study population. This criterion had been similarly applied in EPA's *Air Quality Criteria for Ozone and Related Photochemical Oxidants* (U.S. EPA, 2006d) for pulmonary function.

[5] According to the regression model in Table 5 in Krzyzanowski et al. (1990), the coefficient ± standard error for formaldehyde (in ppb) is –1.28 ± 0.46 and the background PEFR is 349.6 L/minute. Thus, a 10% reduction in PEFR is –35 L/minute and the 95% (one-sided) upper bound on the slope for PEFR as a function of formaldehyde exposure is –1.28 – (1.645 × 0.46), or –2.04 L/minute-ppb. Dividing 35 L/minute by 2.04 L/minute-ppb yields 17 ppb as the BMCL.

Human variability UF = 3: The study was designed to include homes with children, and a POD can be established based on reduced PEFR in children, who were more sensitive to the health effects than the adults in the study. Therefore, the POD represents data for a sensitive life stage, an aspect of human (intraindividual) variability. With respect to the human (interindividual) variability UF, although environmental tobacco smoke and socioeconomic status did not affect the formaldehyde results in children, asthmatic children were more sensitive to the effects of formaldehyde exposure on PEFR; thus, asthmatic children represent a population with increased susceptibility for this effect. The prevalence rate for physician-diagnosed asthma in the children was 15.8% in this study, which is higher than the national prevalence of about 5.9% for ages 5 to 17 years.[6] Thus the BMCL based on all children may be influenced by a higher prevalence of susceptible children for the critical effect. The authors do report that the PEFR was reduced to a greater degree in asthmatic children (as shown in Figure 5-4), and a lower BMC of 17 ppb can be calculated in this subgroup versus a BMC of 27 ppb for all children. However, the published regression statistics do not provide sufficient detail to calculate a BMCL specific for asthmatic children. In addition, other potentially sensitive populations (for example, elderly individuals or individuals with respiratory diseases) may not be adequately represented in the study. Therefore, an UF for human variability of 3 is applied to address the observed increased sensitivity of asthmatic children in lieu of a calculated BMCL

$$RfC = \frac{BMCL_{10}}{\left(UF_A \times UF_L \times UF_S \times UF_H\right)} = \frac{17\ ppb}{\left(1 \times 1 \times 1 \times 3\right)} = 5.6\ ppb \qquad (5\text{-}1)$$

UF_A = 1 (interspecies UF)
UF_L = 1 (LOAEL-to-NOAEL UF)
UF_S = 1 (subchronic-to-chronic UF)
UF_H = 3 (human variability UF)

[6] The national prevalence rate of asthma in children ages 5–17 is according to the Centers for Disease Control and Prevention (CDC) (MMWR 49(40):908-911, 2000). Although the Krzyzanowski et al. (1990) study was conducted in the late 1980s, prevalence data from the National Health Interview Survey for 1997 were used for comparison because that is the earliest year for which data are available after a 1997 redesign of the survey. Previously, the survey asthma question was not specific for physician-diagnosed asthma, so the redesigned results were considered to be more comparable to the physician-diagnosed asthma definition in the Krzyzanowski et al. (1990) study.

specific to asthmatic children and to ensure adequate protection for other potentially sensitive populations.

5.1.2.2.2. ***Candidate RfC derivation for Rumchev et al. (2002) (Asthma).***

Residential formaldehyde exposure was associated with an increased risk of asthma in a population-based case-control study of 192 children aged 6 months to 3 years (Rumchev et al., 2002). While it is acknowledged that accurately diagnosing asthma in young children is difficult, as the diagnosing physician was unaware of the formaldehyde level in the children's home, any diagnostic error would be unrelated to formaldehyde concentrations and would not induce a spurious association. It is noted that the endpoint is physician-diagnosed asthma. The study, which comprises 88 cases of children discharged from the emergency department of a children's hospital in Perth, Australia, with a primary diagnosis of asthma and 104 controls, provides a positive exposure-response relationship adequate for RfC derivation. Seasonal in-home formaldehyde measurements taken in the living room and subject's bedroom were used to assess exposure (8-hour passive sampler). The ORs for risk of asthma by formaldehyde exposure level category were adjusted for numerous risk factors, both familial and environmental, including familial history of asthma, age, sex, socioeconomic status, smoking, presence of pets, air conditioning, humidifier, and gas appliances. Of these, age, allergic sensitization to common allergens, and family history of allergy were independent risk factors for asthma (OR = 1.09, 2.57, and 2.66, respectively). Odds ratios were further adjusted for the effects of the measured indoor air pollutants (see Rumchev et al., 2004), indoor allergen levels of dust mites, relative humidity, and indoor temperature. Categorical analysis of the data indicates that the ORs for asthma were increased in the two highest formaldehyde exposure groups, reaching statistical significance for household exposures > 60 $\mu g/m^3$ (48 ppb) (OR = 1.39) (see Figure 5-5). Analysis of the data with formaldehyde as a continuous variable provides a statistically significant increase in the risk of asthma (3% increase in risk per every 10 $\mu g/m^3$ increase in formaldehyde level.)

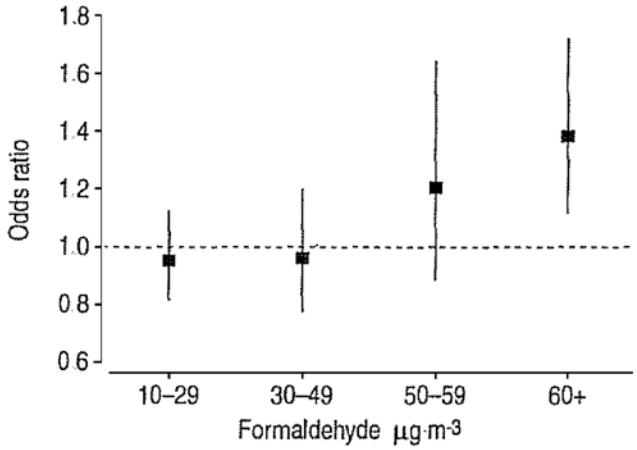


Figure 5-5. Odds ratios for physician-diagnosed asthma in children associated with in-home formaldehyde levels in air.

Source: Rumchev et al. (2002).

Candidate RfC derivation based on Rumchev et al. (2002):

Critical effect: Diagnosis of childhood asthma (case-control study).

Point of departure: A NOAEL of 33 ppb (40 μg/m^3; midpoint of the 30–49 μg/m^3 category) was selected because the OR for asthma in the next highest exposure category was considered to be part of an exposure-related trend of increasing asthma risk and, therefore, biologically significant.

Application of Study-Specific Uncertainty Factors (UFs):

Interspecies UF = 1: No interspecies adjustment is needed as this is a human study.

LOAEL-to-NOAEL UF = 1: No LOAEL-to-NOAEL UF was needed because the POD was a NOAEL (UF_L = 1).

Subchronic to chronic UF = 3: The study addresses ongoing residential exposure to formaldehyde. Although information on the duration of exposure for each participant is not provided, the residential nature of the study suggests a longer term exposure than the duration of the study. Study participants were 3 years or younger, therefore the duration of exposure could not meet the expected definition for a chronic study of one-tenth the lifespan. However, asthma often develops during childhood, indicating a less-than chronic duration of exposure.

Since asthma may develop throughout childhood it is unclear whether a study of children under 3 years of age would be of adequate duration for this developmental window. Therefore, an uncertainty factor of 3 was applied as a subchronic to chronic adjustment.

Human variability UF = 1 or 3: As a case-control study, all new cases of childhood asthma which met the study criteria were eligible for inclusion and the cases likely included children predisposed to asthma. Individuals with a family history of asthma and/or genetic markers for genes believed to predispose individuals to asthma would represent a susceptible population. Therefore, the cases in this study address children as a susceptible population for first diagnosis of asthma. Additionally, there was an association of a familial history of asthma with the diagnosis of children's asthma in this cohort (OR = 2.66). Not all sources of human variability which may contribute to a diagnosis of asthma are known, and there are likely additional sources of interindividual variability among children and among individuals with a family history of asthma, thus it is unlikely that all sources of human variability were adequately represented in the study population.

The two alternatives are described below and cRfCs are derived for each alternative.

Alternative A: Rumchev et al. (2002)
Human variability UF = 3:

To account for potentially susceptible individuals beyond those represented in the study population, an uncertainty factor of 3 for human variability is applied.

$$RfC = \frac{NOAEL}{(UF_A \times UF_L \times UF_S \times UF_H)} = \frac{33\ ppb}{(1 \times 1 \times 3 \times 3)} = 3.3\ ppb$$

UF_A = 1 (interspecies UF)
UF_L = 1 (LOAEL-to-NOAEL UF)
UF_S = 3 (subchronic-to-chronic UF)
UF_H = 3 (human variability UF)

Alternative B: Rumchev et al. (2002)
Human variability UF = 1:

EPA's Technical Report of the RfD and RfC Processes Technical Report (US EPA, 2002a) indicates that UF_H of 1 has been applied in cases where there are data "very specific about the particular vulnerability of infants and children within certain age ranges to an agent." Asthma and allergic sensitization to common allergens develop during childhood and young adulthood defining a developmental window during which individuals are most susceptible to the development of asthma. Since this study includes only children up to 3 years of age, the UF for subchronic exposure is applied above acknowledging that this study does not cover the susceptible developmental window. No additional adjustment is applied for inter-individual variability among children. It is acknowledged that additional sources of human variability are possible—but it is believed that childhood is a key developmental window for initial diagnosis of asthma. The technical report acknowledges that applying a UF_H of 1 may be appropriate where "even within these populations it is possible that some variability still exists.

$$RfC = \frac{NOAEL}{\left(UF_A \times UF_L \times UF_S \times UF_H\right)} = \frac{33\ ppb}{\left(1 \times 1 \times 3 \times 1\right)} = 11\ ppb$$

UF_A = 1 (interspecies UF)
UF_L = 1 (LOAEL-to-NOAEL UF)
UF_S = 3 (subchronic-to-chronic UF)
UF_H = 1 (human variability UF)

5.1.2.2.3. ***Candidate RfC derivation for Garrett et al. (1999 a,b) (Asthma, respiratory symptoms, atopy and severity of allergic sensitization).***

Garrett et al. (1999 a,b) reported on the risk of allergy and asthma-like respiratory symptoms due to formaldehyde exposure in a cross-sectional survey of households with children 7–14 years old with (n = 53) or without (n = 95) doctor-diagnosed asthma. Formaldehyde exposure was characterized by four seasonal in-home sampling events using 4-day passive samples collected in bedrooms, living rooms, kitchens, and outdoors. In logistic regressions, both the prevalence and severity of allergic sensitization to 12 common allergens increased with increasing formaldehyde concentration in the home. Additionally, a calculated respiratory symptom score was increased and demonstrated a significant relationship with increased formaldehyde concentration in a multiple linear regression after adjusting for multiple risk factors and interactions. For each of these endpoints, severity/incidence was increased in the medium (20–50 μg/m^3) and high (>50 μg/m^3) exposure groups relative to the low (<20 μg/m^3)

exposure group, based on the highest of four seasonal 4-day formaldehyde measurements in the home (see Figures 5-6 and 5-7).

The findings of Garrett et al. (1999 a,b) are supported by the observation of an increased bronchial responsiveness to mite allergen in a chamber study of 19 sensitized adult asthmatics exposed to formaldehyde at a concentration of 100 μg/m^3 for 30 minutes (Casset et al., 2006). Additionally, inhalation exposures to formaldehyde have been shown to increase an animal's response to other common allergens via inhalation (Fujimaki et al., 2004b; Sadakane et al., 2002; Riedel et al., 1996; Tarkowski and Gorski, 1995).

Candidate RfC derivation for increased allergic sensitization from Garrett et al. (1999 a,b***):***

Critical effects: ***Allergic sensitization—***Increase in allergic sensitization (proportion of atopic children). Severity of allergic sensitization measured both as number of positive skin tests to common allergens and the recorded allergen wheal ratio for those tests. ***Asthma—***increase in proportion of asthmatic children. ***Respiratory symptoms—***Increased respiratory symptom score.

Point of departure: For all critical effects, categorical analyses are presented that show an increase in the midexposure group (16–40 ppb) and high exposure group (>40 ppb) relative to the low-exposure group (<16 ppb) (see Figures 5-6 and 5-7). However, it is unknown if the findings in the low-exposure group are comparable to the responses that would be observed in an unexposed population. Therefore, the low-exposure group cannot be considered a NOAEL but rather serves as a referent group for the two other exposure groups. Thus, the LOAEL is based on health effects observed in the midexposure group (16−40 ppb) for all three critical effects. As neither the mean or median exposure levels are provided for the exposure categories used to analyze the health effects data, the midpoint of the exposure category is selected for the LOAEL: 28 ppb.

Application of study-specific Uncertainty Factors (UFs)*:*

Interspecies UF = 1: No interspecies adjustment is needed as this is a human study.

LOAEL-to-NOAEL UF = 3: As discussed, the midexposure group is selected as the LOAEL since the low-exposure group is the referent group; there is no true unexposed control. It is unclear whether or not a full LOAEL to NOAEL uncertainty factor is warranted for these data. The authors did provide evidence for increased atopy for every increase of 16 ppb of exposure with borderline statistical significance when adjusted for several potential confounders (OR = 1.4;

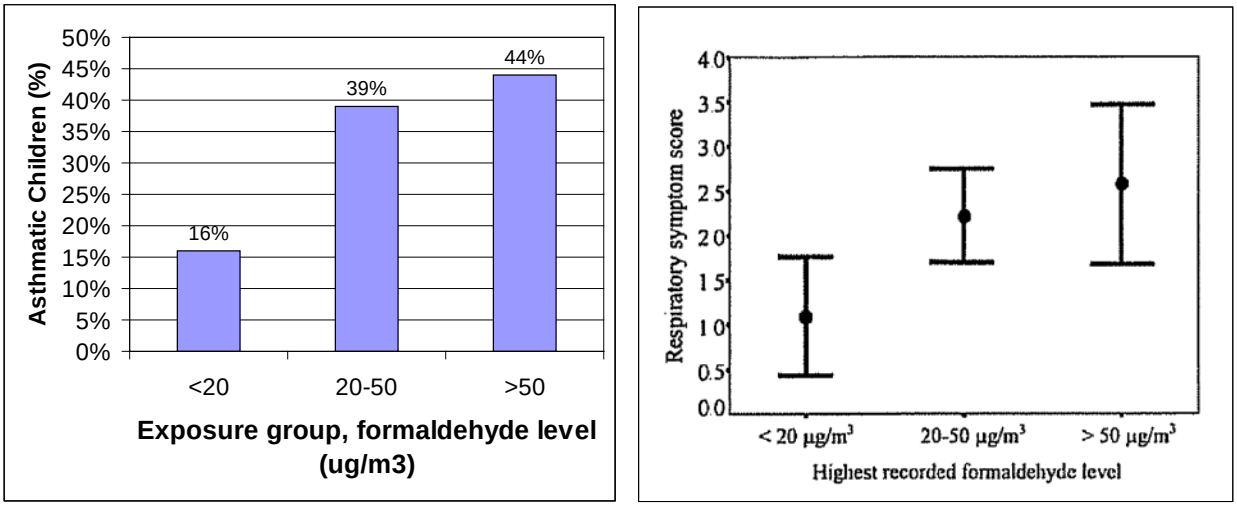


Figure 5-6. Prevalence of asthma and respiratory symptom scores in children associated with in-home formaldehyde levels. Trend analysis indicates statistical significance in these increases {percent asthmatic children, unadjusted (p=0.03) and respiratory symptom score (p=0.03)}.

Source: Garrett et al. (1999a) ; Garrett et al., 1999b (errata).

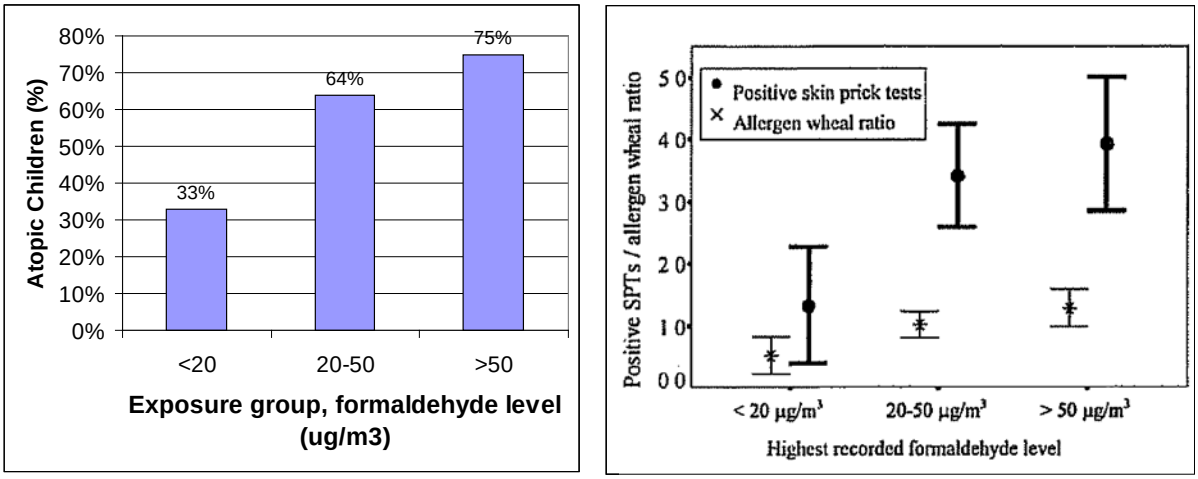


Figure 5-7. Prevalence and severity of allergic sensitization in children associated with in-home formaldehyde levels. Trend analysis indicates statistical significance in these increases {percent atopic children (p = 0.002), positive skin prick tests (p = 0.001) and severity as allergen wheal ratio (p = 0.004)}.

Note: Skin prick tests included 12 environmental allergens (cat, dog, grass [two types], house dust, dust mite [two strains] and fungi [five strains]).
Source: Garrett et al. (1999a) ; Garrett et al., 1999b (errata).

95% CI: 0.98–2.00). An UF of 3 adjusts the LOAEL to a similar range and is consistent with this alternative presentation of the data.

Subchronic to chronic UF = 1: The study addresses ongoing residential exposure to formaldehyde. Although information on the duration of exposure for each participant is not provided, the residential nature of the study suggests a longer term exposure than the duration of the study. It is judged that a population-based study of residential exposures is sufficient for derivation of a chronic RfC without adjusting for a subchronic observation period.

Human variability UF = 1 or 3: This study was designed to assess allergic sensitization, asthma prevalence and respiratory symptoms in children with relation to in-home formaldehyde levels. The recruitment of participants was designed to include households (50%) with asthmatic children, resulting in 43 households with at least one asthmatic child and 37 without asthmatic children for a total of 148 children (35% asthmatic). Parental allergy and asthma were also assessed and included as adjustment variables in the data evaluation. Therefore the study population includes individuals reflecting several key aspects of human variability for asthma and allergic sensitization (age, familial history of disease), and addresses the links between allergic sensitization and asthma. Both asthma and allergic sensitization are risk factors for increased respiratory symptoms.

The two alternatives are described below and cRfCs derived for each alternative

Alternative A: Garrett et al. (1999)

Human variability UF = 3: It is unclear whether the effect levels in the study truly reflect the effect levels in sensitive populations, since study findings controlled for both asthma and family history. Therefore, a value of three was used for the human variability UF.

$$RfC = \frac{LOAEL}{(UF_A \times UF_L \times UF_S \times UF_H)} = \frac{28\ ppb}{(1 \times 3 \times 1 \times 3)} = 2.8\ ppb$$

UF_A = 1 (interspecies UF)
UF_L = 3 (LOAEL-to-NOAEL UF)
UF_S = 1 (subchronic-to-chronic UF)
UF_H = 3 (human variability UF)

Alternative B: Garrett et al. (1999)

Human variability UF = 1:

Individuals with a family history of asthma and/or genetic markers for genes are believed to be predisposed to asthma and this would define a susceptible population within children. In this study parental disease status is a marker for potential genetic susceptibility. Although exposure-response relationships are not provided for individuals with a familial history of disease, analyses provided suggest the results reflect responses from these individuals. Among children with parental allergy, allergic children were exposed to higher formaldehyde levels than non-allergic children (p = 0.02), relating higher formaldehyde exposure to sensitization even among those with a likely genetic susceptibility. As shown in Figure 5-8, formaldehyde levels are related to increased asthma incidence with a significant linear trend (p = 0.02), yet this relationship loses significance when controlling for parental allergy and asthma, suggesting the measured response on which the POD is based is driven by children with a potential for genetic susceptibility.

An EPA Technical Report of the RfD and RfC Processes (US EPA, 2002a) indicates that a UF_H of 1 can be applied in cases where data are "very specific about the particular vulnerability of infants and children within certain age ranges to an agent." Asthma and allergic sensitization to common allergens develop during childhood and young adulthood. Therefore no additional adjustment is applied for human variability. The technical report acknowledges that "even within these populations it is possible that some variability still exists", but that a UF_H of 1 is still applied.

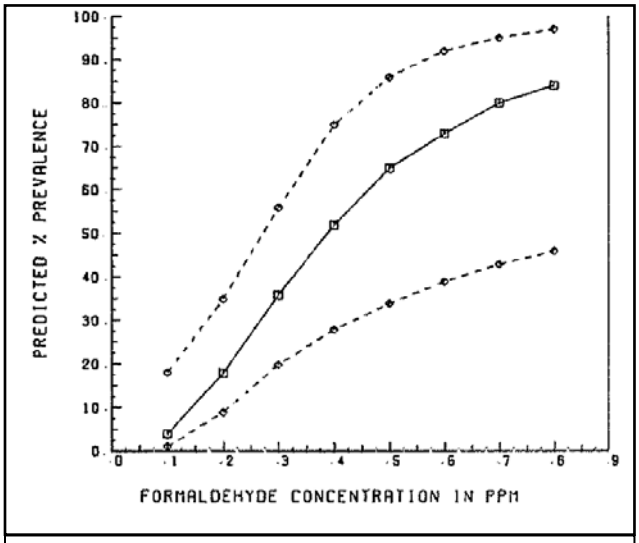
$$RfC = \frac{LOAEL}{\left(UF_A \times UF_L \times UF_S \times UF_H\right)} = \frac{28\ ppb}{\left(1 \times 3 \times 1 \times 1\right)} = 9.3\ ppb$$

UF_A = 1 (interspecies UF)
UF_L = 3 (LOAEL-to-NOAEL UF)
UF_S = 1 (subchronic-to-chronic UF)
UF_H = 1 (human variability UF)

5.1.2.2.4. ***Candidate RfC derivation for Ritchie and Lehnen, 1987; Hanrahan et al., 1984 and Liu et al., 1991 (Sensory irritation).***

There are three studies that report sensory irritation in humans from chronic exposures in a residential environment and provide sufficient exposure data to support quantitative assessment (Liu et al., 1991; Ritchie and Lehnen, 1987; Hanrahan et al., 1984). Each study reports site-specific exposure measurements and presents some metric of individual exposure. These residential studies employ in-home measurements for each study participant, either as average exposure level (Ritchie and Lehnen, 1987; Hanrahan et al., 1984) or as calculated cumulative exposure based on the time in the home (Liu et al., 1991). Eye irritation is reported at similar levels of residential formaldehyde exposure in the three studies (see Figures 5-8 and 5-9). Each

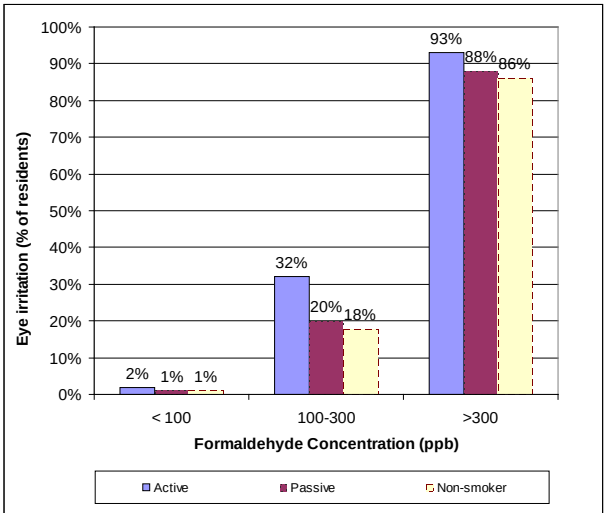
study provides an exposure-response relationship for prevalence of sensory irritation in relation to in-home formaldehyde exposure based on individual level data.



Panel A: Regression of prevalence of eye irritation versus indoor formaldehyde concentration (ppm) in mobile homes (30–60 minute air sample in each home).

Note: Dashed lines show upper and lower 95th percentile confidence intervals on model results. Model based on reported eye irritation from individuals in 42 mobile homes.

Source: Hanrahan et al. (1984).



Panel B: Prevalence of eye irritation in groups defined by in-home formaldehyde exposure (30–60 minute air sample in each mobile home).

Note: Eye irritation rate is given by smoking status: active smokers (n = 143), passive exposure to smoke (n = 133) and nonsmokers (n = 180).

Data source: Ritchie and Lehnen (1987).

Figure 5-8. Positive exposure-response relationships reported for in-home formaldehyde exposures and sensory irritation (eye irritation).

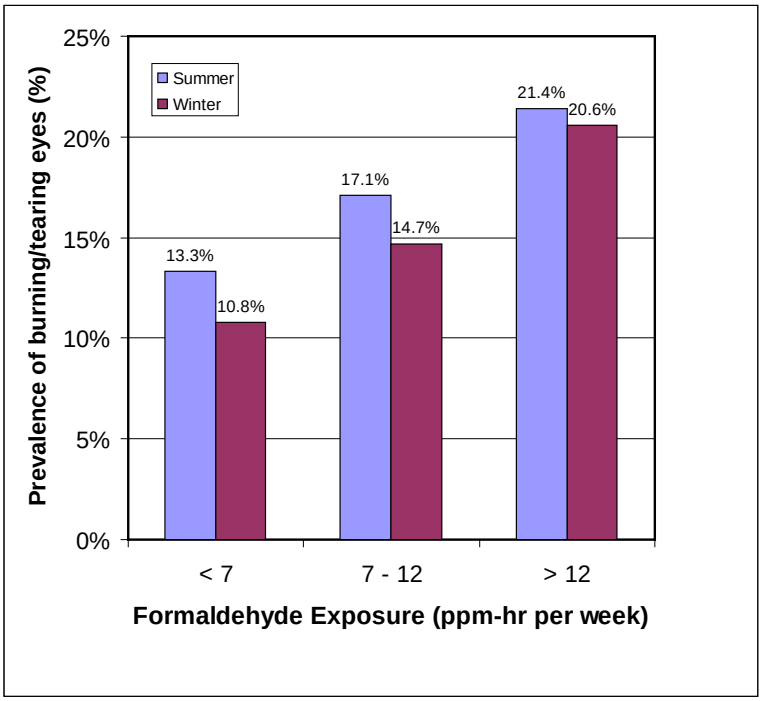


Figure 5-9. Positive exposure-response relationships reported for in-home formaldehyde exposures and sensory irritation (burning eyes).

Note: Cumulative formaldehyde exposure was estimated for each participant from measured in-home formaldehyde levels (7-day passive air sample) and reported hours spent in the home. Prevalence rates are given for both summer (*n* = 1,388) and winter (*n* = 1,093) survey periods.
Data source: Liu et al. (1991).

Ritchie and Lehnen (1987) examined formaldehyde-associated effects on eye, nose, and throat irritation in a large residential study with 2,007 participants from 841 homes. Based on in-home measurements of formaldehyde concentration, participants were categorized into three exposure groups: low (<100 ppb), mid (100–300 ppb) and high (>300 ppb) (average of two 30−60 minute air samples per home). Ritchie and Lehnen (1987) observed clear exposure-response relationships in the percentage of residential occupants reporting eye, nose, and throat irritation. For example, in nonsmoking mobile home residents, incidence scores for eye irritation were 1,–18% and 86%, and for nose/throat irritation were 5–17% and 78%, respectively, for the three exposure groups. The exposure-response relationships were similar regardless of type of home, mobile (*n* = 851) or conventional (*n* = 1,156). Although smoking status was also a predictor of irritation, in-home formaldehyde concentrations were a stronger predictor of health

effects. The study included children and the elderly and results were consistent across age groups. Children <7 years of age were only included in the eye irritation analyses because of concerns about the quality of parental reporting for nose and throat effects in young children. The selection criteria for participants indicate that more sensitive individuals may have been over-represented in the study population.[7] All study participants were self-selected, with a physician's approval, perhaps resulting in a higher proportion of individuals experiencing various irritant and upper respiratory tract symptoms, which may represent a sensitive population for eye, nose, or throat irritation.

Hanrahan et al. (1984) reported an exposure-response relationship for burning eyes and eye irritation in a study of 61 teenage and adult residents of mobile homes. As in the Ritchie and Lehnen (1987) study, in-home formaldehyde measurements were obtained for all participants and measured formaldehyde levels were used to characterize average in-home exposures (30−60 minute air sample). Eye irritation was associated with in-home formaldehyde exposures ($p < 0.05$) (both as "burning eyes" and "eye irritation"), and the authors provided a graphical representation of the best-fitting regression model for exposures between 100 and 800 ppb. From inspection of this graph, the prevalence of eye irritation predicted at 100 ppb is approximately 4% with an upper bound of 18% (95th percentile CI) (see Figure 5-8, Panel A). Because the limit of detection for formaldehyde in indoor air was 100 ppb, data or model results are not provided below 100 ppb.

The third residential study is a random-sample study of over 1,000 mobile home residents (1,394 in the summer; 1,096 in the winter) that included both young children and the elderly (Liu et al., 1991). Cumulative weekly exposures were based on in-home formaldehyde sampling and a participant survey of time spent at home. Air sampling was conducted for a 7-day period using a passive sampler in each home (summer and winter). The resulting estimates of cumulative exposure assumed no formaldehyde exposure outside of the home. Cumulative formaldehyde exposure was a significant predictor of numerous irritant symptoms in a multivariate linear logistic regression, including "burning eyes" ($p < 0.05$). The prevalence of eye irritation increased with increasing cumulative exposure in a categorical analysis of participants 20−64 years old for both summer and winter exposure estimates (see Figure 5-9). Eye irritation was above 10% in the lowest exposure group (0–7.0 ppm-hours/week) and increased to 17.1% and 21.4 % in the mid- and high-exposure group, respectively, for the summer survey time;

[7] Participants in this study were self-selected residents who were concerned about possible formaldehyde exposure and had obtained a written request from a physician to have the Minnesota Department of Health test their homes as part of a free program; thus, people with symptoms may be overrepresented in this study compared with the general population. This potential overrepresentation does not necessarily imply a selection bias because it is unlikely that it was associated with the measured formaldehyde exposure levels in participants' homes.

winter rates were slightly lower but showed a similar increase with increasing cumulative exposure.

Taken together, these three studies report increased eye irritation from residential exposures that are below the BMCLs calculated from acute exposures in the laboratory. Each study has the strength of having individual in-home exposure measurements and demonstrates a positive exposure-response relationship for sensory irritation within a range of residential formaldehyde exposures (both conventional and mobile homes). Potentially confounding factors (such as allergens and some other in-home exposures) have been taken into account and statistical analyses of the data include relevant covariates (e.g., age, sex, smoking status). As such, these studies provide a basis for development of a cRfC for sensory irritation. Additionally, the study populations have been drawn from the general population, including children and the elderly, and have not been limited to those healthy enough for full-time employment (as is often the case in occupational cohorts).

All three studies support a finding of increased eye irritation for exposures above 100 ppb (see Figures 5-8 and 5-9). However, the shape of the exposure-response curve below 100 ppb, or an indication of a no-effect level, is less clear. Two of the studies indicate 1–4% eye irritation in residents where formaldehyde exposures were measured at 100 ppb or less (Ritchie and Lehnen, 1987; Hanrahan et al., 1984). Thus, there is uncertainty in considering 100 ppb as a no-effect level for increased eye irritation for these studies. When modeled, the 95% CIs around the point estimate of 4% eye irritation were 1–18% eye irritation, illustrating the range of response rates at 100 ppb that are consistent with the observed data (Hanrahan et al., 1984). Additionally, the presentation of results by exposure category in Ritchie and Lehnen (1987) is inexact and has individuals with exposures at the low end of the categorical range being grouped with those at higher exposures in the range, obscuring any exposure-response relationship within the categorical range. For these reasons, a POD for RfC derivation from either of these studies should reflect these uncertainties. Therefore, for the NOAEL representing the category of individuals with ≤100 ppb, in which 1–2 % eye irritation was observed, the upper end of this exposure category is not used, but rather the midpoint, 50 ppb (Ritchie and Lehnen, 1987). Although Hanrahan et al. (1984) provided no model results below 100 ppb, an extrapolation of

the graphical results (see Figure 5-8, Panel A) provides an estimated $BMCL_{10}$ of 70 ppb[8]. No additional duration adjustments were made from the in-home exposure measurements to continuous exposure because neither time away from the home, nor potential exposures outside of the home, were characterized in either study.

Of the three studies, only Liu et al. (1991) provides exposure measurements below 100 ppb, with a reported detection limit of 10 ppb formaldehyde for the in-home air monitoring. Additionally, air samples were collected using a 7-day passive sampler which is more representative of average residential exposures than a one-time, 30–60 minute, air sample. Therefore, the data collected by Liu et al. (1991) are more suited to understanding the exposure-response relationship for eye irritation of exposures below 100 ppb. In addition to controlling for age, gender, and smoking status, Liu et al. (1991) controlled for the presence of chronic respiratory disease when assessing the effects of formaldehyde on symptoms of sensory irritation. Finally, this study provides results for both summer and winter survey periods, addressing seasonal variation in both formaldehyde levels and sensory irritation. The use of the cumulative exposure metric considers not only the concentration of formaldehyde but also the number of hours during the week each participant spent in their residence. Linear logistic regression indicates that cumulative formaldehyde exposure was a statistically significant predictor of burning eyes for both winter and summer survey periods. However, no BMCL can be calculated because no regression coefficients were provided in the report. Data were provided for the categorical analysis illustrating a positive exposure-response relationship (redrawn in Figure 5-9). Based on the categorical results, the midexposure group (7−12 ppm-hours/week) demonstrated an increased response compared with the low-exposed group. Since the prevalence rate in the low-exposed group was above 10% for burning eyes, this exposure group does not represent a NOAEL, but rather serves as a referent for the midexposure group. Therefore, the POD is derived from the midpoint of 7−12 ppm-hours/week, 9.5 ppm-hours/week. Using a conversion factor applied by the authors, the cumulative exposure of this midexposure group

[8] Figure 1 of Hanrahan et al. (1984) shows predicted values and 95% confidence intervals (CIs) for the percent prevalence of a burning-eyes response for formaldehyde concentrations ≥100 ppb (See Panel A in Figure 5-9 above). A short extension of the upper 95% CI to the concentration associated with 13% prevalence (i.e., a 10% increased prevalence above an assumed background response rate of 3%; this assumed background rate was chosen to be conservatively high to err on the side of not underestimating the actual value, given that the value was approximated from a visual extension of the upper 95% CI curve) suggests a BMCL of approximately 70 ppb for 10% increased prevalence. The actual value is unknown but is clearly below 100 ppb, which is the minimum exposure concentration depicted in the figure.

corresponds to a continuous home exposure of 70–120 ppb for an individual who spends 60% of the week in the home, with a midpoint of 95 ppb.

Candidate RfC derivation for sensory irritation:

Critical effect: Prevalence of sensory irritation (eye irritation, burning eyes).

Point of departure: Each of the studies discussed above has different strengths and weaknesses for the determination of a POD for sensory irritation. Nevertheless, the effect levels and PODs derived from each study are in relatively close agreement with less than a twofold span from lowest to highest. Therefore each POD is carried through to calculate a cRfC:

NOAEL = 50 ppb (Ritchie and Lehnen, 1987)
$BMCL_{10}$ = 70 ppb (Hanrahan et al., 1984)
LOAEL = 95 ppb (Liu et al., 1991)

Application of Uncertainty Factors (UFs)

Interspecies UF = 1: No interspecies adjustment is needed as this is a human study.

LOAEL-to-NOAEL UF: An uncertainty factor of 1 is applied to the NOAEL and $BMCL_{10}$ established as PODs from Ritchie and Lehnen, (1987) Hanrahan et al. (1984) studies. An uncertainty factor of three is applied to the LOAEL of 95 ppb based on the Liu et al. (1991) study, as the prevalence rates for this exposure level are below 20% for an effect that is of relatively low severity. In addition, the LOAEL is not significantly above the NOAEL and $BMCL_{10}$ from the other studies that evaluated the same endpoint.

Subchronic to chronic UF = 1: These studies address ongoing residential exposure to formaldehyde. Although information on the duration of exposure for each participant is not provided, the residential nature of the study suggests a longer term exposure than the duration of the study. It is judged that a population-based study of residential exposures is sufficient for derivation of a chronic RfC without adjusting for a subchronic observation period.

Human variability UF = 1 or 3: All three studies were population-based and included children, the elderly and both sexes. Sample sizes for two of the studies were

very large (1,394 for Liu et al. [1991]; 2,007 for Ritchie and Lehnen [1987]), increasing the likelihood that sensitive populations were included. Analysis of the data controlled for sex, smoking status, and age group.

The two alternatives are described below and cRfCs derived for each alternative

Alternative A

Sensory irritation studies:

Human variability UF = 3: For all studies, the analysis was based on prevalence rates, decreasing the likelihood that effects on sensitive individuals would be lost due to response averaging. For Ritchie and Lehnen (1987), the prevalence rate in the <100 ppb exposure group (represented by a NOAEL of 50 ppb, the midpoint) was 1−4%. For Hanrahan et al. (1984), the POD is a BMCL corresponding to a 10% response rate. Given these prevalence rates and the fact that the sensory irritation effects assessed are considered minimally adverse, a human variability UF of 3 was considered adequate for this endpoint.

Ritchie and Lehnen (1987):

$$RfC = \frac{NOAEL}{(UF_A \times UF_L \times UF_S \times UF_H)} = \frac{50\ ppb}{(1 \times 1 \times 1 \times 3)} = 17\ ppb$$

Hanrahan et al. (1984):

$$RfC = \frac{BMCL_{10}}{(UF_A \times UF_L \times UF_S \times UF_H)} = \frac{70\ ppb}{(1 \times 1 \times 1 \times 3)} = 23\ ppb$$

Liu et al. (1991):

$$RfC = \frac{LOAEL}{(UF_A \times UF_L \times UF_S \times UF_H)} = \frac{95\ ppb}{(1 \times 3 \times 1 \times 3)} = 9.5\ ppb$$

Alternative B

Sensory irritation studies

Human variability UF = 1: Two studies included a broad age range allowing some assessment of human variability due to life stage. Ritchie and Lehnen (1987) evaluated the influence of age on sensory irritation in the following age groups <1year, 2–6 years, 7–14 years, 15–20 years, 21–54 years, 55–64 years, and ≥65 years. An age effect for eye irritation was not evident in these data and pooled data are presented for this endpoint. Liu et al. (1991) report that greater eye irritation was reported in participants of 20–64 years than in those younger than 20 or older than 65 years. The elderly population (≥65 years) was well-represented in this study (39% of participants in the summer and 34% in the winter). The modeled results on which the $BMCL_{10}$ is based for Hanrahan et al. (1984) are normalized to 48 years of age (the mean age of respondents), which is consistent with the age group considered the most responsive in the Liu et al. (1999) study. Therefore the PODs derived from these studies do account somewhat for human variability across the life stage.

The critical effects of sensory irritation (eye, nose, and throat irritation) are considered minimally adverse health effects. The nominal response rates for eye irritation of 1−4% for in-home exposures below 100 ppb from which the PODs were derived suggest that the PODs are below significant response levels. Additionally, as the data are reported as prevalence rates, there is no masking of effect from sensitive individuals (as may occur when benchmark responses are average values of biometric parameters).

Finally, sensory irritation is a POE effect. Therefore, sources of human variability such as absorption, distribution, and metabolism of a compound are unlikely to influence incidence rates for this endpoint. There may be human variability in the sensitivity of the trigeminal nerve to formaldehyde binding and stimulation.

Taken together, these studies address many potential sources of human variability. Therefore, it is judged that further adjustment to address human variability is not warranted for the minimally adverse health effect of sensory irritation. Thus a UF_H of 1 is applied to all three studies. It is acknowledged that there is the potential for sources of variability not captured in these studies.

Ritchie and Lehnen (1987):

$$RfC = \frac{NOAEL}{\left(UF_A \times UF_L \times UF_S \times UF_H\right)} = \frac{50\ ppb}{\left(1 \times 1 \times 1 \times 1\right)} = 50\ ppb$$

Hanrahan et al. (1984):

$$RfC = \frac{BMCL_{10}}{\left(UF_A \times UF_L \times UF_S \times UF_H\right)} = \frac{70\ ppb}{\left(1 \times 1 \times 1 \times 1\right)} = 70\ ppb$$

Liu et al (1991):

$$RfC = \frac{LOAEL}{\left(UF_A \times UF_L \times UF_S \times UF_H\right)} = \frac{95\ ppb}{\left(1 \times 3 \times 1 \times 1\right)} = 32\ ppb$$

5.1.2.2.5. ***Candidate RfC derivation for Taskinen et al. (1999) (Fecundity density ratio).***

On review of the candidate developmental and reproductive toxicity studies in humans and animals (presented in Section 5.1.3.2.7), the Taskinen et al. (1999) epidemiology study was considered to be the strongest for the purpose of deriving a chronic RfC. This study was a well-designed population-based case-control study of women who were occupationally exposed to formaldehyde. The study population was well defined and adequately selected to allow for meaningful comparisons of health effects among individuals with different levels of exposure to formaldehyde. Potential selection bias and the self-reporting of spontaneous abortion are not considered to have had a significant influence on the study findings. Additionally, the decreased FDR and increased risk of spontaneous abortion observed in Taskinen et al. (1999) are internally consistent and coherent with other reports of increased risk of pregnancy loss associated with exposure to formaldehyde (John et al., 1994; Taskinen et al., 1994; Seitz and Baron, 1990; Axelsson et al., 1984) and is supported by animal data (Kitaev et al., 1984; Sheveleva, 1971).

The Taskinen et al. (1999) study allows the consideration of three potential critical effects: endometriosis, increased spontaneous abortion, and decreased fecundity density ratio (FDR). However, there is little independent support for the finding of increased risk of endometriosis and the ORs for organic solvent exposure within this study (OR = 14.7; 95% CI: 3.1–70) were much greater than for formaldehyde (OR = 4.5, 95% CI: 1.0–20), indicating a reasonable potential for confounding of the formaldehyde association. The finding of increased risk of spontaneous abortions is supported by independent findings in other formaldehyde-exposed cohorts (John et al., 1994; Taskinen et al., 1994; Seitz and Baron, 1990; Axelsson et al., 1984). As this study was designed to examine the effect of workplace formaldehyde exposures on FDR, the study design and data collection best support this finding. The exposure estimates were assessed to represent what the researchers considered the relevant exposures for evaluating risk factors that might influence time-to-pregnancy. Although data on miscarriages were collected to control the time-to-pregnancy findings for potential confounding from formaldehyde-related spontaneous abortions, it is less certain that the exposure measurements coincide with the defined spontaneous abortion cases. Spontaneous abortions were only included in calculations of exposure-specific ORs if a participant indicated that she was employed at the same location when she had the spontaneous abortion and when the time-to-pregnancy exposure assessment was done. The analysis showed that there were statistically significantly increased risks of spontaneous abortion in the lowest exposure group. While this finding was consistent with other studies showing adverse reproductive effects of formaldehyde and appears to be causal, the Taskinen et al. (1999) spontaneous abortion results did not clearly control for all the potential confounders that were controlled for in the FDR analyses (i.e.,

organic solvents and phenols). While the other coexposures were not associated with FDR and therefore not confounders, endometriosis was strongly associated with organic solvents. Therefore, for these endpoints, the study design and strength of results best support the use of decreased FDR in formaldehyde-exposed women as the critical effect for this study.

It is preferable that the critical effect for a specific study be the most sensitive of the effects which is well supported by the study. As spontaneous abortions are significantly increased in the low-exposure group and the response in the midexposure group is considered a no-effect level for decreased FDR, there is uncertainty that an RfC based on the FDR NOAEL would be protective for the more sensitive effect. Although the finding of increased risk of spontaneous abortion is qualitatively convincing, there is more uncertainty in the applicability of the exposure assessment for quantitative risk assessment. Additionally, there is greater uncertainty in the use of the exposure adjustments for the low-exposure group on which the LOAEL for spontaneous abortion is based because the exposure adjustments account for more of the work time in the low-exposure group than the medium and high exposure groups (see Table 5-5).

There are several sources of uncertainty in the exposure estimates for use in RfC derivation. As discussed above, the average exposure estimate for the low exposure group includes a greater proportion of nonassessed background exposures. This is evidenced in part by the reported average exposure being below background levels for these workers, even with exposure measurements as high as 300 ppb. The unaccounted for nontask exposures may represent time during the day spent in the work facility, or time in a different job or work environment. Additionally, task-level exposure measurements were available for only 27% of women in the low exposure group, versus 38% and 69% of women in the medium and high exposure groups, indicating less certainty in exposure classification for the low exposure group.

Duration adjustment for candidate study points of departure. Normally, exposures from occupational studies are adjusted to account for the daily breathing volume appropriate to an environmental (versus occupational) setting and for exposure every day of the year (U.S. EPA, 1993). However, with formaldehyde, there is potential for exposure outside of work from in-home and environmental sources of formaldehyde (Chapter 2). A contemporaneous study of formaldehyde exposures in Finland reports average exposure of 21.4 ppb (measured over 48 hours with a personal monitor) (Jurvelin et al., 2001). Furthermore, both the mean exposure (18 ppb 8hr TWA) and lowest reported exposure (10 ppb 8hr TWA) of the 'low exposed' category are below the reported average ambient exposures for Finland (21.4 ppb). Thus, it is

Table 5-5. Adjustment for nonoccupational exposures to formaldehyde.

Panel A: Proportion of workshift corresponding to the exposure group mean task-level formaldehyde exposure (ppb) and the exposure group daily exposure index (8 hour-TWA).

Exposure group (*n*)	Reported mean exposure (ppb, 8 hr-TWA)		Measured task-level exposures (ppb)		Estimate of time during workday for formaldehyde related tasks assuming mean exposure levels.	
	Mean	Range	Mean	Range	% of worktime[a]	Hours per 8 Hr workshift
Low (119)	18	1–39	70	10–300	26%	2
Medium (77)	76	40–129	140	50–400	54%	4.3
High (39)	219	130–630	330	150–1,000	66%	5.3

[a]Calculated as mean exposure (ppb 8 hour-TWA) divided by mean task-level exposures for the exposure group.

Panel B: Recalculation of daily exposure index (8 hour-TWA) where background formaldehyde exposure is estimated for worktime spent on tasks considered unrelated to occupational use of formaldehyde.

Exposure group (*n*)	Estimate of formaldehyde exposure during formaldehyde-related work tasks		Estimate of formaldehyde exposure from background levels during the workshift		Alternative daily exposure index (ppb, 8 Hr-TWA)
	Mean task level exposure (ppb)	% of worktime in formaldehyde task	Background formaldehyde (ppb)	% of time in nonformaldehyde-related task	
Low (119)	70	26%	21.4	74%	34
Medium (77)	140	54%	21.4	46%	86
High (39)	330	66%	21.4	34%	226

likely that exposure estimates for study participants include time during the workday when women reported no formaldehyde exposure and a zero exposure was assessed for a nonformaldehyde related task. Additionally, participants may have qualified for the study based on employment date but may not have been working with formaldehyde during the entire time-to-pregnancy period. In both cases, the investigators in Taskinen et al. (1999) appear to have

assumed that, while the women were away from their "exposed" workplace, their exposure to formaldehyde was zero, not accounting for background occupational exposures and ambient levels of formaldehyde. This explains why both the mean exposure as well as lower end of workshift exposures for women in the low exposure group were reported at and below expected ambient levels. The women in the low exposure category had task-level workplace exposures of up to 300 ppb in addition to experiencing some work time at background exposure levels. Compared to women who only experienced background exposure levels, those in the low exposure category were at significantly higher risk of spontaneous abortion.

The reported data do not provide information to correct for background formaldehyde exposure during the workday for each participant. However, the published mean exposure values may be used to provide some idea of the impact of including background exposures on the study PODs. Comparison of the values listed in Table 4 of Taskinen et al. (1999) allows for the estimation of the percentage of work time spent performing tasks involving formaldehyde exposure (see Table 5-5, Panel A). For the women in the low exposure category, this percentage is 26% (mean of measured workplace exposures of 70 ppb times 26% equals the mean of the TWA exposure of 18 ppb). Using the same method, the women in the "medium" and "high" exposure category were performing tasks involving formaldehyde exposure approximately 54% and 66% of their work time, respectively. Assuming that the women spent the remainder of their work time at the background concentration of 21.4 ppb (Jurvelin et al., 2001), a more appropriate estimate of the women's 8-hour TWA formaldehyde exposures would be 34 ppb for the low category, 86 ppb for the medium category, and 226 ppb for the high category (see Table 5-5, Panel B).

Candidate RfC derivation for Taskinen et al. (1999):

Critical effect: Decreased FDR.

Point of departure: For decreased FDR, the midexposure level is considered a NOAEL. The mean exposure as an 8-hour TWA for the workday is reported as 76 ppb. EPA has adjusted this POD to account for potential background formaldehyde exposures during the workshift (see Table 5-5) resulting in an adjusted POD of 86 ppb. No further duration adjustment is made to this POD to account for background levels of formaldehyde exposure outside of the workplace.

Application of study-specific Uncertainty Factors (UFs):

Interspecies UF = 1: No interspecies adjustment is needed as this is a human study.

LOAEL-to-NOAEL UF = 1: Selection of an NOAEL as the POD.

Subchronic to chronic UF = 1: The study design represents a study population with a range of exposure durations, including chronic exposures. By drawing the study population from full-time employees and members of the wood-working union, there is an expectation that the study population reflects the demographic of that group as a whole. Although specific summary information is not published for this study group (e.g., average length of employment), the lack of this reporting in itself does not seem to justify an UF for subchronic-to-chronic exposure given the overall study design. As a study adequate for assessing reproductive effects in a chronically exposed cohort, no further adjustment was considered needed.

Human variability UF = 10: The study population included women employed in the wood-working industry who were healthy enough to be gainfully employed. Additionally, study inclusion criteria ensured that all study participants had at least one pregnancy resulting in a live birth during the study period (1985–1995). Therefore, these women were reproductively successful. The authors judged that selective participation did not influence potential confounders such as irregular menstruation or earlier miscarriages, which could impact the time to pregnancy results. Susceptible populations were not addressed and, in fact, the women in the study may be considered healthier than the general population in terms of reproductive health. Therefore, an uncertainty factor of 10 for human variability was applied.

$$RfC = \frac{NOAEL}{\left(UF_A \times UF_L \times UF_S \times UF_H\right)} = \frac{86\ ppb}{\left(1 \times 1 \times 1 \times 10\right)} = 8.6\ ppb \qquad (5\text{-}2)$$

UF_A = 1 (interspecies UF)

UF_L = 1 (LOAEL-to-NOAEL UF)

UF_S = 1 (subchronic to chronic UF)

UF_H = 10 (human variability UF)

5.1.2.3. *Evaluation of the Study-Specific Candidate RfCs*

Seven studies were selected as key studies for consideration in RfC derivation (see Section 5.1.2, Table 5-4). Candidate RfCs from these studies address various health effects including: sensory irritation, respiratory effects, asthma, increased allergic sensitization, and decreased fecundity (see Table 5-6).

Three of the seven studies address sensory irritation of the eye, nose, and throat (Liu et al., 1991; Ritchie and Lehnen, 1987; Hanrahan et al., 1984). The PODs for sensory irritation range from 50 to 95 ppb for a health effect that is considered minimally adverse. Two alternatives are presented for the human variability uncertainty factor in RfC derivation based on these SI studies. Alternative A ($UF_H = 3$) results in cRfCs from 9.5 to 23 ppb. Alternative B ($UF_H = 1$) results in cRfCs from 32 to 70 ppb.

A cRfC of 9 ppb is derived for decreased FDR in an occupational study of women in the wood-working industry (Taskinen et al., 1999). This endpoint is supported by four other epidemiologic studies and is considered a potential health concern for occupationally exposed women (John et al., 1994; Taskinen et al., 1994; Seitz and Baron, 1990; Axelsson et al., 1984). However, there is some uncertainty regarding the influence of peak exposures in the work place on the apparent exposure-response relationship based on average workday exposures calculated for study participants. It is unknown if the observed decreased FDR can be attributed to the average exposures from which the cRfC is derived or if it is a result of the measured exposures (as high as 1,000 ppb). If this were the case the cRfC of 9 ppb, based on the average time-weighted exposures, would be protective for decreased fecundity.

Three studies identify adverse health effects in residential populations including children: increased incidence of asthma, decreased pulmonary function, increase in respiratory symptoms, and increased allergic sensitization (Rumchev et al., 2002; Garrett et al., 1999 a,b; Krzyzanowski et al., 1999). Asthma, allergic sensitization, pulmonary function, and symptoms of respiratory disease are not only clinically related, but etiologically related, and it is reasonable that they are considered together from a public health perspective. These health effects are observed below the exposure levels that result in sensory irritation and the resulting cRfCs are correspondingly lower, in a range between 2.8 and 11 ppb, depending on the study, endpoint considered, and the application of alternative uncertainty factors for human variability (see Table 5-6).

These three studies of related health effects: asthma, allergic sensitization, pulmonary function, and symptoms of respiratory disease in children from in-home exposure to formaldehyde (Rumchev et al., 2002; Garrett et al., 1999 a,b; Krzyzanowski et al., 1999) were chosen as the basis for the derivation of the RfC. These cocritical studies are mutually

Table 5-6. Summary of reference concentration (RfC) derivation from critical study and supporting studies

Endpoint	Study	Study size	Homes	Children	POD (ppb)	Application of study-specific UF			cRfC (ppb)
						UF_L	UF_S	UF_H	
Respiratory effects/asthma and sensitization									
Reduction of PEFR in children (10%)	Krzyzanowski et al. (1990)	208	Yes	Yes	$BMCL_{10}$ = 17	1	1	3	**5.6**
Asthma incidence	Rumchev et al. (2002)	192	Yes	Yes	NOAEL = 33	1	3	**Alternative A**	
								3	**3.3**
								Alternative B	
								1	**11**
Increased asthma; allergic sensitization	Garrett et al. (1999 a,b)	148	Yes	Yes	LOAEL = 28	3	1	**Alternative A**	
								3	**2.8**
								Alternative B	
								1	**9.3**
Sensory Irritation									
Eye irritation, burning eyes	Ritchie and Lehnen (1987)	2,007	Yes	Yes	NOAEL = 50	1	1	**Alternative A**	
								3	**17**
								Alternative B	
								1	**50**
	Hanrahan et al. (1984)	61	Yes	Some teenagers	$BMCL_{10}$ = 70	1	1	**Alternative A**	
								3	**23**
								Alternative B	
								1	**70**

Table 5-6. Summary of reference concentration (RfC) derivation from critical study and supporting studies (continued)

Endpoint	Study	Study size	Homes	Children	POD (ppb)	Application of study-specific UF			cRfC (ppb)
						UF_L	UF_S	UF_H	
Eye irritation, burning eyes (continued)	Liu et al. (1991)	1,394	Yes	Yes	LOAEL = 95	3	1	**Alternative A**	
								3	**9.5**
								Alternative B	
								1	**32**
Reproductive/Developmental									
Decreased fecundability density ratio (FDR)	Taskinen et al., 1999	602	No	No	NOAEL= 86	1	1	10	**8.6**

Notes: 1: The final RfC will be rounded to one significant digit per EPA policy. Since the Candidate RfC is an interim calculation, two-significant digits are retained as common practice in mathematics {i.e., one significant digit more that the final result, to avoid rounding errors compounding across multiple mathematical manipulations}.

supportive and provide similar cRfCs. Therefore, the RfC is taken as the mean of the cRfCs of the cRfCs of the three cocritical studies. For two of these studies (Rumchev et al., 2002; Garrett et al., 1999 a,b), EPA is providing alternatives for the application of the UF addressing human variability. These alternatives result in a threefold difference in cRfCs for each study when considering the critical effects of childhood asthma and allergic sensitization (see Table 5-6). Alternative A, described above for each study, acknowledges that evaluation of these effects in children does address some aspects of human variability, but there remains the potential for additional interindividual variability within the studied population, thus a UF of 3 is warranted. Alternative B, described above for each study, also acknowledges that these studies address human variability and susceptible populations. However in alternative B it is judged that since children are a sensitive lifestage for these effects (asthma and atopy), and are likely the most sensitive population, an UF of 1 may be applied. It is acknowledged that some degree of interindividual variability may remain.

Alternative A: Application of a UF of 3 for human variability

Co-critical studies: Rumchev et al. (2002); Krzyzanowski et al. (1999); Garrett et al. (1999)

Critical endpoints: Asthma, allergic sensitization, pulmonary function, and symptoms of respiratory disease in children.

Candidate RfCs:
cRfC = 5.6 ppb—decreased PEFR (Krzyzanowski et al., 1999)
cRfC = 3.3 ppb—increased physician-diagnosed asthma (Rumchev et al., 2002)
cRfC = 2.8 ppb—increased asthma, atopy and respiratory symptoms (Garrett et al., 1999)

RfC: $$RfC = \frac{5.6\ ppb + 3.3\ ppb + 2.8\ ppb}{3} = \frac{11.7\ ppb}{3} = 4\ ppb$$

Alternative B: Application of a UF of 1 for human variability
(UF_H = 3 remains for Krzyzanowski et al., 1999)

Co-critical studies: Rumchev et al. (2002); Krzyzanowski et al. (1999); Garrett et al. (1999)

Critical endpoints: Asthma, allergic sensitization, pulmonary function, and symptoms of respiratory disease in children.

Candidate RfCs:
cRfC = 5.6 ppb—decreased PEFR (Krzyzanowski et al., 1999)
cRfC = 11 ppb—increased physician diagnosed asthma (Rumchev et al., 2002)
cRfC = 9.3 ppb—increased asthma, atopy and respiratory symptoms (Garrett et al., 1999)

RfC: $$RfC = \frac{5.6\ \text{ppb} + 11\ \text{ppb} + 9.3\ \text{ppb}}{3} = \frac{25.9\ \text{ppb}}{3} = 9\ \text{ppb}$$

5.1.3. Database Uncertainties in the RfC Derivation

The database of available laboratory animal studies, human clinical and epidemiological studies, and supporting mechanistic information for formaldehyde is substantial. Many of the health effects are well studied in animals and humans, especially those endpoints related to sensory irritation and respiratory effects at the POE, such as respiratory tract pathology, asthma and reduced pulmonary function. This is reflected in the number and high quality of human studies presented in Table 5-4 and supporting data summarized in Chapter 4.

The data also indicate effects in other health effect categories, specifically neurotoxic effects, reproductive toxicity, and developmental effects (see Section 5.1.2). These are areas where additional research are needed to reduce uncertainty and better characterize the potential for health effects and the concentrations at which they might occur in humans.

The existing toxicological study database strongly supports the potential for formaldehyde to cause both reproductive and developmental toxicity (see Chapter 4; Tables 4-69 and 4-72). There is, however, no assessment of these endpoints from a satisfactory two-generation toxicity study to fully evaluate the effect of formaldehyde exposure on reproductive and developmental endpoints. Data are adequate to derive a cRfC of 9 ppb for decreased fecundability density ratio (FDR) from a human occupational study (Taskinen et al., 1999). This study also reports an increase in spontaneous abortions, although there is uncertainty on the exposure levels of concern for this endpoint; spontaneous abortions may also contribute to the decreased FDR on which one of the cRfCs is based. The greatest uncertainty in

the cRfC for decreased FDR is the use of a time-weighted exposure metric which does not address possible contributions of peak exposure levels to the observed health effect. As such, it is possible that this cRfC is lower than is needed for protection against decreased FDR. The cRfC for decreased FDR does suggest that the RfC derived from the better studied respiratory effects would be protective of that reproductive/developmental endpoint, but there remain uncertainties as to the full range of potential reproductive and developmental effects. No data exist to sufficiently inform the exposure-response relationship for other reproductive and developmental endpoints as they relate to RfC derivation (see Section 5.1.2.6). For example, male reproductive effects and structural and behavioral developmental effects (including postnatal development) are not addressed by a study of decreased FDR. This is a database deficiency. A survey of the currently available data indicates observed effect levels of 5,000−10,000 ppb for male reproductive endpoints and 400 ppb and above for growth retardation and structural anomalies in animal studies. However, these studies employed only one treatment level, precluding the ability to establish a dose-response relationship, thus limiting the strength of the studies for use in RfC derivation.

Similarly, there is evidence that formaldehyde can cause neurotoxic effects. There is a deficit of studies with appropriate exposure scenarios to support derivation of an RfC reflecting the potential for observed neurotoxicity due to formaldehyde exposure. None of the available human studies that evaluated neurological effects were adequate for use in quantitative risk assessment, although they did identify neurological effects of concern, including changes in memory and concentration (e.g., Bach et al. [1990]; Kilburn et al. [1987, 1985]) and increased risk of mortality from amyotrophic lateral sclerosis (ALS) with increasing duration of exposure to formaldehyde (Weisskopf et al., 2009). The human and animal data indicate the potential for serious neurological and behavioral effects from short-term formaldehyde exposure (see Section 5.1.2.6). Limited studies in humans, as well as controlled studies in established animal models, confirm the neurotoxic effects of formaldehyde at exposure levels of 100–170 ppb (Malek et al., 2003a, c; Bach et al., 1990) (see Table 5-1). For example, an adverse effect level of 100 ppb for impaired learning is reported for short-term exposures (2 hours/day for 10 days) in rats (Malek et al., 2003a). For this effect, appropriate duration adjustment for extrapolation of a 2-hour repeated exposure over a limited number of days is uncertain. Given the nature of these health effects, and the potential for children to be exposed in the home to levels as high as 100 ppb (the level at which effects were seen in animals following a single exposure), this is a significant data gap. Studies are inadequate to determine whether exposure to levels of formaldehyde at or below those that impact children's respiratory health and sensitization will cause neurotoxicity in humans, including endpoints such as impaired learning and memory.

Approaches to the application of a database uncertainty factor:
Options EPA is considering include:

(1) Provide an RfC derived from studies of respiratory and allergenic responses and protective of sensory irritation effects with a database uncertainty factor of one given significant data on formaldehyde, but noting that further research reproductive, developmental and neurotoxic effects would be valuable.

(2) Provide an RfC with a database uncertainty factor of one, with this RfC explicitly identified as being protective of the well-studied effects.

(3) Apply a database UF of 3 to the RfC derived from studies of respiratory and allergenic responses to reflect the potential that reproductive, developmental, or neurotoxic effects might occur at lower doses:

(3) Provide both an RfC identified as protective of the better-studied effects and an RfC with a database uncertainty factor of 3 incorporated to account for limits to the data on reproductive, developmental and neurotoxic effects.

It is unclear what uncertainty factors are appropriate to account for human variability and deficiencies in the overall database. For this reason, several alternatives have been presented.

5.1.4. Uncertainties in the RfC Derivation

By design, the RfC is an estimate of an exposure level at which it is unlikely there would be deleterious effects to the human population (including sensitive subgroups) during a lifetime of exposure. Although the RfC is derived from the best available studies, there are a number of uncertainties that underlie the RfC. Some of these uncertainties are addressed quantitatively by applying UFs on a study-specific a basis for RfCs based on animal studies, less-than-chronic exposures, use of a LOAEL as the POD, and to address human variability for the relevant endpoint (see Section 5.1.3). This section elaborates on some of the sources of uncertainty in the final RfC.

As the RfC is derived from human studies, the majority in a residential setting, study aspects that are often a great source of uncertainty are of no concern (e.g., use of animal studies, study of a worker population). The uncertainties discussed below apply specifically to the database of formaldehyde studies and the process to derive the RfC.

5.1.4.1. *Point of Departure*

Most of the studies considered for RfC derivation did not provide enough data to support BMD modeling, which is generally the preferred approach for obtaining a POD for a given dataset (the preference for a POD based on BMD modeling does not, as a general rule, apply to comparisons across datasets). Rather, the PODs for most studies were LOAELs or NOAELs, which have a number of shortcomings relative to a POD obtained from BMD modeling (i.e., a BMCL or BMDL):

- LOAELs and NOAELs are a reflection of the particular exposure/dose levels used in a study, contributing some inaccuracy to the POD determination.
- LOAELs and NOAELs are often determined based on statistical significance and, thus, reflect the number of study subjects or test animals. Studies are typically dissimilar in detection ability and statistical power, with smaller studies tending to identify higher exposure levels as NOAELs compared with larger but otherwise similarly designed studies.
- Different LOAELs and NOAELs represent different response rates, so direct qualitative and quantitative comparisons are not possible.

PODs identified from BMD models overcome some of the deficiencies associated with LOAELs and NOAELs. Benchmark models were used for two inhalation data sets, Hanrahan et al. (1984) and Krzyzanowski et al. (1990).

It should also be noted, however, that even for BMCLs/BMDLs there is often uncertainty, in particular for continuous responses, about what response level to select as the BMR, i.e., where to define the cut-off point between a level of change that is not adverse and one that is adverse. In addition, BMD models currently in use are purely mathematical models and are not intended to accurately reflect the biology of the effect being modeled.

Another source of uncertainty in the POD is the adjustment for continuous exposure. RfCs are meant to apply to continuous (24 hour/day) exposures. Exposure patterns in human and laboratory animal inhalation studies are typically not continuous and assumptions must be made in converting reported exposure levels to equivalent continuous exposures. Similarly, there are uncertainties about potential dose rate effects, in particular the effect of peak exposures in occupational studies.

5.1.4.2. *Extrapolation from Laboratory Animal Data to Humans*

Because the inhalation database for formaldehyde contains many human studies for a variety of health effects, it was not necessary to rely on animal data for the endpoints from which

to derive the RfC. Thus, unlike for most RfCs, this is not a source of uncertainty in the RfC for formaldehyde.

5.1.4.3. *Human Variation*

Heterogeneity among humans is another uncertainty associated with extending results observed in a limited human study population or laboratory animal experiment to a larger, more diverse human population.

For three of the studies used to derive the RfC, a value of 3 was used for the human variability UF (rather than the default value of 10) because the studies had an apparent over-representation of populations expected to have increased susceptibility (see Section 5.5.3.1):

- The residential study by Ritchie and Lehnen (1987) evaluated eye, nose, and throat irritation in a large number of subjects, including children and the elderly. As a result of the study's participation criteria, individuals with greater sensitivity were potentially over-represented.
- Thirty percent of the subjects in the residential study by Krzyzanowski et al. (1990) were children who are more sensitive to formaldehyde-associated decreases in PEFR than adults. The cRfC determination for this study focused on the results in the children, among whom asthmatics were over-represented (roughly three times) compared with the national average of 9.4% in 2008 (Bloom et al., 2009).
- Garrett et al. (1999 a,b) conducted a cross-sectional survey of allergy and asthma-like symptoms in children with or without a doctor's diagnosis of asthma. The study was designed to include a high proportion of asthmatic children, a sensitive population for the effects being studied.

EPA notes, however, that, while a human variability UF of 3 rather than 10 was used to account for certain special attributes of these studies/effects, there is still uncertainty about how much of the overall population heterogeneity is actually reflected even in these relatively diverse residential studies.

5.1.4.4. *Subchronic-to-Chronic Extrapolation*

RfCs are intended to apply to chronic lifetime exposures. If a study is subchronic (typically less than 10% of a lifetime), a UF for subchronic-to-chronic extrapolation is generally applied to the cRfC for that study. For the key human residential and occupational studies used to derive the RfC in this assessment, the average durations of exposure in the households or workplaces under study are unknown. In this assessment, these studies were considered chronic in nature and no subchronic-to-chronic UF was applied. However, there is uncertainty about

whether or not the responses observed fully reflected the potential effects of chronic exposure, especially in children, where, for example, impacts on the developing respiratory and immune systems could be predisposing the children to further adverse effects later in life.

5.1.5. Previous Inhalation Assessment

There is no previous EPA RfC assessment for formaldehyde with which to compare and contrast the RfC developed in this assessment.

5.2. QUANTITATIVE CANCER ASSESSMENT BASED ON THE NATIONAL CANCER INSTITUTE COHORT STUDY

For quantitative assessment of cancer risk, it is generally preferable to use good-quality epidemiologic data, when available, over laboratory animal data. The follow-up studies by Hauptmann et al. (2004) and Beane Freeman et al. (2009) of the large National Cancer Institute (NCI) retrospective cohort mortality study of U.S. workers involved in the production or use of formaldehyde, with quantitative exposure estimates for the individual workers, present an opportunity to perform quantitative cancer risk assessments of nasopharyngeal cancer (NPC) and lymphohematopoietic cancers (Hodgkin lymphoma and leukemia) based on human data. Although other upper respiratory tract cancers were also identified as being causally associated with formaldehyde exposure in the weight-of-evidence analysis in Section 4.5, NPC was the only upper respiratory tract cancer with exposure-response data adequate for the derivation of unit risk estimates in the Hauptmann et al. (2004) follow-up study of solid tumors. Similarly, the weight-of evidence analysis in Section 4.5 concluded that there were causal relationships between formaldehyde exposure and all lymphohematopoietic cancers as a group, leukemias as a group and myeloid leukemia (see Section 4.1.2.2.1.4). Overall the epidemiologic evidence was considered supportive of a causal association between formaldehyde exposure and both Hodgkin lymphoma and multiple myeloma (see Section 4.1.2.2.1.4 and Section 4.5). However, from the Beane Freeman et al. (2009) follow-up study of lymphohematopoietic malignancies, only all leukemias combined and Hodgkin lymphoma were judged to have exposure-response data adequate for the derivation of unit risk estimates (see Section 5.2.3.1 below).

5.2.1. Choice of Epidemiology Study

Several follow-up studies of formaldehyde exposure in industrial workers with some exposure-response information have recently become available. These studies are discussed in more detail in chapter 4 and the appendix (Human Health) and are reviewed only briefly here. Hauptmann et al. (2004) and Beane Freeman et al. (2009) presented follow-ups of the NCI study (originally described by Blair et al. [1986]) of workers at 10 U.S. plants producing or using formaldehyde. Marsh et al. (2007, 2002) focused on pharyngeal cancer and, in particular, NPC mortality in sequential follow-up analyses of the Marsh et al. (1996) cohort study, which examined 1 of the 10 plants studied by NCI. Pinkerton et al. (2004) presented a follow-up of the National Institute for Occupational Safety and Health (NIOSH) study of workers exposed to formaldehyde in three U.S. garment plants (originally described by Stayner et al. [1988]). Coggon et al. (2003) presented an extended follow-up of a study of workers in six British factories where formaldehyde was produced or used (originally described by Acheson et al. [1984] and previously followed up by Gardner et al. [1993]). In addition, Hauptmann et al. (2009) recently conducted a case-control study of lymphohematopoietic and brain cancers, with exposure-response analyses, nested in the cohorts of "professional" workers (funeral industry workers, in this case) studied by Hayes et al. (1990) and Walrath and Fraumeni (1983, 1984).

The analyses presented here are based on the NPC (Hauptmann et al., 2004) and lymphohematopoietic cancer (Beane Freeman et al., 2009) results from the NCI follow-up studies. The NCI cohort study is the largest of the three independent industrial worker studies and is the only one with sufficient individual exposure data for exposure-response modeling. In addition, the NCI study is the only one of the three studies that used internal comparisons rather than standardized mortality ratios (SMRs), thus minimizing the impact of the healthy worker effect, which can attenuate observed effect estimates. The NCI cohort consists of 25,619 workers (88% male) employed in any of the 10 plants prior to 1966. A follow-up through 1994 presented exposure-response analyses for nine NPC deaths as well as analyses of deaths from other solid cancers based on 865,708 person-years of follow-up (Hauptmann et al., 2004). The most recent follow-up based on 998,106 person-years of observation (through 2004) analyzed 319 deaths attributed to lymphohematopoietic malignancy from a total of 13,951 deaths (Beane Freeman et al., 2009). The results for solid cancers from this recent follow-up had not yet been published at the time of this draft assessment. A detailed exposure assessment was conducted for each worker in the NCI cohort, based on exposure estimates for different jobs held and tasks performed (Stewart et al., 1986). Exposure estimates were made using several different metrics—peak exposure, average intensity, cumulative exposure, and duration of exposure. Respirator use and exposures to formaldehyde-containing particulates and other chemicals were

also considered. For the NPCs, significant trends were observed for the cumulative and peak exposure metrics (Hauptmann et al., 2004). For the lymphohematopoietic cancers, significant trends were observed primarily for all lymphohematopoietic cancers and for Hodgkin lymphoma with the peak exposure metric (Beane Freeman et al., 2009).

The NIOSH follow-up study (Pinkerton et al., 2004) analyzed mortality data (2,206 deaths; 59 from lymphatic and hematopoietic cancers) from their cohort of 11,098 workers (82% female). Leukemia and aleukemia were elevated for workers with >10 years of exposure and for workers with ≥20 years since first exposure. However, since no historical exposure level data were available for this cohort, individual worker exposures could not be estimated and exposure-response modeling was not conducted. The British cohort updated by Coggon et al. (2003) consisted of 14,014 male workers, and the follow-up included 5,185 deaths (83 from lymphohematopoietic cancers). In this cohort, lung cancer mortality was statistically significantly increased, especially in workers in the high-exposure category; however, actual exposure estimates were not available for exposure-response modeling (worker exposures were categorized as nil/background, low, moderate, or high, depending on the job considered to have had the highest exposure). Lymphohematopoietic cancers were not elevated in the British cohort, although, as discussed above, the results were based on external comparisons against national mortality statistics. Neither the NIOSH nor the British study reported increased risks of NPC, although only 1 case (0.96) was expected in the NIOSH cohort (Pinkerton et al., 2003) and only 2.0 cases were expected in the British cohort (Coggon et al., 2003). 95% confidence intervals for the relative risk of NPC from these studies (0.07–3.55 and 0.00–3.00, respectively) were estimated by Bosetti et al. (2008) and are not inconsistent with the NPC findings of Hauptmann et al. (2004).

In the Hauptmann et al. (2009) nested case-control study, exposures were estimated for each case and control using multiple exposure metrics. Because of limitations in the exposure assessment, however, this study, while useful for hazard assessment, was not used by EPA to derive quantitative risk estimates. Of primary concern, the worker histories were obtained from surrogate responders (next of kin and coworkers). While this approach can produce good quality results for general metrics such as ever embalming or years of embalming, which yielded statistically significant associations (for ever embalming) and trends (for years in jobs with embalming) for lymphohematopoietic cancer of nonlymphoid origin and, in particular, myeloid leukemia, validity declines for more specific variables such as number and duration of embalmings per calendar time period and frequency of spills per calendar time period. These latter variables are needed in the exposure model used to estimate exposures for metrics such as cumulative exposure. Where information on a particular variable was obtained from multiple

respondents, Hauptmann et al. (2009) reported a substantial amount of discordance for variables such as number of any embalmings and number of autopsied embalmings. Furthermore, considerable amounts of data were missing. For example, Hauptmann et al. (2009) reported that all but 16 of 44 cases of lymphohematopoietic cancer of nonlymphoid origin had 30% or more of their work history missing. Moreover, although of lesser concern in and of itself, even where good retrospective data on the model variables may have been available, there was additional uncertainty in the estimates resulting from the exposure model. In a validation phase, using real measurements from independent embalmings, the final exposure model, after modifications to correct for initial overestimation, explained 74% of the variability.

In addition to limitations in the exposure assessment used in the Hauptmann et al. (2009) study, there were substantial uncertainties about the quantitative precision of the exposure-response relationship observed for myeloid leukemia, which was the one cancer type examined in this study for which there was consistent evidence of an association with formaldehyde exposure. Of the 34 myeloid leukemia cases in the Hauptmann et al. (2009) study, there was only one unexposed case for all of the exposure metrics. Thus, the relative risk estimates (odds ratios) derived in comparison to the referent group are unstable. To address this problem, Hauptmann et al. (2009) created an alternate referent group comprised of workers with <500 lifetime embalmings. As might be expected, since this alternate referent group is no longer an unexposed referent group, odds ratios for the various levels of the different exposure metrics declined considerably (e.g., for the cumulative exposure metric, odds ratios based on the unexposed referent group were 4−5 times higher than those based on the <500-embalmings referent group), although they remained increased relative to the referent group. Thus, although the results of the Hauptmann et al. (2009) study were supportive of the hazard assessment, the overall uncertainty in the quantitative exposure-response data, particularly in the exposure assessment, from the study was considered prohibitive for the development of quantitative cancer risk estimates.

5.2.2. Nasopharyngeal Cancer

5.2.2.1. *Exposure-Response Modeling of the National Cancer Institute Cohort*

A detailed exposure assessment was conducted for the NCI cohort, and quantitative exposure estimates were generated for each worker (Stewart et al., 1986). Formaldehyde exposure estimates, including 8-hour time-weighted average (TWA) exposures and level and frequency of peak exposures, were derived for each job, work area, and calendar year combination. A peak was defined as a short-duration exposure (typically <15 minutes) above the TWA. Cumulative exposures (in ppm × years) were estimated by multiplying the time a

worker spent in a specific job by the TWA exposure for that job and summing over all the jobs held by the worker. Duration was the total time spent in jobs with formaldehyde exposure, and average intensity was the ratio of cumulative exposure to duration. Formaldehyde exposures after 1980 were not taken into account in the follow-up study, but this was considered to have a minimal impact on the results (see Section 5.2.2.4).

The results of NCI's internal analyses for NPC, using the peak exposure, average intensity, cumulative exposure, and duration of exposure metrics, are presented in Table 5-7. The relative risks (rate ratios) (RRs) were estimated using log-linear Poisson regression models stratified by calendar year, age, sex, and race and adjusted for pay category (salary/wage/unknown). The NCI investigators used the low-exposure category as the reference category to "minimize the impact of any unmeasured confounding variables since nonexposed workers may differ from exposed workers with respect to socioeconomic characteristics" (Hauptmann et al., 2004). A 15-year lag interval was used in estimating exposures in order to account from a minimal latency period for the development of solid cancers, including NPCs.

As can be seen in Table 5-7, peak exposure is the exposure metric that provides the strongest exposure-response relationship with NPC. However, it is not clear how to extrapolate RR estimates based on these peak exposure estimates to meaningful estimates of lifetime extra risk of cancer from environmental exposures, where the risk is usually considered to be from continuous lifetime exposures to low environmental levels. In addition, peak exposure is a more subjective measure than the other metrics, it is not based on actual measurements, and it is a categorical rather than continuous measure. Furthermore, the "true" exposure metric best describing the biologically relevant delivered dose of formaldehyde is unknown. The cumulative exposure metric provides a good fit to the data (p trend = 0.029 for all person-years), and, since this is generally the preferred metric for quantitative risk assessment for environmental exposure to carcinogens, cumulative exposure was chosen as the exposure metric for the risk estimate calculations for NPC in this assessment.

The nonexposed person-years were included in the primary cancer risk analyses presented here in order to be more inclusive of all the exposure-response data. Such data are typically included in exposure-response modeling. Furthermore, the data were stratified by pay category, which should alleviate some concerns about the nonexposed workers having different socioeconomic characteristics. Final results for the exposed person-years only are presented for comparison.

As described above, Hauptmann et al. (2004) investigated the relationship between formaldehyde exposure and NPC mortality using log-linear Poisson regression models. They also conducted log-linear trend tests using the general model $RR = e^{\beta X}$, where β represents the

regression coefficient for exposure and X is exposure as a continuous variable. The trend models were stratified by calendar year, age, sex, and race and adjusted for pay category. Dr. Hauptmann

Table 5-7. Relative risk estimates for mortality from nasopharyngeal malignancies (ICD-8 code 147) by level of formaldehyde exposure for different exposure metrics

Relative risk (number of deaths)				*p* trend[b]	*p* trend[c]
Peak exposure (ppm)					
0	**>0 to <2.0[a]**	**2.0 to <4.0**	**≥4.0**		
1.00[d] (2)	–(0)	–(0)	1.83 (7)	0.044	<0.001
Average intensity (ppm)					
0	**>0 to <0.5**	**0.5 to <1.0**	**≥1.0**		
1.00[d] (2)	–(0)	0.38 (1)	1.67 (6)	0.126	0.066
Cumulative exposure (ppm × years)					
0	**>0 to <1.5**	**1.5 to <5.5**	**≥5.5**		
2.40 (2)	1.00 (3)	1.19 (1)	4.14 (3)	0.029	0.025
Duration of exposure (years)					
0	**>0 to <5**	**5 to <15**	**≥15**		
1.77 (2)	1.00 (4)	0.83 (1)	4.18 (2)	0.206	0.147

[a]Reference category for all categories.
[b]Likelihood ratio test (1 degree of freedom) of zero slope for formaldehyde exposure (continuous variable, except for peak exposure metric) among all (nonexposed and exposed) person-years.
[c]Likelihood ratio test (1 degree of freedom) of zero slope for formaldehyde exposure (continuous variable, except for peak exposure metric) among exposed person-years only.
[d]Reference category due to no cases in the low-exposure category.

Source: Hauptmann et al. (2004).

provided EPA with the β estimates (and their standard errors) from the trend tests for NPC and the cumulative exposure metric for all person-years and for exposed person-years only (personal communication from Michael Hauptmann, NCI, to Jennifer Jinot, EPA, March 29, 2004). These estimates are presented in Table 5-8.

Table 5-8. Regression coefficients from NCI log-linear trend test models for NPC mortality from cumulative exposure to formaldehyde[a]

Person-years	β (per ppm × year)	Standard error (per ppm × year)
All	0.05183	0.01915
Exposed only	0.05318	0.01914

[a]Models stratified by calendar year, age, sex, and race and adjusted for pay category; cumulative exposures calculated using a 15-year lag interval.

Source: Personal communication from Michael Hauptmann to Jenifer Jinot (March 29, 2004).

5.2.2.2. *Prediction of Lifetime Extra Risk of Nasopharyngeal Cancer Mortality*

The regression coefficients presented in Table 5-8 were used to predict the extra risk of NPC mortality from environmental exposure to formaldehyde.

Extra risk = (Rx–Ro)/(1–Ro),

where Rx is the lifetime risk in the exposed population and Ro is the lifetime risk in an unexposed population (i.e., the background risk). Extra risk estimates were calculated using the β regression coefficients and a life-table program that accounts for competing causes of death.[9] U.S. age-specific 1999 all-cause mortality rates for all race and gender groups combined (National Center for Health Statistics [NCHS], 2002) were used to specify the all-cause background mortality rates in the life-table program. NCHS 1996–2000 age-specific background mortality rates for NPC were provided by Dr. Eisner of NCI's Surveillance, Epidemiology and End Results (SEER) program (personal communication from Milton Eisner, SEER, to Jennifer Jinot, EPA, December 19, 2003). Risks were computed up to age 85 because cause-specific mortality (and incidence) rates for ages above 85 years are less reliable. Conversions between occupational formaldehyde exposures and continuous environmental exposures were made to account for differences in the number of days exposed per year (240 versus 365) and in the amount of air inhaled per day (10 versus 20 m^3). An adjustment was also

[9]This program is an adaptation of the approach that was previously used in BEIR IV, "Health Risks of Radon and Other Internally Deposited Alpha Emitters." National Academy Press, Washington, DC, 1988, pp. 131–134. The same methodology was also used more recently in EPA's 1,3-butadiene health risk assessment (U.S. EPA, 2002). A spreadsheet illustrating the life table used for the extra risk calculation for the derivation of the LEC_{0005} for NPC incidence (see Section 5.2.2.3) is presented in Appendix C.

made for the 15-year lag period. The reported standard errors for the regression coefficients were used to compute the one-sided 95% upper confidence limits (UCLs) for the extra risks based on a normal approximation.

Point estimates and one-sided 95% UCLs for the extra risk of NPC mortality associated with varying levels of continuous exposure to formaldehyde are presented in Table 5-9. The model predicts extra risk estimates that are fairly linear for exposures below about 0.001 to 0.01 ppm but not for exposures above 0.01 ppm.

Table 5-9. Extra risk estimates for NPC mortality from various levels of continuous exposure to formaldehyde

Exposure concentration (ppm)	Extra risk	95% UCL on extra risk
0.0001	1.69×10^{-7}	2.71×10^{-7}
0.001	1.69×10^{-6}	2.73×10^{-6}
0.01	1.76×10^{-5}	2.90×10^{-5}
0.1	2.63×10^{-4}	5.75×10^{-4}
1	6.22×10^{-1}	9.00×10^{-1}
10	9.82×10^{-1}	9.85×10^{-1}

Consistent with EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the same data and methodology were also used to estimate the exposure level (effective concentration [EC_x]) and the associated (one-sided) 95% lower confidence limit (LEC_x) corresponding to an extra risk of 0.05% (x = 0.0005). Although EPA guidelines emphasize the use of exposure levels associated with a 10% extra risk level for the POD for low-dose extrapolation, that would not be appropriate in this instance. A 10% extra risk level is very high for responses generally observed in epidemiology studies; thus, a 1% extra risk level is typically used for epidemiologic data to avoid upward extrapolation. For NPC, however, even the 1% level of risk is associated with RR estimates that are substantially higher than those observed in the epidemiology study. Hence, even a 1% extra risk level would be an upward extrapolation. Based on the life-table program, the RR estimate for an extra risk of 1% for NPC mortality is 46. Even 0.1% yields an RR estimate on the high end of the observable range of the epidemiology study (RR = 5.5). A 0.05% extra risk level yields an RR estimate of 3.27, which better corresponds to the RRs in the range of the data. Thus, 0.05% extra risk was selected for determination of the POD, and, consistent with EPA's *Guidelines for Carcinogen Risk*

Assessment (U.S. EPA, 2005a), the LEC value corresponding to that risk level was used as the POD. While this may appear to be an inordinately low response level, it must be recognized that NPC has a very low background mortality rate (e.g., lifetime background risk is about 0.00022); therefore, a 1% extra risk (i.e., 0.01) would be a huge increase relative to the background risk. This is consistent with the fact that, even with a large cohort followed for a long time, only nine NPC deaths were observed in the NCI follow-up through 1994.[10]

Because formaldehyde is a mutagenic carcinogen and the weight of evidence supports the conclusion that formaldehyde carcinogenicity can be attributed, at least in part, to a mutagenic MOA (see Section 4.5), a linear low-dose extrapolation was performed in accordance with EPA's carcinogen risk assessment guidelines (U.S. EPA, 2005a). The EC_{0005}, LEC_{0005}, and inhalation unit risk estimates for NPC mortality are presented in Table 5-10.

Table 5-10. EC_{0005}, LEC_{0005}, and inhalation unit risk estimates for NPC mortality from formaldehyde exposure based on the Hauptmann et al. (2004) log-linear trend analyses for cumulative exposure

Person-years	EC_{0005} (ppm)	LEC_{0005} (ppm)	Unit risk[a] (ppm^{-1})
All	0.15	0.093	5.4×10^{-3}
Exposed only	0.15	0.091	5.5×10^{-3}

[a]Unit risk = $0.0005/LEC_{0005}$.

5.2.2.3. *Prediction of Lifetime Extra Risk of Nasopharyngeal Cancer Incidence*

EPA cancer risk estimates are typically derived to represent a plausible upper bound on increased risk of cancer *incidence*, as from experimental animal incidence data. Cancer data from epidemiology studies are more often mortality data, as is the case in the NCI study. For cancers with low survival rates, mortality-based estimates are reasonable approximations of cancer incidence risk. However, for NPC, the survival rate is substantial (51% at 5 years in the 1990s in the United States, according to Lee and Ko [2005]), and incidence-based risks are preferred because EPA is concerned with cancer occurrence, not just cancer mortality.

Therefore, an additional calculation was done using the same regression coefficients provided by Dr. Hauptmann (see Table 5-8) but with age-specific NPC incidence rates for

[10] Ten NPCs were reported on death certificates and included in NCI's SMR analysis, but one of these cases was apparently misclassified on the death certificate, so only nine cases were used to estimate the RRs in the internal comparison analysis, as discussed by Hauptmann et al. (2004).

1996−2000 from SEER in place of the NPC mortality rates in the life-table program. SEER collects cancer incidence data from a variety of geographical areas in the United States. The incidence data used here are from SEER 12, a registry covering about 14% of the U.S. population, which was the most current SEER registry at the time this analysis was done. SEER 1996–2000 age-specific background incidence rates for NPC were provided by Dr. Eisner of NCI's SEER program (personal communication from Milton Eisner, SEER, to Jennifer Jinot, EPA, December 18, 2003). The incidence-based calculation relies on the reasonable assumptions that NPC incidence and mortality have the same exposure-response relationship for formaldehyde exposure and that the incidence data are for first occurrences of NPC or that relapses provide a negligible contribution. The calculation also relies on the fact that NPC incidence rates are small compared with the all-cause mortality rates.

The resulting EC_{0005}, LEC_{0005}, and inhalation unit risk estimates for NPC incidence are presented in Table 5-11. The unit risk estimate for cancer incidence is twofold higher than the corresponding mortality-based estimate, for all person-years. This sizeable discrepancy can be attributed to the high survival rates for NPC.

Table 5-11. EC_{0005}, LEC_{0005}, and inhalation unit risk estimates for NPC incidence from formaldehyde exposure based on the Hauptmann et al. (2004) trend analyses for cumulative exposure

Person-years	EC_{0005} (ppm)	LEC_{0005} (ppm)	Unit risk[a] (ppm^{-1})
All	0.074	0.046	1.1×10^{-2}
Exposed only	0.072	0.045	1.1×10^{-2}

[a]Unit risk = $0.0005/LEC_{0005}$.

The preferred estimate for the inhalation cancer unit risk for NPC is the estimate of 1.1×10^{-2} per ppm derived using incidence rates for the cause-specific background rates, for all person-years. The results from the exposed person-years are essentially identical.

Because NPC is a rare cancer, with a relatively low number of cases occurring per year in the United States, a rough calculation was done to assure that the unit risk estimate derived for NPC incidence is not implausible in comparison to actual case numbers. For example, assuming an average constant lifetime formaldehyde exposure level of 5 ppb for the U.S. population, the inhalation unit risk estimate for NPC equates to a lifetime extra risk estimate of 5.5×10^{-5}. Assuming an average lifetime of 75 years (this is not EPA's default average lifetime of 70 years

but rather a value more representative of actual demographic data) and a U.S. population of 300,000,000, this lifetime extra risk estimate suggests a crude upper-bound estimate of 220 incident cases of NPC attributable to formaldehyde exposure per year. Alternatively, assuming an average constant lifetime formaldehyde exposure level of 20 ppb, the calculation suggests a crude upper-bound estimate of 880 incident cases of NPC per year. Both upper bound estimates, using different assumed lifetime exposure levels, are well below the estimated 2,100 total incident NPC cases per year calculated from a published NPC incidence rate for the United States of 0.7/100,000 person-years (Lee and Ko, 2005).[11]

5.2.2.4. *Sources of Uncertainty*

The two major sources of uncertainty in quantitative cancer risk estimates are generally interspecies extrapolation and high-to-low dose extrapolation. The risk estimates derived from the Hauptmann et al. (2004) analyses of the NCI cohort are not subject to interspecies uncertainty since they are based on human data. However, substantial uncertainty remains in the extrapolation from occupational exposures to lower environmental exposures. Although the actual exposure-response relationship at low exposure levels is unknown, the linear low-dose extrapolation that was used is warranted by the strong support for formaldehyde carcinogenicity having a mutagenic MOA (see Section 4.5). The linear low-dose extrapolation from the 95% lower bound on the exposure level associated with the extra risk level serving as the benchmark response is generally considered to provide a plausible upper bound on the risk at lower exposure levels. Actual low-dose risks may be lower to an unknown extent.

Other sources of uncertainty emanate from the epidemiologic study and its analysis (Hauptmann et al., 2004), including the retrospective estimation of formaldehyde exposures in the cohort, the modeling of the epidemiologic exposure-response data, the appropriate exposure metric for exposure-response analysis, and potential confounding or modifying factors.

The same team of investigators (Stewart et al., 1986) conducted a detailed retrospective exposure assessment to estimate the individual worker exposures. Formaldehyde exposures were estimated for specific jobs/tasks based on monitoring data, discussions with workers and plant managers, and assessment by industrial hygienists. Individual worker estimates were derived for a variety of exposure metrics based on work histories. This exposure assessment was a major undertaking, involving over 100 person-months. Hauptmann et al. (2004) suggested that

[11] With the application of age-dependent adjustment factors (see Section 5.4.4), the lifetime unit risk estimate for NPC would increase by a factor of 1.66, and the crude upper-bound estimates of the incident cases per year attributable to formaldehyde exposure would similarly increase by a factor of 1.66. The resulting adjusted estimates of 365 and 1460 for 5 ppb and 20 ppb exposure levels, respectively, are still well below the estimated total number of 2100 incident cases per year in the United States.

employment of such a detailed exposure assessment would tend to minimize exposure misclassification for average and cumulative exposure and duration of exposure but that peak exposure estimates could be more susceptible to misclassification because they were not based on actual measurements. In addition, the follow-up study did not take into account exposures after 1980. Hauptmann et al. (2003) stated that any underestimation of (total) exposure resulting from the 1980 cutoff "would be small because only 3.7% of all person-years were contributed by workers who were 65 years or younger and in exposed jobs in 1980" and because exposure levels were believed to have been much lower after 1980 than in earlier years.

As discussed in Chapter 4 and the appendix (Human Health), Marsh et al. (1996) also estimated individual worker exposures at 1 of the 10 plants (Wallingford, Connecticut) studied by the NCI team, and 5 of the 9 NPC deaths were from that plant. The Marsh et al. (1996) exposure estimates were about 10-fold lower than those derived by the NCI team for the workers at the Wallingford plant. Marsh et al. (2002) hypothesized that "the NCI used data from several facilities to estimate exposures in a single facility." However, the NCI investigators maintained that they estimated exposures for each plant separately. While the exact reasons for such a large discrepancy are unclear, some differences in the assessment procedures which could have resulted in substantial differences in the estimates are apparent. First, according to Marsh et al. (1996), 91.7% of the white male Wallingford plant workers were specified as being exposed to formaldehyde in the NCI study, while only 83.3% were considered to have been exposed in the Marsh et al. (1996) analysis (it should be noted that these two cohorts of the Wallingford plant are not identical). Second, the NCI investigators (Stewart et al., 1987, 1986) did their own exposure monitoring at all the plants, including the Wallingford facility, in order to standardize the data provided by the plants as well as to fill data gaps for certain jobs. There is no indication that Marsh et al. (1996) made any additional measurements themselves. Third, although the Marsh et al. (2002, 1996) papers are not entirely consistent on this point, those investigators apparently assumed that the job-specific exposures at the plant were essentially constant over the history of the plant, whereas the NCI team, based on interviews with plant personnel knowledgeable about equipment and process changes, assumed that past exposures were higher.

In any event, despite the discrepancies in the absolute exposure values, the relative exposures for both the Marsh et al. (2002, 1996) and NCI studies, as reflected in the exposure-response relationships, are less subject to misclassification and are considered to be reliable. The Wallingford plant is just 1 of the 10 plants in the NCI study (representing 4,389 of the 25,619 workers in the NCI cohort), but if the Marsh et al. (1996) exposure estimates, which are roughly 10-fold lower than the NCI estimates, are closer to the actual exposures for those workers, then the true potency of formaldehyde could be greater than that suggested by the unit

risk estimates calculated above based on the NCI data. Furthermore, if the NCI exposure values were significantly overestimated across all 10 plants, then the actual potency could be higher still.

With respect to the exposure-response model, the log-linear model used by Hauptmann et al. (2003) for their trend tests (i.e., $RR = e^{\beta X}$) is a commonly used model for epidemiologic data with exposure as a continuous variable. However, the actual exposure-response relationship is unknown. Moreover, even if the correct exposure-response model were known, there would be substantial uncertainty in estimating the model parameters because there are only nine NPC deaths to model. Furthermore, Beane Freeman et al. (2009) reported that in the follow-up through 2004 it was discovered that 1,006 deaths that occurred during the 1980 to 1994 follow-up period had not been included in the analyses of the 1994 follow-up study (Hauptmann et al., 2004, 2003), for reasons that have not been identified. Because NPC is such a rare cancer, it is not expected that many, if any, NPC deaths were among the 1,006 excluded deaths; however, it is unknown how inclusion of the 1,006 deaths would have altered the overall exposure-response relationship and, hence, the regression coefficient. Additionally, a 15-year lag was used for all the NCI solid cancer models. The actual minimum latency is unknown; however, the investigators reported that lag intervals between 2 and 20 years yielded similar results.

Another potentially significant source of uncertainty is associated with the exposure metrics. With the log-linear model used for modeling the occupational data, the peak exposure metric gave the strongest exposure-response relationship between formaldehyde exposure and increased risk of NPCs. However, it is unclear how to extrapolate RR estimates based on peak exposure estimates to meaningful estimates of lifetime extra risk of cancer from environmental exposure (i.e., extra risk from lifetime continuous low-level environmental exposures). The cumulative exposure metric also yielded a statistically significant exposure-response relationship and was used for the primary cancer risk calculations in this assessment. The "true" exposure metric best describing the toxicologically relevant dose of formaldehyde for nasopharyngeal carcinogenesis is unknown. If a peak-exposure type of metric is the best representative of the toxicologically relevant dose, this suggests that there are dose-rate effects in the exposure-response relationship for formaldehyde and NPC. If this is the case, the unit risk estimates presented here, which are based on a linear low-dose extrapolation, may overestimate the true risks to an unknown extent.

Hauptmann et al. (2004) gave a lot of consideration to potential confounding and modifying factors in their analyses. The important factors of age, race, sex, calendar year, and pay category were taken into account in their Poisson regression and trend analyses.

Furthermore, they used the low-exposure person-years, rather than the unexposed person-years, as their referent group in an effort to minimize any potential confounding effects resulting from differences in socioeconomic or other characteristics between exposed and unexposed workers. When the slope estimate (i.e., regression coefficient) for the exposed person-years only was used in the analyses presented here, the unit risk estimate was essentially identical to that calculated from the slope estimate for all person-years (see Tables 5-10 and 5-11).

In addition, these investigators evaluated routine respirator use, exposure to formaldehyde-containing particulates, durations of exposure to 11 other chemicals/substances in the plants (antioxidants, asbestos, carbon black, dyes and pigments, hexamethylenetetramine, melamine, phenol, plasticizers, urea, wood dust, and benzene), and duration of employment as a chemist or laboratory technician. Only 133 workers ever routinely used a respirator (Hauptmann et al., 2003). Hauptmann et al. (2004) reported that RR estimates for NPC changed when adjusted for duration of melamine exposure, although trend tests remained significant for cumulative formaldehyde exposure ($p = 0.006$). The investigators suggested that the association with melamine may be spurious, and the regression coefficients (i.e., β estimates) used in this assessment were not adjusted for melamine. RR estimates reportedly did not change substantially when adjusted for exposure to any of the other 10 chemicals/substances. None of the workers who died of NPC was identified as being exposed to wood dust. On the other hand, each of the seven formaldehyde-exposed workers who died of NPC was also exposed to particulates, and neither of the two workers who died of NPC but were not exposed to formaldehyde was exposed to particulates. However, for those workers exposed to particulates, NPC risk increased with increasing formaldehyde exposure, suggesting a formaldehyde-associated effect. Nonetheless, because of the correspondence between formaldehyde and particulate exposures within the workers who died of NPC, there is uncertainty as to whether or not particulates were acting as a modifying factor. Adjusting for duration of time spent working as a chemist or laboratory technician did not substantially alter the results (Hauptmann et al., 2004).

Adjusting for plant may result in overadjustment because plant is highly correlated with exposure. Moreover, Hauptmann et al. (2004) adjusted for important plant-related factors by adjusting for the 11 chemicals/substances. Nonetheless, these investigators conducted analyses adjusted for plant to address potential unmeasured confounders associated with plant, and they reported that the association with NPC remained. As noted above, five of the nine NPC deaths were from the Wallingford plant also studied by Marsh et al. (2006, 2002). Marsh et al. (2007) hypothesized that the excess NPCs in the Wallingford plant could be due to external employment

in metal-working industries, but we found no evidence to support this supposition (see Section 4.1.1.1).

Although smoking data were not available for the cohort, smoking is unlikely to explain the excesses in NPCs because there was no consistent increase for tobacco-related diseases, including lung cancer, across the same exposure metrics. No information was available on Epstein-Barr virus, a major risk factor for NPC, in the cohort.

Despite inevitable uncertainties, it is important not to lose sight of the strengths of the NCI study. In addition to the use of internal analyses and the extensive exposure assessment and consideration of potential confounding or modifying variables, the NCI study has a large cohort that has been followed for a long time. The cohort included 25,619 subjects, 75% of whom entered before 1960, contributing a total of 865,708 person-years (730,312 for the exposed workers) to the 1994 follow-up. Duration of follow-up in 1994 ranged up to 58 years, with a median of 35 years. Duration of exposure ranged up to 46 years, with a median of 2 years.

Additional uncertainties are not so much inherent in the exposure-response modeling or in the epidemiologic data themselves but rather stem from the process of obtaining more general EPA risk estimates from these specific results. EPA cancer risk estimates typically represent a plausible upper bound on increased risk of cancer incidence in the general population for all tissue sites potentially affected by an agent. For experimental animal studies, this is accomplished by using tumor incidence data and summing across all the tumor sites that demonstrate significantly increased incidences, generally using data from the most sensitive sex and species. However, in estimating comparable risks from the NCI epidemiologic data, certain limitations are encountered. First, the NCI study is a retrospective mortality study, and cancer incidence data are unavailable for the cohort. Second, these occupational epidemiology data represent a worker cohort that is generally healthier than the general population (e.g., SMRs < 1) (see Table 2 of Hauptmann et al. [2004]).

The first limitation was addressed quantitatively in the calculation of cancer incidence risk estimates from the mortality results, and, even though there are assumptions made in using incidence data this way, the incidence-based estimates are believed to be better estimates of cancer incidence risk than the mortality-based estimates. With respect to the second limitation, the healthy worker effect is often an issue in occupational epidemiology studies, and it is difficult to know to what extent there is a healthy worker effect with respect to the development of NPC in this study. As discussed above, Hauptmann et al. (2004) sought to minimize potential confounding effects resulting from differences in socioeconomic or other characteristics between exposed and unexposed workers by using the low-exposure person-years, rather than the unexposed person-years, as their referent group. Nonetheless, when the slope estimates for the

exposed person-years only were used in the analyses in this assessment, unit risk estimates essentially identical to those calculated from the slope estimates for all person-years were obtained (see Tables 5-10 and 5-11). In terms of representing the general population, the NCI cohort was somewhat diverse, but the workers were predominantly white males (81%) then white females (12%), black males (7%), and black females (<1%), and they were all adults.

Finally, NPC is just one of the upper respiratory tract cancers concluded to be causally associated with formaldehyde exposure (see Section 4.5). These upper respiratory tract cancers are rare cancers and are difficult to detect in cohort studies. Thus, although NPC was the only such cancer with an exposure-response relationship amenable to the derivation of a unit risk estimate, additional, unquantified risk may exist for the other upper respiratory tract cancers. If there was a strong exposure-response relationship between these cancers and formaldehyde exposure, a more apparent association in the Hauptmann et al. (2004) study might have been expected, as was seen for NPC, despite the rare nature of these cancers. Thus, the exposure-response relationship for these other upper respiratory tract cancers is likely modest, at best, and, because these are rare cancers, the contribution of the risk for these cancers to the total cancer risk from formaldehyde exposure is not expected to be large. Nonetheless, with such rare cancers, there is uncertainty regarding the extent to which the estimate based on NPC may underestimate the risk for all upper respiratory tract cancers.

In summary, the inhalation cancer unit risk estimate of 1.1×10^{-2} per ppm for NPC is based on human data from a high-quality epidemiologic study with individual exposure estimates for each worker. A major uncertainty is the appropriate model/exposure metric for extrapolation to environmental exposures.

5.2.3. Lymphohematopoietic Cancer

5.2.3.1. *Exposure-Response Modeling of the National Cancer Institute Cohort*

The results of NCI's internal analyses for lymphohematopoietic cancers using the peak exposure, average intensity, and cumulative exposure metrics from the follow-up through 2004 are reported by Beane Freeman et al. (2009). There was reportedly no evidence of associations with duration of exposure, and those results were not presented. For the peak exposure metric, statistically significant log-linear trends were observed for all lymphohematopoietic cancers, Hodgkin lymphoma, and leukemia (the latter only when the unexposed person-years were included). There was also evidence for potential associations with myeloid leukemia specifically, especially when risks were viewed over time, and with multiple myeloma. Using the average exposure metric, there was a significant trend for Hodgkin lymphoma. With the cumulative exposure metric, there were no statistically significant trends; however, the Hodgkin

lymphoma trend results had *p*-values not much greater than 0.05 (*p* trends = 0.06 and 0.08 with and without the unexposed person-years, respectively), as did the leukemia trend results (*p* trends = 0.08 and 0.12 with and without the unexposed person-years, respectively). As discussed above with NPC, it is not clear how to extrapolate RR estimates based on the peak exposure estimates to meaningful estimates of lifetime extra risk of cancer from environmental exposures. The average exposure metric is also problematic because it suggests that duration of exposure is not important (e.g., exposure to a given exposure level for 1 year conveys the same amount of risk as exposure to the same level for 70 years). Cumulative exposure is generally the preferred metric for quantitative risk assessment for environmental exposure to carcinogens, and, because the Hodgkin lymphoma and leukemia trend results had *p*-values not much greater than 0.05 using the cumulative exposure metric and the elevations in risk with that metric were consistent with significant elevations observed with the peak exposure (for Hodgkin lymphoma and leukemia) and average exposure (for Hodgkin lymphoma) metrics (see Table 5-12), a determination was made to calculate unit risk estimates for Hodgkin lymphoma and leukemia based on cumulative exposure. There is also support for associations between formaldehyde exposure and both Hodgkin lymphoma and leukemia from other studies (see Section 4.5.2). No other lymphohematopoietic cancer responses provided adequate exposure-response data with the cumulative formaldehyde exposure metric in the NCI cohort from which to derive unit risk estimates.

As for the NPC results discussed in Section 5.2.2, the RR estimates in Table 5-12 were derived using log-linear Poisson regression models stratified by calendar year, age, sex, and race and adjusted for pay category (salary/wage/unknown). The NCI investigators used the low-exposure category as the reference category to "minimize the impact of any unmeasured confounding variables since nonexposed workers may differ from exposed workers with respect to socioeconomic characteristics" (Hauptmann et al., 2004). A 2-year lag interval was used to determine exposures in order to account for a minimal latency period for lymphohematopoietic cancers.

Dr. Beane Freeman provided EPA with the regression coefficient estimates for Hodgkin lymphoma and leukemia mortality from the log-linear trend test models for cumulative exposure (i.e., RR = $e^{\beta X}$, with exposure [X] as a continuous variable) used in the NCI analyses (personal communication from Laura Beane Freeman, NCI, to John Whalan, EPA, August 26, 2009). These estimates are presented in Table 5-13. As with the NPC calculations in Section 5.2.2, the nonexposed person-years were included in the primary unit risk estimate derivations in order to

Table 5-12. Relative risk estimates for mortality from Hodgkin lymphoma (ICD-8 code 201) and leukemia (ICD-8 codes 204–207) by level of formaldehyde exposure

for different exposure metrics

Cancer type	Relative risk (number of deaths)				*p* trend[b]	*p* trend[c]
Peak exposure (ppm)						
	0	**>0 to <2.0[a]**	**2.0 to <4.0**	**≥4.0**		
Hodgkin lymphoma	0.67 (2)	1.0 (6)	3.30 (8)	3.96 (11)	0.004	0.01
Leukemia	0.59 (7)	1.0 (41)	0.98 (27)	1.42 (48)	0.02	0.12
Average intensity (ppm)						
	0	**>0 to <0.5**	**0.5 to <1.0**	**≥1.0**		
Hodgkin lymphoma	0.53 (2)	1.0 (10)	3.62 (9)	2.48 (6)	0.03	0.05
Leukemia	0.54 (7)	1.0 (67)	1.13 (25)	1.10 (24)	0.50	>0.50
Cumulative exposure (ppm × years)						
	0	**>0 to <1.5**	**1.5 to <5.5**	**≥5.5**		
Hodgkin lymphoma	0.42 (2)	1.0 (14)	1.71 (7)	1.30 (4)	0.06	0.08
Leukemia	0.53 (7)	1.0 (63)	0.96 (24)	1.11 (29)	0.08	0.12

[a]Reference category for all categories.
[b]Likelihood ratio test (1 degree of freedom) of zero slope for formaldehyde exposure (continuous variable, except for peak exposure metric) among all (nonexposed and exposed) person-years.
[c]Likelihood ratio test (1 degree of freedom) of zero slope for formaldehyde exposure (continuous variable, except for peak exposure metric) among exposed person-years only.

Source: Beane Freeman et al. (2009).

Table 5-13. Regression coefficients for Hodgkin lymphoma and leukemia mortality from NCI trend test models[a]

Cancer type	Person-years	β (per ppm × year)	Standard error (per ppm × year)
Hodgkin lymphoma	All	0.02959	0.01307
	Exposed only	0.02879	0.01333
Leukemia	All	0.01246	0.006421
	Exposed only	0.01131	0.00661

[a]Models were stratified by calendar year, age, sex, and race and adjusted for pay category; exposures included a 2-year lag interval.

Source: Personal communication from Laura Beane Freeman to John Whalan (August 26, 2009).

be more inclusive of all the exposure-response data. Final results for the exposed person-years only are presented for comparison.

5.2.3.2. *Prediction of Lifetime Extra Risks for Hodgkin Lymphoma and Leukemia Mortality*

Extra risk estimates for Hodgkin lymphoma and leukemia mortality were calculated using the same general methodology described above for the NPC mortality estimates (see Section 5.2.2.2), with the following exceptions. U.S. age-specific 2006 all-cause mortality rates (NCHS, 2009) and NCHS age-specific 2002–2006 background mortality rates for Hodgkin lymphoma and leukemia (http://seer.cancer.gov/csr/1975_2006/) for all race and gender groups combined were used in the life-table programs. In addition, a 2-year lag period was used instead of a 15-year lag period.

The resulting point estimates and one-sided 95% UCLs for the extra risk of Hodgkin lymphoma mortality associated with varying levels of continuous exposure to formaldehyde are presented in Table 5-14. The results for leukemia are shown in Table 5-15. In both cases, the models predict extra risk estimates that are fairly linear for exposures below about 0.01–0.1 ppm but not for exposures above 0.1 ppm.

As discussed in Section 5.2.2.2 above, 1% extra risk levels are typically used as the basis for the POD for low-dose extrapolation from epidemiologic data. As for NPC, however, Hodgkin lymphoma has a very low background mortality rate (e.g., lifetime background risk is about 0.00038), and the 1% level of risk is associated with RR estimates that are substantially higher than those observed in the epidemiology study. Hence, a 1% extra risk level would be an

Table 5-14. Extra risk estimates for Hodgkin lymphoma mortality from various levels of continuous exposure to formaldehyde

Exposure concentration (ppm)	Extra risk	95% UCL on extra risk
0.0001	2.04×10^{-7}	3.53×10^{-7}
0.001	2.05×10^{-6}	3.55×10^{-6}
0.01	2.10×10^{-5}	3.71×10^{-5}
0.1	2.79×10^{-4}	6.17×10^{-4}
1	1.63×10^{-1}	8.36×10^{-1}
10	9.89×10^{-1}	9.90×10^{-1}

Table 5-15. Extra risk estimates for leukemia mortality from various levels of continuous exposure to formaldehyde

Exposure concentration (ppm)	Extra risk	95% UCL on extra risk
0.0001	1.64×10^{-6}	3.02×10^{-6}
0.001	1.64×10^{-5}	3.03×10^{-5}
0.01	1.66×10^{-4}	3.10×10^{-4}
0.1	1.87×10^{-3}	3.90×10^{-3}
1	8.07×10^{-2}	5.19×10^{-1}
10	9.80×10^{-1}	9.89×10^{-1}

upward extrapolation. Based on the life-table program, the RR estimate associated with an extra risk of 1% for Hodgkin lymphoma mortality is 27. Even 0.1% yields an RR estimate at the higher end of what was observed in the epidemiology study (RR = 3.6) (note that our primary analyses include the nonexposed workers, and thus the 0-exposure group becomes the referent group and the RR estimates presented for Hodgkin lymphoma and cumulative exposure in Table 5-12 would be adjusted upward [about 2.4-fold] relative to the 0-exposure group). A 0.05% extra risk level yields an RR estimate of 2.3, which better corresponds to the RRs at the lower end of the observable range. Thus, 0.05% extra risk was selected for determination of the POD for Hodgkin lymphoma, and, consistent with EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the LEC value corresponding to that risk level was used as the POD.

RR estimate (2.5) that would be above the highest categorical result reported, even after adjusting the RR estimates upward relative to the 0-exposure group (see above paragraph). A 0.5% extra risk level yields an RR estimate of 1.8, which better corresponds to the RRs in the range of the data. Thus, the LEC value corresponding to 0.5% extra risk was selected for the POD for leukemia.

Because formaldehyde is a mutagenic carcinogen and the weight of evidence supports the conclusion that formaldehyde carcinogenicity can be attributed, at least in part, to a mutagenic MOA (see Section 4.5), a linear low-dose extrapolation was performed, also in accordance with EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). The EC_{0005}, LEC_{0005}, and inhalation unit risk estimates for Hodgkin lymphoma mortality are presented in Table 5-16, and the EC_{005}, LEC_{005}, and inhalation unit risk estimates for leukemia mortality are presented in Table 5-17.

Table 5-16. EC_{0005}, LEC_{0005}, and inhalation unit risk estimates for Hodgkin lymphoma mortality from formaldehyde exposure based on Beane Freeman et al. (2009) log-linear trend analyses for cumulative exposure

Person-years	EC_{0005} (ppm)	LEC_{0005} (ppm)	Unit risk[a] (ppm^{-1})
All	0.151	0.0875	5.7×10^{-3}
Exposed only	0.155	0.0881	5.7×10^{-3}

[a]Unit risk = $0.0005/LEC_{0005}$.

Table 5-17. EC_{005}, LEC_{005}, and inhalation unit risk estimates for leukemia mortality from formaldehyde exposure based on Beane Freeman et al. (2009) log-linear trend analyses for cumulative exposure

Person-years	EC_{005} (ppm)	LEC_{005} (ppm)	Unit risk[a] (ppm^{-1})
All	0.224	0.121	4.1×10^{-2}
Exposed only	0.246	0.126	4.0×10^{-2}

[a]Unit risk = $0.005/LEC_{005}$.

5.2.3.3. *Prediction of Lifetime Extra Risks for Hodgkin Lymphoma and Leukemia Incidence*

As for NPC, both Hodgkin lymphoma and leukemia have substantial survival rates (84.7% at 5 years for Hodgkin lymphoma [http://seer.cancer.gov/statfacts/html/hodg.html] and 53.1% at 5 years for leukemia [http://seer.cancer.gov/statfacts/html/leuks.html], based on 1999−2005 SEER data); thus, it is preferable to derive incidence estimates. Unit risk estimates for Hodgkin lymphoma and for leukemia incidence were calculated as described above for the NPC incidence estimates (see Section 5.2.2.3). Age-specific background incidence rates for 2002−2006 for Hodgkin lymphoma and for leukemia from SEER17, a registry covering about 26% of the U.S. population, were obtained from the SEER Web site (http://seer.cancer.gov/csr/1975_2006/). The incidence-based calculation relies on the assumptions that Hodgkin lymphoma (and leukemia) incidence and mortality have the same exposure-response relationship for formaldehyde exposure and that the incidence data are for first occurrences of Hodgkin lymphoma (and leukemia) or that relapses provide a negligible contribution. The first assumption is more uncertain for leukemia because it is a grouping of subtypes with different survival rates (see Section 5.2.3.4 for further discussion). The calculation also relies on the fact that Hodgkin lymphoma (and leukemia) incidence rates are small compared with the all-cause mortality rates. The resulting EC_{0005}, LEC_{0005}, and inhalation unit risk estimates for Hodgkin lymphoma incidence are presented in Table 5-18, and the EC_{005}, LEC_{005}, and inhalation unit risk estimates for leukemia incidence are presented in Table 5-19. The unit risk estimate for Hodgkin lymphoma incidence is about threefold higher than the corresponding mortality-based estimate, for all person-years. This sizeable discrepancy can be attributed to the high survival rates for Hodgkin lymphoma. For leukemia, the incidence unit risk estimate is about 40% higher than the mortality-based estimate. This difference is lower than the twofold

Table 5-18. EC_{0005}, LEC_{0005}, and inhalation unit risk estimates for Hodgkin lymphoma incidence from formaldehyde exposure, based on Beane Freeman et al. (2009) log-linear trend analyses for cumulative exposure

Person-years	EC_{0005} (ppm)	LEC_{0005} (ppm)	Unit risk[a] (ppm^{-1})
All	0.0515	0.0298	1.7×10^{-2}
Exposed only	0.0529	0.0301	1.7×10^{-2}

[a]Unit risk = $0.0005/LEC_{0005}$.

Table 5-19. EC_{005}, LEC_{005}, and inhalation unit risk estimates for leukemia incidence from formaldehyde exposure based on Beane Freeman et al. (2009) log-linear trend analyses for cumulative exposure

Person-years	EC_{005} (ppm)	LEC_{005} (ppm)	Unit risk[a] (ppm^{-1})
All	0.162	0.0875	5.7×10^{-2}
Exposed only	0.178	0.0909	5.5×10^{-2}

[a]Unit risk = $0.005/LEC_{005}$.

difference seen with NPC estimates, despite comparable survival rates, probably because of different age distributions of the mortality and incidence rates.

The preferred estimate for the inhalation cancer unit risk for Hodgkin lymphoma is the estimate of 1.7×10^{-2} per ppm derived using incidence rates for the cause-specific background rates, for all person-years. Similarly, the preferred estimate for leukemia is the estimate of 5.7×10^{-2} per ppm derived using incidence rates, for all person-years. In both cases, the results from the exposed person-years only are essentially identical.

Because Hodgkin lymphoma is a rare cancer, with a relatively low number of cases occurring per year in the United States (according to SEER statistics, an estimated 8,510 people were diagnosed with Hodgkin lymphoma in the United States in 2009 [http://seer.cancer.gov/statfacts/html/hodg.html]), a rough calculation was done to assure that the unit risk estimate derived for Hodgkin lymphoma incidence is not implausible in comparison to actual case numbers. For example, assuming an average constant lifetime formaldehyde exposure level of 5 ppb for the U.S. population, the inhalation unit risk estimate for Hodgkin lymphoma equates to a lifetime extra risk estimate of 8.5×10^{-5}. Assuming an average lifetime

of 75 years (this is not EPA's default average lifetime of 70 years but rather a value more representative of actual demographic data) and a U.S. population of 300,000,000, this lifetime extra risk estimate suggests a crude upper-bound estimate of 340 incident cases of Hodgkin lymphoma attributable to formaldehyde exposure per year. Alternatively, assuming an average constant lifetime formaldehyde exposure level of 20 ppb, the calculation suggests a crude upper-bound estimate of 1,360 incident cases of Hodgkin lymphoma per year. Both upper bound estimates, using different assumed lifetime exposure levels, are well below the estimated 8,510 total incident Hodgkin lymphoma cases diagnosed per year in the United States.[12]

5.2.3.4. *Sources of Uncertainty*

By and large, the sources of uncertainty discussed above (see Section 5.2.2.4) for the NPC risk estimates, such as high-to-low dose extrapolation, retrospective exposure estimation, exposure metric/model uncertainties, and application of data from a "healthy" worker cohort to the more diverse general population also apply to the Hodgkin lymphoma and leukemia risk estimates. The Hodgkin lymphoma risk estimates are based on 27 deaths, which is more than were available for the NPC risk estimates, but 27 is still a small number for exposure-response modeling. The leukemia risk estimates are based on 123 deaths, so there is less uncertainty with the parameter estimation from the exposure-response modeling for that cancer type, although uncertainties still exist about the general model form. A 2-year lag interval was used for lymphohematopoietic cancers versus the 15-year lag for NPC. Beane Freeman et al. (2009) evaluated lag intervals between 2 and 25 years and reported that lag intervals of about 18 years provided the best fit to the lymphohematopoietic cancer data but did not change the risk estimates; thus, they retained the 2-year lag interval that was used in the previous follow-up (Hauptmann et al., 2003). The most appropriate lag intervals for Hodgkin lymphoma and leukemia are unknown, but alternate lags are unlikely to have a large impact on the results.

[12] With the application of age-dependent adjustment factors (see Section 5.4.4), the lifetime unit risk estimate for Hodgkin lymphoma would increase by a factor of 1.66, and the crude upper-bound estimates of the incident cases per year attributable to formaldehyde exposure would similarly increase by a factor of 1.66. The resulting adjusted estimates of 564 and 2,260 for 5 ppb and 20 ppb exposure levels, respectively, are still well below the estimated total number of 8,500 incident cases per year in the United States.. Similar calculations for leukemia yield even lower relative upper-bound estimates of cases attributable to formaldehyde exposure, in comparison to estimated total incident cases, because, although the unit risk estimate for leukemia is about 3.3 times the unit risk estimate for Hodgkin lymphoma, the total estimated number of incident leukemia cases in the United States. is 5.3 times the estimate for Hodgkin lymphoma (an estimated 44,790 cases diagnosed in the U.S. for 2009, according to SEER [http://seer.cancer.gov/statfacts/html/leuks.html]). For leukemia, crude upper-bound ADAF-adjusted estimates of the incident cases per year attributable to formaldehyde exposure levels of 5 ppb and 20 ppb are 1900 and 7,580, respectively, which are well below the estimated total number of 44,790 incident cases per year in the United States.

The same potential confounding or modifying factors that were investigated for NPC and the other solid cancers, as discussed in Section 5.2.2.4 above, were evaluated for the lymphohematopoietic cancers. Beane Freeman et al. (2009) reported that controlling for duration of exposure to the 11 other substances that they considered (see Section 5.2.2.4) or for working as a chemist or laboratory technician "did not meaningfully change results"; results were not shown. The investigators also reported that excluding the 586 individuals with exposure to benzene, a known leukemogen, did not change the RR estimates for myeloid or lymphoid leukemia in the highest peak exposure category. Furthermore, Beane Freeman et al. (2009) found no evidence of heterogeneity of RR estimates for lymphohematopoietic cancers by race, sex, or pay category, and adjusting for plant reportedly did not substantively change results.

A further uncertainty is which lymphohematopoietic cancer types are linked to formaldehyde exposure. As discussed in Section 4.5.2, lymphohematopoietic cancers are a diverse group of cancers with different etiologies, and the epidemiologic database suggests associations with multiple different subtypes of these cancers. Section 4.5 concludes that formaldehyde is causally associated with all lymphohematopoietic cancers as a group and with leukemias as a group (with the strongest evidence for myeloid leukemia). However, at present, exactly which subtypes are etiologically linked to formaldehyde exposure is unknown. Cancer risk estimates were derived for Hodgkin lymphoma and leukemia because, in addition to support for an association between these lymphohematopoietic cancer subtypes and formaldehyde exposure with other exposure metrics and from other studies, these had the strongest associations with cumulative exposure in the Beane Freeman et al. (2009) update of the large, high-quality NCI study. However, it is unknown whether these two subtypes best represent the total lymphohematopoietic cancer risk.

In addition, leukemia itself is a grouping of diverse (e.g., acute lymphocytic, chronic lymphocytic, acute myeloid, chronic myeloid) subtypes, and using this grouping injects additional uncertainty into the derivation of cancer incidence estimates. One of the assumptions that the incidence-based calculation relies on is that the cancer incidence and mortality have the same exposure-response relationship for formaldehyde exposure. This assumption may be problematic for the leukemia incidence estimates if not all of the leukemia subtypes represented in the grouping are associated with formaldehyde exposure to the same extent. This is because different leukemia subtypes have different survival rates, so if a subtype with a relatively high survival rate is included in the background incidence rates while not actually being associated with formaldehyde exposure or being associated to a lesser extent than other subtypes, then the incidence risk will be overestimated. The mortality risk calculations are not similarly affected

by including subtypes that may not actually be associated with formaldehyde exposure because background mortality for the subtypes is already taken into account in the regression coefficient. Figure 5-10 shows the mortality versus incidence rates for all leukemia and the two main subtypes, myeloid leukemia and lymphoid leukemia. This figure does not show the acute versus chronic myeloid and leukemia subtypes or the monocytic or other leukemia subtypes; however, it serves to illustrate the impact of using rates for groupings that contain subtypes with different survival rates. For example, if lymphoid leukemia is the predominant subtype associated with formaldehyde exposure, then using the leukemia grouping for the incidence rates may underestimate the cancer incidence risk because the incidence rates for leukemia (relative to the mortality rates) are diluted with inclusion of the incidence rates for myeloid leukemia, which has a smaller incidence-to-mortality ratio (i.e., poorer survival). On the other hand, if myeloid leukemia is the predominant subtype associated with formaldehyde exposure, then using the leukemia grouping for the incidence rates may overestimate cancer incidence risk. If incidence risks are being overestimated, the effect should be minimal because the incidence risk estimates for leukemia calculated in Section 5.2.3.3 are not that much greater (about 40%) than the mortality-only estimates.

Finally, as for the NPC risk estimates, when the slope estimates for the exposed person-years only were used for the Hodgkin lymphoma and leukemia risk calculations, unit risk estimates similar to those calculated from the slope estimates for all person-years were obtained (see Tables 5-16 to 5-19); thus, the impacts of including the unexposed person-years are minimal.

As discussed in Section 5.2.2.4, despite inevitable uncertainties, it is important not to lose sight of the strengths of the NCI study. In addition to the use of internal analyses and extensive exposure assessment and consideration of potential confounding or modifying variables, the NCI study has a large cohort that has been followed for a long time. With the additional follow-up through 2004, reflected in the lymphohematopoietic cancer results of Beane Freeman et al. (2009), the median duration of follow-up was 42 years, and the 25,619 cohort members had accrued 998,106 person-years of follow-up. Over half of the cohort was deceased, and there was a substantial number of lymphohematopoietic deaths (319 total; 286 in the exposed workers).

In summary, the inhalation cancer incidence unit risk estimates of 1.7×10^{-2} per ppm for Hodgkin lymphoma and 5.7×10^{-2} per ppm for leukemia are based on human data from a high-quality epidemiologic study with individual exposure estimates for each worker. The major source of uncertainty in both risk estimates is the extrapolation to environmental exposures.

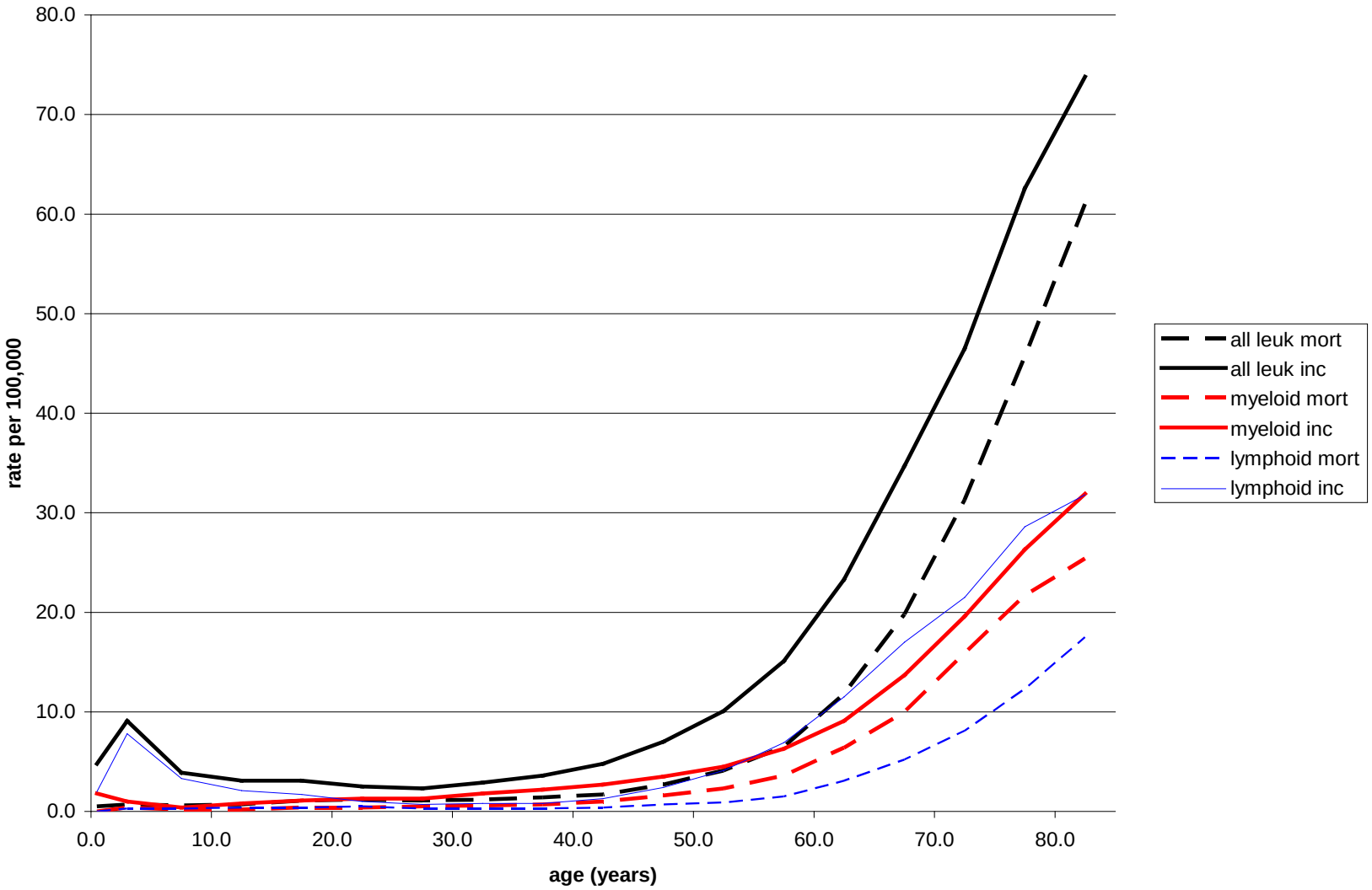


Figure 5-10. Age-specific mortality and incidence rates for myeloid, lymphoid, and all leukemia.

5.2.4. Conclusions on Cancer Unit Risk Estimates Based on Human Data

In this assessment, a (plausible upper bound) lifetime extra cancer unit risk of 5.4×10^{-3} per ppm of continuous formaldehyde exposure was estimated using a life-table program and linear low-dose extrapolation of the excess NPC mortality and log-linear modeling results (for cumulative exposure) reported in a high-quality occupational epidemiologic study (based on nine NPC deaths). Applying the same regression coefficient and life-table program to background NPC incidence rates yielded a lifetime extra cancer unit risk estimate of 1.1×10^{-2} per ppm (8.8×10^{-6} per μg/m^3).

Using similar methods and data for Hodgkin lymphoma (27 deaths) and leukemia (123 deaths) mortality based on the cumulative exposure metric, from a further follow-up of the same cohort study, (plausible upper bound) lifetime extra cancer risk estimates of 1.7×10^{-2} per ppm (1.4×10^{-5} per μg/m^3) and 5.7×10^{-2} per ppm (4.6×10^{-5} per μg/m^3) for Hodgkin lymphoma incidence and leukemia incidence, respectively, were derived.

To estimate the total cancer risk from formaldehyde exposure, risk estimates for these three cancer types (NPC, Hodgkin lymphoma, and leukemia) were combined, although, as discussed above, these three cancer types may not fully reflect the total cancer risk for all cancers thought to be causally associated with formaldehyde exposure. For an approximate estimate of the combined (upper bound) risk, risk estimates were combined assuming a normal distribution. For comparability, risk estimates for formaldehyde were combined at a common level of 0.1 ppm. This level was selected because it is close to the PODs (LEC_{005}s) used above for leukemia mortality (0.121 ppm) and leukemia incidence (0.0875 ppm), and leukemia is the predominant cancer type in terms of extra risk. Note that unit risk estimates for the different cancer types calculated at 0.1 ppm will differ slightly from those reported above (see Sections 5.2.2 and 5.2.3) because they are calculated at a level other than the PODs used in the above calculations. To derive the combined risk, maximum likelihood estimates (MLEs) of risk and their 95% upper bounds (UCLs) were calculated for each cancer type using the same methods and life-table programs employed in sections 5.2.2 and 5.2.3. The standard errors (SEs) were then estimated from the risk estimates using the equation: UCL = MLE + 1.645 × SE. The variances can then be calculated from the SEs according to the equation: Variance = SE^2. The sum of the variances then provides an estimate of the variance for the sum of the MLEs, and the 95% upper bound on the sum of the MLEs can be estimated by applying the above equations in reverse. Tables 5-20 and 5-21 provide a summary of the results of these calculations for the combined cancer mortality and incidence risks, respectively.

Table 5-20. Calculation of combined cancer mortality unit risk estimate at 0.1 ppm

Cancer type	MLE of risk	95% upper bound on risk	SE	Variance
NPC	2.63×10^{-4}	5.75×10^{-4}	1.90×10^{-4}	3.60×10^{-8}
Hodgkin lymphoma	2.79×10^{-4}	6.17×10^{-4}	2.05×10^{-4}	4.22×10^{-8}
Leukemia	1.87×10^{-3}	3.90×10^{-3}	1.23×10^{-3}	1.52×10^{-6}
Sum	2.41×10^{-3}	5.09×10^{-3}		1.60×10^{-6}
Combined risk		4.49×10^{-3}	1.27×10^{-3}	
Combined unit risk[a] (per ppm)		4.49×10^{-2}		

[a]Unit risk = 95% upper bound on combined risk/0.1 ppm.

Table 5-21. Calculation of combined cancer incidence unit risk estimate at 0.1 ppm

Cancer type	MLE of risk	95% upper bound on risk	SE	Variance
NPC	7.56×10^{-4}	1.62×10^{-3}	5.25×10^{-4}	2.76×10^{-7}
Hodgkin lymphoma	1.10×10^{-3}	2.35×10^{-3}	7.60×10^{-4}	5.77×10^{-7}
Leukemia	2.84×10^{-3}	5.89×10^{-3}	1.85×10^{-3}	3.44×10^{-6}
Sum	4.70×10^{-3}	9.86×10^{-3}		4.29×10^{-6}
Combined risk		8.10×10^{-3}	2.07×10^{-3}	
Combined unit risk[a] (per ppm)		8.10×10^{-2}		

[a]Unit risk = 95% upper bound on combined risk/0.1 ppm.

As can be seen from the results in Table 5-20, the upper bound risk estimates for cancer mortality for the individual cancer types at 0.1 ppm are within 10% of the values that would be obtained from the unit risk estimates derived in sections 5.2.2 and 5.2.3 (see Tables 5-10, 5-16, and 5-17). Furthermore, the combined unit risk estimate for mortality for the three cancer types (4.5×10^{-2} per ppm) is appropriately bounded by the mortality unit risk estimate for leukemia (4.1×10^{-2} per ppm), which has the highest individual mortality unit risk estimate, and by the sum (5.2×10^{-2} per ppm) of the individual unit risk estimates presented in sections 5.2.2 and

5.2.3. Similarly, the combined risk calculated at 0.1 ppm is necessarily bounded by the sum of the MLEs and the sum of the 95% upper bounds for the individual risks calculated at 0.1 ppm. Thus, the value of 4.5×10^{-2} per ppm (3.7×10^{-5} per µg/m^3) calculated at 0.1 ppm for the combined unit risk is a reasonable estimate for the total cancer mortality unit risk (based on the three cancer types considered).

As can be seen from the results in Table 5-21, the upper bound risk estimates for cancer incidence for the individual cancer types at 0.1 ppm are within 33% of the values that would be obtained from the unit risk estimates derived in sections 5.2.2 and 5.2.3 (see Tables 5-11, 5-18, and 5-19). Furthermore, the combined (incidence) unit risk estimate for the three cancer types (8.1×10^{-2} per ppm) is appropriately bounded by the unit risk estimate for leukemia (5.7×10^{-2} per ppm), which has the highest individual unit risk estimate, and by the sum (8.6×10^{-2} per ppm) of the individual unit risk estimates presented in sections 5.2.2 and 5.2.3. Similarly, the combined risk calculated at 0.1 ppm is necessarily bounded by the sum of the MLEs and the sum of the 95% upper bounds for the individual risks calculated at 0.1 ppm. Thus, the value of 8.1×10^{-2} per ppm (6.6×10^{-5} per µg/m^3) calculated at 0.1 ppm for the combined unit risk is a reasonable estimate for the total cancer unit risk (based on the three cancer types considered).

As documented in Section 4.5, formaldehyde is a mutagenic carcinogen and the weight of evidence supports the conclusion that formaldehyde carcinogenicity can be attributed, at least in part, to a mutagenic MOA. Therefore, since there are no chemical-specific data to evaluate susceptibility of different life stages, increased early-life susceptibility should be assumed, and, if there is early-life exposure, the age-dependent adjustment factors (ADAFs) should be applied in accordance with EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b). See Section 5.4.4 below for more details on the application of the ADAFs.

5.3. DOSE-RESPONSE MODELING OF RISK OF SQUAMOUS CELL CARCINOMA IN THE RESPIRATORY TRACT USING ANIMAL DATA

In the previous section, dose-response analyses based on human data for lymphohematopoietic cancer and NPC were presented. The dose-response analyses of cancer risk presented in this section are based on nasal tumor data from laboratory bioassays using F344 rats. Because the analyses involved are extensive, most of the details are provided in the appendices.

An increased incidence of nasal squamous cell carcinoma (SCC) was seen in two long-term bioassays using F344 rats (Monticello et al., 1996; Kerns et al., 1983). Although other

studies in laboratory animals exist, these two studies, when combined, provide the most robust data for analyses. These inhalation data on nasal SCC tumor incidence were used to estimate human respiratory cancer risk in the nose and were also extrapolated to the entire respiratory tract; in other words, a site concordance between rat and human is not assumed. This is reasonable because the respiratory and transitional epithelial cell types considered to be at risk of SCC in the upper respiratory tract are also prevalent in the lower human respiratory tract, and there is greater penetration of formaldehyde flux posteriorly in the nose and in the rest of the human respiratory tract relative to that of the rat. These considerations are strengthened by the findings of DNA-protein cross-links (DPXs) in the proximal portions of the rhesus monkey lower respiratory tract (Casanova et al., 1991). In addition, some epidemiologic studies (Gardner et al., 1993; Blair et al., 1990, 1986) reported an increase in lung cancer associated with formaldehyde exposure, while others (Collins et al., 1997; Stayner et al., 1988) reported no such increases.

EPA's cancer guidelines (U.S. EPA, 2005a) suggest using a BBDR model for extrapolation when data permit. A BBDR model for formaldehyde was developed by scientists at the CIIT Centers for Health Research (see Appendix D) (Conolly et al., 2004, 2003, 2000; Kimbell et al., 2001a, b; Overton et al., 2001; CIIT, 1999), which interfaced several models to combine the extensive mechanistic information available in studies involving the F344 rat and rhesus monkey and time-to-tumor incidence data in long-term bioassays, as shown by the schematic in Figure 5-11. This mechanistic information included formaldehyde and DPX dosimetry in the F344 rat, rhesus monkey, and human airways and cell proliferation data in the F344 rat nasal lining. This document presents extensive evaluation of the underlying models and data and of the alternative parametrizations of the models that were also explored for the purpose of the current assessment (see Appendix E, Appendix F). A summary of conclusions is presented in Section 5.3.3. In particular, the following conclusions by EPA were critical in determining how the models could be used to inform the quantitative dose-response assessment:

- When used to model the dose-response in the range of the available data, the BBDR models were judged to have the advantage of being more accurate and biologically based (than purely statistical descriptions such as the multistage-weibull model) and allowing utilization of various data in an integrated manner.
- Variations to modeling the F344 rat tumor incidence data in Conolly et al. (2003) were examined. Given the data, each of these models, including the modeling in Conolly et al. (2003), was judged to be just as biologically plausible as the other. Each of the models described the rat tumor incidence equally well, was based on different characterizations

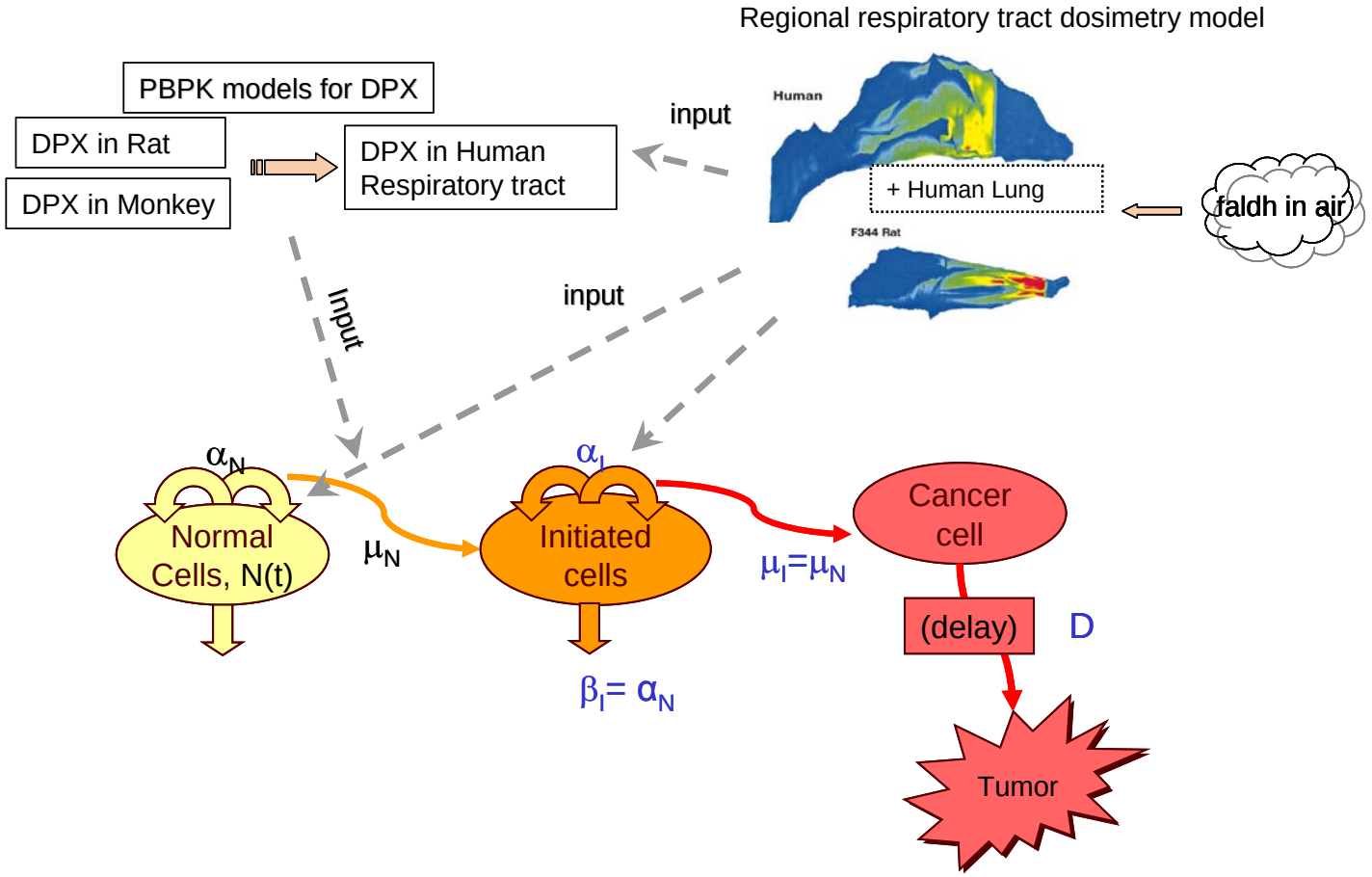


Figure 5-11. Schematic of integration of pharmacokinetic and pharmacodynamic components in the CIIT model.

Note: β = death rate; μ = mutation rate per cell division; α_N, N(t), μ_N are informed (partially or fully) by empirical data; other parameters are estimated by fitting to tumor incidence data.

of the same empirical cell kinetic data, and was based on the same empirical data on DPX measurements. However, the added human risk over baseline levels estimated by these models (including the original model) were extremely different, and ranged from negative to large positive values at environmental exposure concentrations.

- When used for the purpose of extrapolating risk, the BBDR models did not appear to reasonably constrain either risk estimates extrapolated to human exposures or risk estimates for the F344 rat when they were extrapolated below the range of observable data.
- Human respiratory cancer risk calculated in Conolly et al. (2004) was numerically unstable. Therefore, clonal growth modeling was not found to be a useful approach for human extrapolation of rodent risk estimates.
- Thus, the biologically based derivation of human risk estimates in Conolly et al. (2004) cannot be characterized as a plausible upper bound in the face of model uncertainties (a key conclusion of those authors).

For all these reasons, the BBDR modeling of the rat data

- was employed in this assessment to derive multiple PODs (for SCC in the respiratory tract) in the range of the observed data, using model-derived internal dose estimates,
- but was not used to extrapolate far below the observed data.

The inhalation unit risk estimates of SCC in the human respiratory tract were derived by using multiple methods to model the F344 tumor incidence data as follows:

1. conventional multistage Weibull time-to-tumor modeling
2. variations of the model for rat tumor incidence implemented in Conolly et al. (2003) that were considered in the process of the evaluation.

PODs were calculated as exposure concentrations corresponding to the 95% statistical upper bound extra risks of 0.005, 0.01, and 0.05 (0.005 used only with BBDR modeling). The inhalation unit risk for SCC in the human respiratory tract (upper and lower) derived from the above animal bioassay data was then calculated by linear extrapolation to the origin from the POD. Linear extrapolation is supported in part by the proven genotoxicity of the chemical and the observation of cytogenetic effects in human occupational exposures (see chapter 4). In particular, the formation of DPXs on formaldehyde interaction with DNA has been observed at doses well below those considered cytotoxic (see Section 5.3.1.2). In results obtained in some implementations of the biologically based models, formaldehyde-induced mutagenicity (modeled

as proportional to DPX concentration) was found to be a critical determinant of its tumorigenicity, both at the low dose pertaining to human exposure concentrations as well as in the dose range in which formaldehyde is considered to be cytotoxic.

The human equivalent concentration was calculated by assuming that continuous lifetime exposure to a given steady-state flux of formaldehyde (expressed in pmol/mm^2-hour) leads to equivalent risk of nasal cancer across species. Risk per respiratory or transitional epithelial cell with replicative potential was computed as a function of formaldehyde flux in the nasal region and extrapolated to the rest of the respiratory tract.

5.3.1. Long-Term Bioassays in Laboratory Animals

This section briefly describes the various animal data and dosimetry information utilized in the above (but not in all) models, based on which estimates for the inhalation unit risk are derived later in this chapter.

5.3.1.1. *Nasal Tumor Incidence Data*

Various bioassays have reported the effects of formaldehyde on rats, mice, and rhesus monkeys and have been discussed at length earlier in this document. Two of these bioassays (Monticello et al., 1996; Kerns et al., 1983), when combined, allow for the most robust characterization of the long-term dose response in a laboratory species; therefore, the focus here is on these bioassays, combined. These long-term bioassays found an increased incidence of nasal SCCs in rats exposed to formaldehyde by the inhalation route. In these combined data, rats were exposed to 0, 0.7, 2.0, 6.01, 9.93, and 14.96 ppm (0, 0.86, 2.5, 7.4, 12.2, and 18.4 mg/m^3) exposure concentrations of formaldehyde. SCCs were observed only at 6.01 ppm and higher exposure concentrations. Table 5-22 provides a summary of the tumors from these bioassays, and the time-to-tumor characteristics are as shown by the data in Figure 5-12 (in Section 5.3.3). Other tumor bioassays were also conducted by various researchers and have been detailed in chapter 4.

Table 5-22. Summary of tumor incidence in long-term bioassays on F344 rats

Formaldehyde exposure, ppm	Number of animals	Number with SCC	Percent with SCC
0.0	341	0	0
0.7	107	0	0
2	353	0	0
6.01	343	3	0.87
9.93	103	22	21.4
14.96	386	162	42.0

Sources: Combined data from Monticello et al. (1996) and Kerns et al. (1983).

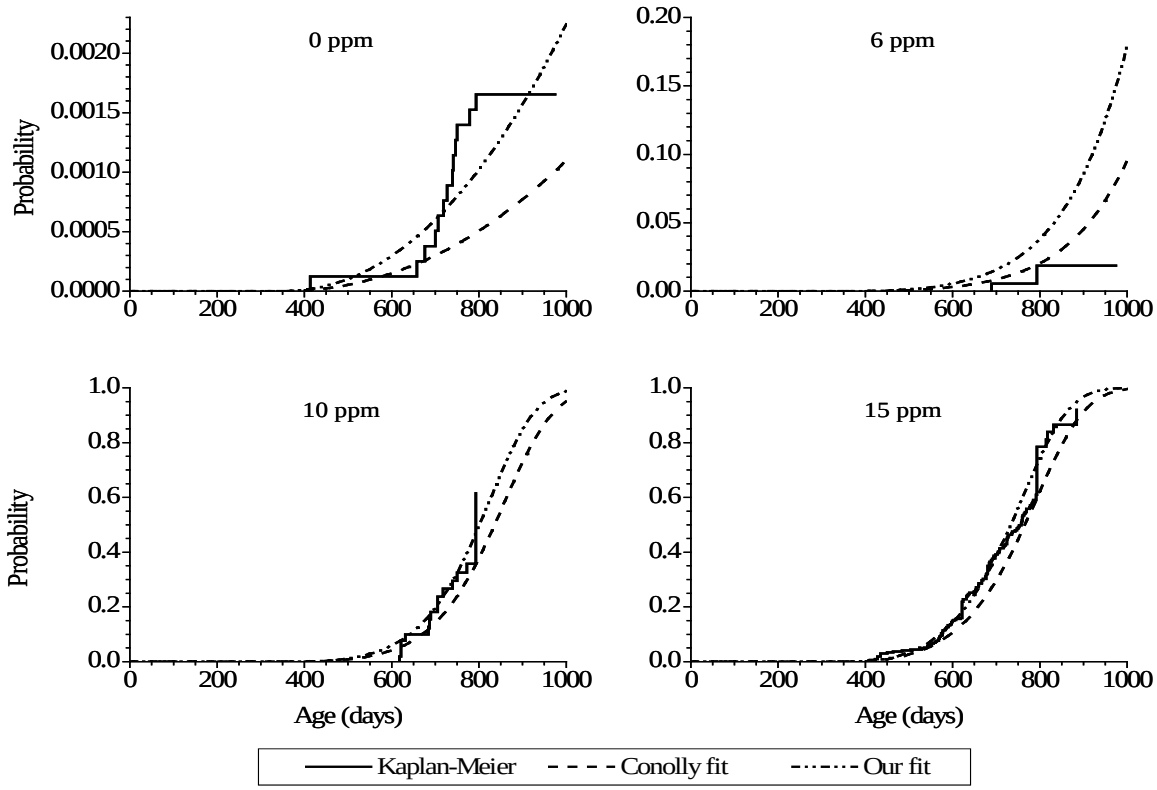


Figure 5-12. Fit to the rat tumor incidence data using the model and assumptions in Conolly et al. (2003).

Note: Fitting was performed on data of Kerns et al. (1983) and Monticello et al. (1996) combined with ALL NTP historical controls under the assumption that all SCCs are fatal. Figure compares the fit obtained by Conolly et al. (2003) with the reproduction of these results under identical conditions, inputs, and assumptions by Subramaniam et al. (2007). There were minor residual differences among the implementations; see the appendix in Subramaniam et al. (2007) for explanation.

Source: Subramaniam et al. (2007). Reprint permission required.

5.3.1.2. *Mechanistic Data*

The Kerns et al. (1983) and Monticello et al. (1996) tumor studies were accompanied or followed by additional studies that provided extensive mechanistic information on both pharmacokinetics and pharmacodynamics. These studies have been summarized elsewhere in this document and in other reviews (CIIT, 1999; Monticello and Morgan, 1997; Morgan, 1997; Heck et al., 1990). In addition to the tumor incidence data, the following data and mechanistic information (some of which were model derived) are used in the quantitative models utilized in

this chapter. Additional data for the rhesus monkey are also available that inform the hazard assessment but which have not been explicitly used in deriving the inhalation unit risk. Rhesus monkey data have been discussed in chapter 4 and chapter 3 (DPX and formaldehyde dosimetry).

- DPX: Formaldehyde interacts with DNA to form DPXs. These cross-links are considered to induce mutagenic as well as clastogenic effects. Casanova et al. (1994, 1989) carried out two studies of DPX measurements in F344 rats. In the first study, rats were exposed to concentrations of 0.3, 0.7, 2, 6, and 10 ppm for 6 hours and DPX measurements were made over the whole respiratory mucosa of the rat, while, in the second study, the exposure was to 0.7, 2, 6, or 15 ppm formaldehyde for 3 hours and measurements were made at "high" and "low" tumor sites. DPX formation was observed at all exposure concentrations in both studies (0.3 ppm–15 ppm); the DPX levels were statistically significantly elevated at concentrations ≥2 ppm, with the trend also indicating elevated DPXs at 0.7 ppm. These data were used in the development of a PBPK model for predicting DPX levels in the nasal lining (see chapters 3 and 4).
- Cell labeling index data: Male F344 rats were exposed to formaldehyde gas over a range of concentrations (0, 0.7, 2, 6, 10, or 15 ppm) in two phases of a labeling study. The first phase (Monticello et al., 1991) employed injection labeling with a 2-hour pulse labeling time, and animals were exposed to formaldehyde for periods of 1, 4, and 9 days and 6 weeks. The second phase (Monticello et al., 1996) used osmotic minipumps for labeling with a 120-hour release time to quantify labeling in animals exposed for 13, 26, 52, and 78 weeks. These data have been analyzed at length in Appendix E.
- Airflow models: Physical and computer models of airflow in anatomically realistic representations of the F344 rat and human upper respiratory tract have been constructed (Kimbell et al., 1993, 1997a; Kepler et al., 1998; Subramaniam et al., 1998; see Chapter 3).
- Formaldehyde dosimetry: Regional uptake of formaldehyde has been calculated for the upper respiratory tract of the rat and human by using the above computer representations and for the lower respiratory tract of the human by using an idealized representation of the human lower respiratory tract (Kimbell et al., 2001a; Overton et al., 2001; also see chapter 3 and further discussion of uncertainties in Appendix F).

5.3.2. The CIIT Biologically Based Dose-Response Modeling

The studies mentioned above in 5.3.1.1 and 5.3.1.2 were generated at the CIIT Centers for Health Research and led to the development of a biologically motivated dose-response model for formaldehyde-induced cancer as represented in a series of papers and in a health assessment report (CIIT model) (Conolly et al., 2004, 2003, 2000; Conolly, 2002; Kimbell et al., 2001a, b; Overton et al., 2001; CIIT, 1999). EPA's cancer guidelines (U.S. EPA, 2005a) suggest using a BBDR model for extrapolation when data permit since it facilitates the incorporation of MOA in

risk assessment. The CIIT modeling and available data were evaluated in a series of peer-reviewed papers (Klein et al., 2010; Crump et al., 2008; Subramaniam et al., 2008, 2007) and debated further in the literature (Conolly et al., 2009; Crump et al., 2009). Alternatives to the parametrization and model structure in the CIIT biological modeling (but based on that original model) are further explored and evaluated in this assessment (Appendix E). Appendix F carries out a sensitivity analysis of the human risk estimates in Conolly et al. (2004) based on key uncertainties evaluated in Appendix E. These BBDR models are used in this assessment to calculate PODs from the dose-response curve for the F344 rat nasal tumor risk; extrapolation to human is then carried out by using EPA's baseline ("default") approach (U.S. EPA, 1994) but using model-derived internal dose metrics for rat and human. See Section 5.3.3 for rationale supporting these decisions.

First, the key features of the BBDR modeling in Conolly et al. (2003, 2004) are briefly described, and the following notation is used throughout this section: N cell = normal cell; I cell = initiated cell; LI = labeling index and is equal to the number of labeled cells/(number labeled + unlabeled cells); ULLI = unit length LI equal to the number of labeled cells/length of basement membrane; α_N = division rate of normal cells ($hour^{-1}$); μ_N = rate at which an initiated cell is formed by mutation of a normal cell (per cell division of normal cells).

In Conolly et al. (2003), tumor incidence data in the Kerns et al. (1983) and Monticello et al. (1996) long-term bioassays were modeled by using an approximation of the two-stage clonal growth model (Moolgavkar et al., 1988) and allowing formaldehyde to have a direct mutagenic action. Conolly et al. (2003) combined these data with historical control data on 7,684 animals obtained from National Toxicology Program (NTP) bioassays. These models are based on the Moolgavkar, Venzon, and Knudson (MVK) stochastic two-stage model of cancer (Moolgavkar et al., 1988; Moolgavkar and Knudson, 1981; Moolgavkar and Venzon, 1979), which accounts for growth of a pool of normal cells, mutation of normal cells to initiated cells, clonal expansion and death of initiated cells, and mutation of initiated cells to fully malignant cells.

The MVK model for formaldehyde accounted for two MOAs as follows that may be relevant to formaldehyde carcinogenicity:

1. An indirect MOA in which the regenerative cell proliferation in response to formaldehyde cytotoxicity increases the probability of errors in DNA replication. This MOA was modeled by using labeling data on normal cells in nasal mucosa of rats exposed to formaldehyde.
2. A possible direct mutagenic MOA, based on information indicating that formaldehyde is mutagenic (Speit and Merk, 2002; Heck et al., 1990; Grafström et al., 1985), was modeled by using rat data on formaldehyde production of DPXs (Monticello et al., 1996,

1991). In Conolly et al. (2003), the intracellular dose that induces mutations is considered proportional to the local DPX dose.

The human model for formaldehyde carcinogenicity (Conolly et al., 2004) is conceptually very similar to the rat model. The model uses, as input, results from a dosimetry model for an anatomically realistic representation of the human upper airways and an idealized representation of the lower airways. However, the model does not incorporate any data on human responses to formaldehyde exposure.

A novel contribution of the CIIT model, described by the schematic in Figure 5-11, is that cell replication rates and DPX concentrations are driven by local dose, which is formaldehyde flux to each region of nasal tissue expressed as pmol/mm^2-hour. This dosimetry is predicted by computational fluid dynamics (CFD) modeling using anatomically accurate representations of the nasal passages of a single F344 rat or Caucasian male human (see chapter 3). Such a feature is important in incorporating site-specific toxicity in the case of a highly reactive gas like formaldehyde for which uptake patterns are spatially localized and significantly different across species (see chapter 3). In the CIIT model, each of these parameters is characterized by local flux (see Figure 5-11). The inputs to the two-stage cancer modeling consisted of results from other model predictions as well as empirical data as follows:

- Regional uptake of formaldehyde in the respiratory tract was predicted by using CFD modeling in the F344 rat and human (Kimbell et al., 1997a, 2001a, b; Overton et al., 2001; Subramaniam et al., 1998).
- Replication rates for normal cells were inferred from LI data on rats exposed to formaldehyde (Monticello et al., 1996, 1991, 1990).
- Concentrations of DPXs linked to the regional flux of formaldehyde were predicted by a PBPK model (Conolly et al., 2000) calibrated to fit the DPX data in F344 rat and rhesus monkey (Casanova et al., 1994, 1991) and subsequently scaled up to humans. The DPX concentration levels were incorporated into the two-stage clonal expansion model by defining mutation rate of normal and initiated cells as the same linear function of DPX. That is,

$$\mu_N = \mu_I = \mu_{Nbasal} + KMU \times DPX \qquad (5\text{-}1)$$

where μ_N is the rate at which an initiated cell is formed by mutation of a normal cell (per cell division of normal cells), and likewise μ_I is the rate at which a malignant cell is formed by mutation of an initiated cell (per cell division of initiated cells). The unknown

constants μ_{Nbasal} (the baseline rate) and KMU were estimated by fitting model predictions to the tumor bioassay data.

The rat model in Conolly et al. (2003) involved six unknown statistical parameters that were estimated by fitting the model to the rat formaldehyde bioassay data shown in Table 5-22 (Monticello et al., 1996; Kerns et al., 1983) plus data from several thousand control animals from all the rat bioassays conducted by the NTP. These NTP bioassays were conducted from 1976 through 1999 and included 7,684 animals with an incidence of 13 SCCs (i.e., 0.17% incidence). The resulting model predicts the probability of a nasal SCC in the F344 rat as a function of age and exposure to formaldehyde. The fit to the tumor incidence data is shown in Figure 5-12 (in Section 5.3.3.). (For later reference in Appendix E, this figure compares the fit to the data obtained by the modeling in Conolly et al. [2003] with that obtained by the reimplementation of this model in Subramaniam et al. [2007].)

Subsequent to the BBDR model for modeling rat nasal cancer, Conolly et al. (2004) developed a corresponding model for humans for the purpose of extrapolating the risk to humans estimated by the rat model. Also, rather than considering only nasal tumors, the model is used to predict the risk of SCC in the entire human respiratory tract. The human model for formaldehyde carcinogenicity in Conolly et al. (2004) is conceptually very similar to the rat model in Conolly et al. (2003) and follows the schematic in Figure 5-11. The following points need to be noted:

- The model does not incorporate any data on human responses to formaldehyde exposure.
- The model is based on an anatomically realistic representation of the human nasal passages (in a single individual) and an idealized representation of the lower respiratory tract. Local formaldehyde flux to respiratory tissue is estimated by a CFD model for humans (Kimbell et al., 2001a; Overton et al., 2001; Subramaniam et al., 1998).
- Rates of cell division and cell death are, with a minor modification, assumed to be the same in humans as in rats.
- The concentration of formaldehyde-induced DPXs in humans is estimated by scaling up from values obtained from experiments in the F344 rat and rhesus monkey (Conolly et al., 2000, and also discussed further in Section 3.6.6 of this document). The human value for KMU in Equation1 is obtained by assuming that the ratio KMU/μ_{basal} is invariant across species. The other statistical parameters for the human model are either: (a) estimated by fitting the model to the human background incidence of tumors, (b) assumed to have the same value as that obtained in the rat model, or, (c) in one case, fixed at a value suggested by the epidemiologic literature.

Some further clarification pertaining to the structure and calibration of the models in Conolly et al. (2004, 2003) that are key to understanding model assumptions is provided in Appendix D.

5.3.2.1. *Major Results of the CIIT Modeling Effort*

Based on the biologically based modeling of the rat SCC data, CIIT (1999) and Conolly et al. (2004, 2003) presented the following major conclusions. The evaluation of the strength of these conclusions is summarized in Section 5.3.3., and as addressed in that section, this current assessment is not in agreement with these conclusions.

- The putative, directly mutagenic action of formaldehyde "does not play a significant role in the tumor response in the rat (and also in the human), [and such a conclusion] should be robust for any potentially mutagenic effect of formaldehyde with a time course similar to that of DPX."
- Respiratory cancer risks associated with inhaled formaldehyde are de minimis (10^{-6} or less) at relevant human exposure levels. This was based on using an upper bound on the model estimate for the directly mutagenic action of formaldehyde.
- Therefore, exposure standards protective of effects of formaldehyde-induced cytotoxicity should be sufficient to protect from its potential carcinogenic effects.
- The human risk estimates in Conolly et al. (2004) were judged by the authors to be conservative in the face of model uncertainties because the model: (a) included a hockey-stick model for normal cell replication rates when the cell replication dose-response curve as averaged by the authors had a J shape, (b) used overall respiratory tract cancer incidence data in humans, and (c) evaluated the model at the statistical upper bound of the proportionality parameter relating DPXs to the probability of mutation.
- The dose-response assessment in Conolly et al. (2004) did not explicitly evaluate the risk of lymphohematopoietic cancers. However, Conolly et al. (2004) argued that formaldehyde was unlikely to cause the cancers reported in Hauptmann et al. (2003). Their reasoning was based on the steepness of the dose-response curve predicted in Conolly et al. (2004) for respiratory cancer at exposures of 1 ppm and above, and the conclusions in Heck and Casanova (2004).

5.3.3. This Assessment's Conclusions from Evaluation of Dose-Response Models of DPX, Cell-Replication and Genomics Data, and of BBDR Models for Risk Estimation

The CIIT modeling of the rat tumor incidence and mechanistic information detailed in Section 5.3.1 and alternative models that were developed based on the conceptual framework in the CIIT modeling were extensively evaluated for this assessment. These results are presented in Appendices D, E (BBDR modeling of the rat data), and F (sensitivity analysis of BBDR model

results for human risk). In particular, Table E-1 in Appendix E and Table F-1 in Appendix F tabulate all the uncertainties and assumptions that were examined along with results of that evaluation. The quantitative and qualitative characterization of the cell replication data from Monticello et al. (1996, 1991) are presented in Appendix E. The most significant conclusions resulting from these various analyses, focusing on the ones that have maximal impact on the dose-response assessment, are presented below.

Description of Time-to-Tumor Data

The overall approach and use of data in Conolly et al. (2004, 2003) have substantial advantages to offer in describing the dose response observed in animal bioassays. The authors' model provides a good statistical description of the time-to-tumor data. The fit to the data was found to be superior to that obtained by using multistage-Weibull time-to-tumor modeling of the tumor incidence data (comparison based on visual inspection [see Figure 5-12 in this section and Figures 5-17, 5-18, 5-19 in Section 5.3.4]).

Integration of Various Relevant Data

The model framework integrates various pharmacokinetic and pharmacodynamic components (regional formaldehyde flux, DPX, cell-replication, and tumor incidence data) within a single conceptual framework and thus facilitates description of the tumor dose response that utilizes the extensive mechanistic information available for formaldehyde.

Regional Dosimetry

Regional (site-specific) dosimetry in the upper respiratory tract is considered important for understanding the tumorigenicity of a reactive chemical like formaldehyde. The regional dosimetry models discussed in chapter 3 compute local formaldehyde flux to the tissue and are based on anatomically realistic constructions of the nasal airways in each species. The other relevant mechanistic data, DPX and cell replication, are expressed as a function of this local formaldehyde flux.

Confidence in Dosimetry

Model predictions of formaldehyde flux to the respiratory lining have not been verified experimentally, and such verification would present formidable experimental challenges. Overall, the formaldehyde dosimetry modeling utilized in the CIIT modeling presents a reasonable level of confidence, as detailed in chapter 3, Section 3.6, by virtue of agreement among multiple model predictions (models that predict airflow profiles as well as a PBPK model

for DPX, which uses the calculated formaldehyde flux as input) and various kinds of available data. These data comprise airflow profiles in physical casts of the nasal cavity of an F344 rat (Kimbell et al., 1997a, 2001a), a human (Subramaniam et al., 1998), and a rhesus monkey (Kepler et al., 1998); DPX data (see discussion of Cohen-Hubal et al. [1997] in chapter 3); and qualitative concordance between uptake patterns and cell proliferation (Morgan et al., 1997; Monticello et al., 1996). The CFD models of formaldehyde flux represent only an individual of each species. However, considerable interindividual differences are to be expected in the regional dosimetry, particularly in the human (Garcia et al., 2009: Subramaniam et al., 2008). This is discussed briefly in Chapter 3 (see Section 3.6) and further in Appendices B and F.

Control Tumor Data

In developing their model, Conolly et al. (2004, 2003) included control rats from all NTP cancer bioassays—a total of 7,684 rats. As elaborated in Appendix E, lumping **all** NTP historical control animals along with the control animals in the Kerns et al. (1983) and Monticello et al. (1996) inhalation bioassays does not appear to be supportable and substantially alters dose-response predictions (Crump et al., 2009, 2008; Subramaniam et al., 2008, 2007). There are legitimate questions regarding comparability of results in rats from different stocks, studied at different times, in different laboratories, and by different routes of exposure and evaluated by using somewhat different pathological procedures (Haseman, 1995; Rao et al., 1987). If historical controls are used from only those inhalation studies that present a low potential for genetic and time-related variations in tumor incidence and survival of animals or if only concurrent controls are used, the model for extrapolation of risk to humans (the human BBDR model) becomes numerically unstable. In such a model, it is not possible to bound human risk by using the extrapolation approach applied in the CIIT model. When the included NTP control data were restricted to those from NTP **inhalation** studies, the upper bound human risk estimate obtained by Conolly et al. (2004) (i.e., with everything else in their modeling retained unchanged) was increased by 50-fold (Crump et al., 2008).

Cell Replication Dose Response

As discussed in chapter 4, characterization of the uncertainties and variability in the cell replication dose response is crucial to understanding formaldehyde carcinogenicity. Analyses of the data in Monticello et al. (1991, 1996) to derive dose response curves for cell replication are presented in Appendix E and are partly published in Subramaniam et al. (2008). The raw individual animal data from this bioassay were made available to EPA. The analyses demonstrate the following:

- Sustained exposure to formaldehyde affects cell division rates (compared to baseline levels) over a continuum of formaldehyde flux to the nasal lining that includes flux levels below those thought to be cytotoxic.
- Given the qualitative and quantitative uncertainties in the data and in their interpretation, a variety of cell replication dose-response models are plausible as reasonable characterization of the data. Cell replication response differs substantially among nasal sites and over time during the course of the bioassay. In consideration of these differences, the dose response for cell replication included shapes that were monotonic increasing as well as nonmonotonic at low dose (also see Meng et al. 2010 and a discussion of their data in Appendix F). For example, rather different statistical descriptions of the data result depending on whether
    i. different sites and exposure times were modeled separately;
    ii. all exposure times were pooled to model the response at each site;
    iii. the labeling index was time-weighted and averaged over all sites;
    iv. flux and labeling index were weighted by the number of cells at a given site;
    v. the short exposure durations in Monticello et al. (1991) were examined separately. In addition, transient increases in cell turnover at subcytotoxic doses are seen in other experiments in rats exposed to formaldehyde (see chapter 4).
- At higher, cytolethal formaldehyde flux levels, regenerative hyperplasia-induced cell proliferation clearly takes over.

Genotoxicity

Chapter 4 provides multiple lines of evidence to characterize formaldehyde as a genotoxicant. Of particular note is the observation of cytogenetic effects at human occupational exposures and the formation of DPXs upon formaldehyde interaction with DNA at doses well below those considered cytotoxic. As noted earlier, DPX formation was detected in rats at exposures ranging from 0.3 ppm to 15 ppm. These DPX levels are seen to be statistically significantly increased over baseline levels at 2 ppm and above. The DPX measured at 0.7 ppm shows a trend that is consistent with an increase at this dose (see chapter 3), and it is critical to consider "trend" when analyzing low-dose data.

Inferences on MOA from Modeling the Data

The highly curvilinear nature of dose responses associated with DPX formation, LI data, and tumor response, as well as mechanistic interpretation of these observed data, have provided grounds for arguments in the literature that formaldehyde tumorigenicity (at exposures ≥6 ppm) should be uncoupled from its potential carcinogenicity in the low-dose region. Furthermore, some researchers have argued that any potential low-dose risk is due to formaldehyde's mutagenicity, that this mutagenic potential is too weak to be of significance, and that the observed risk is entirely due to cell proliferation induced by regenerative hyperplasia in response to cell injury at cytotoxic doses (i.e., without a relevant role for the direct mutagenic action of formaldehyde). Conolly et al. (2004, 2003) represented a quantitative expression of this point of view. However, alternative parametrizations of the model used in Conolly et al. (2004, 2003) have shown that the mutagenic component can be important to explaining the observed tumor incidence and that the risk at low dose due this mutagenicity can be significant (Subramaniam et al., 2007; Appendix E).

As mentioned in 5.3.3.6, analysis of the considerable uncertainty-variability in the cell labeling data indicates that, upon exposure to formaldehyde, cell replication is significantly altered over a continuum that includes low and high concentration levels. At high dose, the effect on cell replication is regenerative. At lower doses, the data indicate that both monotonic and nonmonotonic dose-response curves for cell replication are plausible. Various plausible dose-response curves for cell replication were incorporated into the alternate BBDR models evaluated in this assesment (see Appendix E) and were seen to strongly influence the low-dose response curves for risk. The following exercise was particularly instructive in illuminating the uncertainty in the shape of the dose-response curve at low dose. The BBDR models were exercised with normal cell replication rates considered to be less than (nonmonotonic) or equal to (threshold) baseline rates over a segment of the low-dose range. Such a scenario did not necessarily lead to lower than baseline or threshold in formaldehyde respiratory cancer risk in the rat in that low-dose range[13]. This is partly because there are no data to inform how formaldehyde-induced mutation might alter cell replication and apoptotic rates (in particular if the mutation is to be construed as an initiating event in the carcinogenesis).

Accordingly, the dose-response assessment in this document does not treat formaldehyde as a threshold carcinogen.

[13] all the models reproduced the chronic time-to-tumor data well

Kinetics of Initiated Cells

Modeling results are hypersensitive to the division and death rate of initiated cells that cannot be further inferred by the available empirical cell labeling data (Conolly et al., 2009; Crump et al., 2009, 2008). Several plausible alternate model structures for describing initiated cell kinetics, none of which degrade the agreement of the model with the underlying data used to construct the model originally, led to low-dose risk estimates in the rodent that varied by many orders of magnitude, including negative values (see Figures E-5A,B and E-6A,B in Appendix E).

Extremely small perturbations in the division rate (and, likewise, of death rates) of initiated cells in the model lead to human risk estimates ranging anywhere from negative values to +0.01 at 0.01 ppm (see Crump et al. 2008 and Appendix F, Figure F-5). These perturbations were small compared with the normal variation in the division rates of normal cells.

The sensitivity analyses on the basis of which these conclusions were reached have been criticized as resulting in implausible risk estimates (given the epidemiologic data) as a consequence of implementing model variations that are not biologically reasonable (Conolly et al. 2009). This criticism was rebutted by Crump et al. (2009) on biological and epidemiological grounds. These debates are discussed fully in Appendix F.

In addition, there are major qualitative uncertainties in extrapolating normal cell replication rates from the rat to human (see Table F-1 in Appendix F, and Subramaniam et al. [2008]). Subramaniam et al. (2008) examined the inferences that arise from the assumptions in the CIIT model on initiated cell replication and death rates and concluded that several inferences were not supportable on the basis of available biological information (see Appendix E, Section E.3.3.1 for a summary).

Risk Extrapolation

The modeling approach in the human formaldehyde model of Conolly et al. (2004) and the variations examined showed extreme sensitivity, including numerical instability, to uncertain model assumptions. This model, and the alternative BBDR models examined, were therefore determined not to be informative for extrapolation from animal to human at any exposure concentration. In the face of model uncertainties, the biologically based derivation of human risk estimates of 10^{-6} or less at exposures of 0.1 ppm and below in Conolly et al. (2004) or CIIT (1999) cannot be characterized as a plausible upper bound.

The use of clonal growth modeling for extrapolation of risk from high to low exposures in the rodent followed by a conventional (default) approach to extrapolate the low-dose animal risk to the low-dose human risk was next evaluated. However, as explained earlier, the models do not adequately constrain risk in the rodent. For example, various model representations as

shown in Figure E-6A,B in Appendix E were used to evaluate added MLE risk at the 10^{-5} level (see Figure F-5A,B in Appendix E) in the F344 rat. Human exposures were then calculated that would result in equivalent lifetime risk by using formaldehyde flux estimated in each species as the dosimeter and conventional extrapolation methods (U.S. EPA, 1994b). A 25-fold difference was found between the different models in the equivalent exposure concentration so derived. Model uncertainty was substantially higher than the statistical uncertainty arising out of a given model specification. Therefore, this avenue was also found not to be informative. Consequently, the CIIT model or its variations were not used in this assessment as a biologically-based or biologically-motivated means of extrapolating outside the observed dose-response in the F344 rat.

Thus, in view of all the above considerations and in accordance with EPA's cancer guidelines (U.S. EPA, 2005a), the derivation of unit risk for human respiratory cancer from animal bioassay data in this document is based on a linear extrapolation to the origin from a POD on the dose-response curve. Low-dose linearity was exhibited by the risk estimates from most of the models that were examined in the sensitivity analysis (see discussion surrounding Figure E-5A,B in Appendix E).

BBDR Modeling for Deriving an "Integrated" POD

The CIIT BBDR modeling approach provides a good fit to the time-to-tumor data and therefore allows for an appropriate determination of a POD while at the same time incorporating a large amount of mechanistic information in an integrated manner and allowing the use of model-derived internal dose estimates. Thus, use of this model provides an alternative to developing separate PODs based on several of the underlying components of the data, such as DPX, flux, and labeling data. Accordingly, the model is used in this assessment to derive a POD from a dose response, based on the nasal cancers in rats. Uncertainties in the derivation of the POD were represented by using the variations of the CIIT model examined in this chapter. These POD calculations as well as others are detailed below.

Genomics Data

The genomics data of Thomas et al. (2007) and Andersen et al. (2008) provide additional insight into formaldehyde's biological effects in the URT and the steep dose-response curve for tumorigenesis. However, as summarized in a review by Chiu et al. (2010), there are various limitations in the interpretation of these genomics data and their relevance for the pathways contributing to the disease process in humans. In particular, the data from these studies, as analyzed, do not inform the critical MOA questions pertaining to formaldehyde carcinogenicity. These insights have been elaborated in Section 4.4.5, and the difficulties in the use and interpretation of the quantitative modeling of these data, as presented in these studies, are detailed at length in Appendix G.

5.3.4. Benchmark Dose Approaches to Rat Nasal Tumor Data

This section describes various BMD analyses to determine PODs for low-dose extrapolation of SCC risk in the human respiratory tract (upper and lower).

5.3.4.1. *Benchmark Dose Derived from BBDR Rat Model and Flux as Dosimeter*

5.3.4.1.1. ***Response for benchmark dose.***

Typically, the BMD is calculated at the 5 or 10% response level. However, it appears appropriate to consider the benchmark response (BMR) at lower levels in exceptional cases that are supported by empirical data. In the case of data combined from the Kerns et al. (1983) and Monticello et al. (1996) bioassays, the lowest observed tumor response of SCC was below the 1% level (at 0.85%) (see Table 5-22). Additionally, the BBDR modeling incorporates precursor response in the form of LI data. Therefore, it was determined that it would also be appropriate to evaluate the POD at the 0.5% level while still staying in the neighborhood of the experimentally observed response.

The various data presented earlier in this chapter point to highly curvilinear dose responses for formaldehyde-induced tumor incidence as well as DPX and cell replication. This is also borne out by dose-response information based on gene array data (Thomas et al. 2007; Andersen et al. 2008). Cytotoxicity-driven regenerative replication and epithelial degeneration play a critical role in the steeply rising nature of the tumor dose-response. These observations raise the concern that cancer potency derived by straight-line extrapolation from the low end of observed tumor data (roughly at the 1% response) has the potential to be a significant overestimate for a reasonable upper bound. The pertinent question then is: what is a low-dose linear dose-response modeling of the data that is statistically consistent with the uncertainties in the observed time-to-tumor data. To address this question, the risk estimate based on the linear

extrapolation from a POD (based on the statistical upper confidence bound on risk) to the origin is compared with that predicted at the low-dose end by the Multistage-Weibull model fitted to the observed time-to-tumor data. The unit risk based on this model is obtained by calculating q1*, the 95% statistical upper bound on the coefficient associated with the linear term in the multistage model polynomial. This model fits the data reasonably well, reflects the highly curvilinear shape of the dose-response because of its mathematical flexibility, and allows for the possibility of low-dose linearity. Thus, for comparison the following estimates of unit risk are also presented (in addition to the unit risks calculated at the 1%, 5% and 10% response levels):

1. Unit risk that is based on q1*, which is derived from fitting the multistage Weibull model to the observed data.
2. Unit risk based on low-dose linear extrapolation from a POD at the 0.5% level.

5.3.4.1.2. ***Dose metric.***

The dose metric used for the extrapolation was the average wall mass flux of formaldehyde (expressed in pmol/mm^2-hour to the entire surface of the airway lining but excluding tissue lined by nonmucus-coated squamous tissue, which was considered to not absorb formaldehyde). The use of flux as a dosimeter is similar to the calculation of a regional gas dose ratio (RGDR) as proportional to minute volume divided by the surface area in the given species and is thus in line with EPA's guidance for calculating a dosimetric adjustment factor (DAF) for category 1 gases, whose effects are presumed to be at the POE (U.S. EPA, 1994b) (i.e., ratio of average flux over the same respiratory region in each species = ratio of the quantity [minute volume/surface area of the region] between the two species). This lends support to an interspecies extrapolation based on the equivalence of formaldehyde flux as a determinant of risk.

The spatial distribution of formaldehyde over the nasal lining was characterized by partitioning the nasal surface by formaldehyde flux to the tissue, resulting in 20 "flux bins" (see Figure 5-13). Each bin is comprised of elements (not necessarily contiguous) of the nasal surface that receive a particular interval of formaldehyde flux per ppm of exposure concentration (Kimbell et al., 2001b). The spatial coordinates of elements comprising a particular flux bin are fixed for all exposure concentrations, with formaldehyde flux in a bin scaling linearly with exposure concentration (ppm). The number of cells at risk varies across the bins, as shown in Figure 5-14.

5.3.4.1.3. ***Extrapolation to humans.*** For linear extrapolation from the 0.5 and 1% levels, two alternative versions of the biologically based model in Conolly et al. (2003) for the F344 rat were used. In both cases, only the historical control data from NTP inhalation studies (as opposed to all NTP studies) were added to the concurrent controls and weekly averaged DPX concentrations as calculated by Subramaniam et al. (2007) (who implemented a variant of the PBPK model in Conolly et al. 2000) were used. Both models provided good fits to the tumor incidence data, similar to the fit shown in Figure 5-12. Neither model could be considered better than the other on the basis of model description of tumor incidence data. The values of the parameters in these models and their fit to the data are provided in Tables E-4 and E-5 of Appendix E.

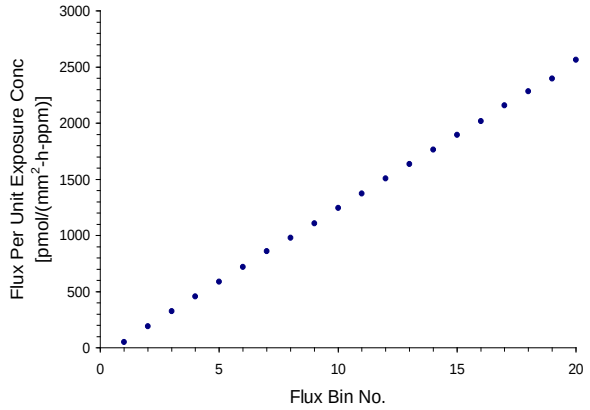


Figure 5-13. Spatial distribution of formaldehyde over the nasal lining, as characterized by partitioning the nasal surface by formaldehyde flux to the tissue per ppm of exposure concentration, resulting in 20 flux bins.

Source: Subramaniam et al. (2008).

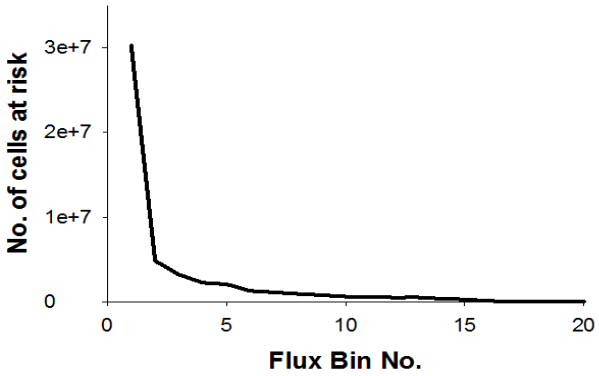


Figure 5-14. Distribution of cells at risk across flux bins in the F344 rat nasal lining.

Source: Subramaniam et al. (2008).

In Model 1 the normal cell replication dose response was described by the same hockey-stick-shaped curve used in Conolly et al. (2003). The form of the dose-response curves for initiated cell kinetics (division and death) was also the same as that considered by Conolly et al. (2003). This model is the same as Model E in Table III of Subramaniam et al. (2007) and values of the parameters and model fit to the data can be obtained from their Table.

Model 2 was an alternative to the Conolly et al. (2003) model, and is denoted as Model 15 in the sensitivity analysis described in Appendix E (see Figures E-6A and Table E-4 for parameter values). The dose response for replication of normal cells was monotone increasing and did not exhibit a threshold in dose. This was obtained by fitting the 13-week cell replication data in Monticello et al. (1996). The raw replicate animal data from this study was provided to EPA by the Hamner Institutes for Health Research. The cell replication dose response for initiated cells was a sigmoidal-shaped curve, increasing monotonically with flux from a background value up to an asymptotic value. The baseline cell-replication for initiated cells was constrained to be equal to or greater than the baseline rate of division of normal cells. Initiated cell death rate was considered proportional to initiated cell birth rate. The biological rationale for these choices is given in Appendix E.

Models 1 and 2 predicted monotonic dose-response curves.

The sequence of steps in arriving at a unit risk for SCC in human nasal airways from a given BBDR modeling of the F344 rat nasal tumor incidence data is outlined below. Extrapolation to the lower respiratory tract is described later.

1. Calculate the MLE risk and 95% upper confidence bound on risk at various exposure concentrations (d_{RAT} in ppm) by exercising the two BBDR models. Here, the POD is defined as d_{RAT} for which the 95% upper bound added risk is either 0.005 or 0.01. These values approximate the 95% lower bounds on the BMD corresponding to the added risks (i.e., the $BMDL_{RAT}$).
2. Using CFD modeling simulations in Kimbell et al. (2001b), calculate the average flux over the entire rat nose at resting breathing rates corresponding to d_{RAT}. Here, the subscript "i" is over flux bins and N is the number of cells at risk in a given bin.

$$AvgFlux(d_{RAT}) = d_{RAT} \times \left[\frac{\sum_i \left(\frac{flux}{ppm}\right)_i \cdot N_i}{\sum_i N_i} \right]_{RAT} \quad (5\text{-}3)$$

3. The experiment exposure was for periods of 5 days/week, 6 hours/day. Therefore, calculate the average daily exposure, obtained by making a 5/7 × 6/24 duration adjustment; that is, 5/7 × 6/24 × AvgFlux(d_{RAT}).
4. Now assume that lifetime exposure to similar levels of average formaldehyde flux to cells at risk leads to similar lifetime risk (MLE or upper bound, respectively) of tumor incidence across animal species. Also, in calculating human equivalent

concentrations, EPA has traditionally assumed chronic animal laboratory exposure scenarios to be equivalent to human lifetime exposures (U.S. EPA, 1994b).

5. Since a CFD model for a human upper respiratory tract is available (Subramaniam et al., 1998), it is possible to determine the average wall mass flux in this particular human nose for any specific breathing scenario. Likewise, a computational "single-path" model to determine average mass flux at any specific lung depth was available (Overton et al. 2001); however, risk in the lower respiratory tract will be addressed later. From the human CFD simulations in Kimbell et al. (2001a, b), the human airborne exposure concentration level that would yield an average wall mass flux in the human nose equal to [(5/7) × (6/24) × AvgFlux(d_{RAT})] is then calculated. In other words, given a risk-specific dose in the rat, the equivalent human exposure concentration is given by

$$d_{HUMAN} = (5/7) \times (6/24) \times AvgFlux(d_{RAT}) \times \left[\frac{\sum_i N_i}{\sum_i (\frac{flux}{ppm})_i \times N_i} \right]_{HUMAN} \quad (5\text{-}4)$$

6. To use this equivalent human exposure concentration, make the following assumption: when humans are exposed to the above concentration of formaldehyde (d_{HUMAN}) throughout the course of a lifetime, the added risks are anticipated to be similar to those experienced by the animal in the chronic bioassay.

7. Let *f* denote the ratio of the average flux per ppm of exposure concentration in the two species:

$$f = \frac{\left[\frac{\sum_i (\frac{flux}{ppm})_i \times N_i}{\sum_i N_i} \right]_{RAT}}{\left[\frac{\sum_i (\frac{flux}{ppm})_i \times N_i}{\sum_i N_i} \right]_{HUMAN}} \quad (5\text{-}5)$$

Now, the olfactory epithelium comprises a substantial fraction of nasal tissue in the rat. Because the olfactory region in the rat projects directly in the path of main airstreams (Kimbell et al., 1997a), a sizable flux of formaldehyde is delivered to this region in the rat. Tumors were not observed in the olfactory tissue of the rat. Therefore, since effects observed in the rat are being extrapolated to the human, cells from olfactory tissue are excluded in calculating average flux in the rat in the Equation 4. For the human, both volumetric flow (2.5%, Subramaniam et al. [1998])

and surface area (~5%, Kelly et al. [2000]) for the olfactory region are relatively small, so inclusion of this region is not likely to make a difference of much significance in the calculation of average flux in the human. Since data on formaldehyde flux delivered to the human olfactory region were not readily available, the olfactory region was not excluded for the human. The average human flux calculated here uses a working level classification for the activity profile where an individual spent equal amounts of time in a day at resting and light and moderate activity levels, corresponding to minute volumes of 7.5, 9, and 25 L/minute, respectively. This resulted in the following ratio[14]:

$$f = 444_{[rat]}/956.4_{[human]} = 0.46 \quad (5\text{-}6)$$

8. The airborne exposure concentrations d_{HUMAN} corresponding to a given MLE and upper bound lifetime added risk levels are the human BMD_{HUMAN} and $BMDL_{HUMAN}$, respectively. These are shown in Figure 5-15. (The rather sudden increase by ~0.0015 in the upper confidence bound on risk for model 1 for exposure exceeding ~0.41 ppm could not be explained. This jump was verified by repeated calculations that used different initial simulation conditions and convergence criteria.)

Extrapolation to the human lower respiratory tract

Next, the human lower respiratory tract is also considered to be potentially at risk. Therefore, the above calculations of BMD and BMDL need to be augmented to include the lower respiratory tract for humans. This calculation was facilitated by dosimetry calculations of formaldehyde wall mass flux to various depths in the lung by using a single path model. Refer to Overton et al. (2001) for details on their dosimetry modeling. The calculations for including the lower respiratory tract in determining an overall BMD and BMDL involved the following steps:

a. As given by Equation 5-3, calculate d_{HUMAN} for various MLE risk levels. This gives a dose-response relationship for lifetime risk of SCC in the human nose due to continuous exposure to airborne formaldehyde.

b. Express this dose-response relationship in terms of average flux over the entire human nasal lining.

c. Next, express this dose-response relationship, calculated here for the entire nose, as risk per nasal cell versus average flux.

[14] This is to be contrasted with a corresponding value of 0.71 in Schlosser et al. (2003) who used only resting inspiratory rates.

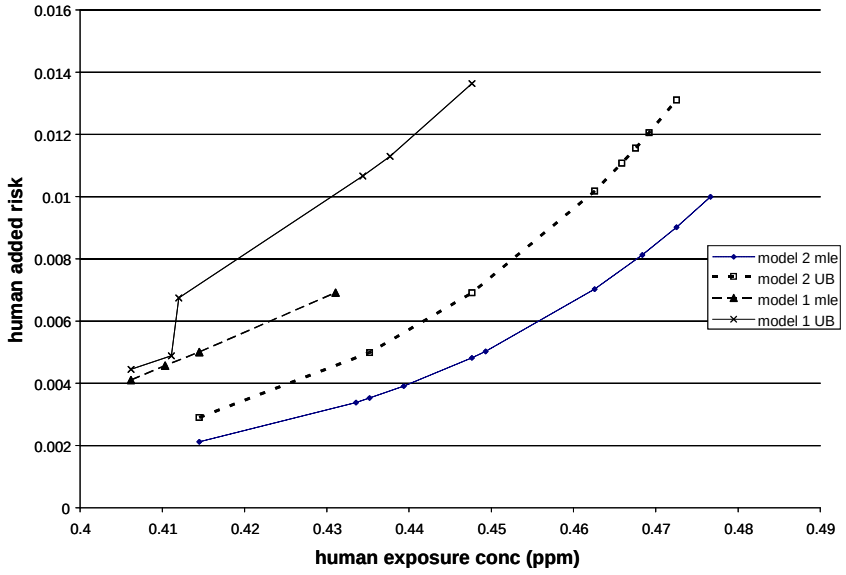


Figure 5-15. MLE and upper bound (UB) added risk of SCC in the human nose for two BBDR models.

Note: Airborne exposure concentrations d_{HUMAN} corresponding to a given MLE and upper bound lifetime added risk levels are the human BMD_{HUMAN} and $BMDL_{HUMAN}$, respectively.

d. Now, if the respiratory and transitional cell types in the human lung and nose are equally susceptible to formaldehyde-induced cancer risk (as is also assumed in Conolly et al. [2004]), then it appears reasonable to assume that MLE risk per cell at a given value of formaldehyde flux is the same in the lung as in the nose.

e. The number of cells and the average flux in a given flux bin in the lung are known (Overton et al., 2001). Thus, at a given air concentration, the MLE risk due to cells in the various flux bins of the lung is obtained.

f. One important feature of Overton et al. (2001) was that their flux bins mapped physically with lung depth. Therefore, in addition to extrapolating risk to the entire human lung, it was also relatively easy to extend the risk calculation in e. above as a function of airway generation in the lung (corresponding to different lung depths).

g. The MLE value risk to the lower respiratory tract (as determined above in steps a.−e.) was a small fraction of risk to the upper respiratory tract. This is because of high formaldehyde reactivity and solubility at the POE. Therefore, it sufficed to assume that the relative increase in upper bound risk for the combined upper respiratory tract + lower respiratory tract compared to that for only the upper respiratory tract would be the same as the corresponding relative increase in the

value of the MLE risk. The upper bound risk to the entire respiratory tract and consequently the BMDL value corresponding to a given response were thus determined.

These calculations indicated that including the risk of SCC in the lower respiratory tract resulted in at most a 3% increase in the added risk at the lower end of the human exposure range in Figure 5-15 (i.e., at 0.42 ppm) and about a 1.5% increase at the higher end of the range in that plot. Therefore, including the lower respiratory tract did not appreciably alter the human BMDs and BMDLs at the 0.5 and 1% response levels. This occurs because of the steepness in the dose-response curve in this exposure range and much lower risk in the lung at any exposure concentration.

Unit risks of SCC in the human respiratory tract extrapolated in this manner are reported in Table 5-23.

Table 5-23. BMD modeling of unit risk of SCC in the human respiratory tract

Extra risk level	Benchmark levels (ppm)		Unit risk[a] (per ppm)
	BMD	BMDL	
0.005	0.415–0.450	0.410–0.435	1.2×10^{-2}
0.010		0.430–0.460	2.2×10^{-2}

[a]Obtained from the mean of the two BMDLs.

Note: Findings are based on nasal tumors in rats and formaldehyde flux to tissue as dosimeter, using dose-response curves for the F344 rat predicted by clonal growth modeling. Two chronic bioassays (Monticello et al., 1996; Kerns et al., 1983) were combined, and control animals from the historical NTP inhalation bioassays were added to the control animals in these bioassays.

5.3.4.2. *Comparison with Other Benchmark Dose Modeling Efforts*

The CIIT assessment (Schlosser et al., 2003; CIIT, 1999) also presented, as their less preferred option, a benchmark approach on the data set obtained by combining the two chronic bioassays with similar protocols (Monticello et al., 1996; Kerns et al., 1983) along with data from 94 animals that had not been previously examined. These authors used two measures of response: tumor incidence and cell proliferation. In each case, they used two dosimeters: DPX and formaldehyde flux to the nasal lining.

The extrapolation to human was carried out by using a hybrid CFD and pharmacokinetic model. The CFD model (Kimbell et al., 2001a, b; Kepler et al., 1998; Subramaniam et al., 1998)

enabled calculation of site-specific flux in the nose of the rat, monkey, and human species for inhaled formaldehyde concentrations, and the PBPK model (Conolly et al., 2000) linked this flux to predicted DPX levels. The models were constructed for anatomically realistic representations of a single individual in each species. The CFD and PBPK modeling and uncertainties in these estimates have been reviewed in the Modeling the Toxicokinetics of Formaldehyde and DPX section of chapter 3.

5.3.4.2.1. ***Benchmark dose using administered concentration.***

Schlosser et al. (2003) fit multistage, Weibull, polynomial, and log-probit quantal models to the tumor data and exercised the models (except the log-probit) with and without requiring that the fits pass through the origin. The log-probit fit passed through the origin (see Figure 5-16). A fifth degree polynomial was used in the multistage model. The best fit was obtained with the polynomial and Weibull models for the tumor incidence data with a nonzero intercept (threshold) on the dose axis. Fits passing through the origin did not pass the statistical goodness-of-fit criteria ($p > 0.01$) for models other than the log-probit. The dose response near the lowest dose was steep, with the LED_{10}s and LED_{01}s for the administered concentrations nearly the same for each model, at least to one significant figure, and ranged from 3.8 to 6.4 ppm.

5.3.4.2.2. ***Benchmark dose derived with internal dose (flux and DPX) as dose metrics in Schlosser et al. (2003).***

Schlosser et al. (2003) used CFD simulations (Kimbell et al., 2001a, b) of mass flux of formaldehyde delivered across the nasal lining. The dose metric used by Schlosser et al. (2003) for the extrapolation was the average flux of formaldehyde, expressed in pmol/cm^2-minute, to the entire surface of the airway lining. This excluded tissue lined by nonmucus-coated squamous tissue, which was considered not to absorb formaldehyde.

In the CFD model, flux in any region is linearly related to the airborne exposure concentration (i.e., flux = $f \times C_{air}$ [ppm], where f is a constant of proportionality and C_{air} is the exposure concentration). The ratio of f (rat)/f (human) was determined as given by Equation 5-4. This ratio was equal to 0.71 and differed from the value of 0.46 used in this document (as presented in Equation 4-5) because Schlosser et al. (2003) used resting inspiratory rates.

In the next level of dosimetric complexity, Schlosser et al. (2003) used DPX as the relevant dosimeter based on values predicted by PBPK models developed by Conolly et al.

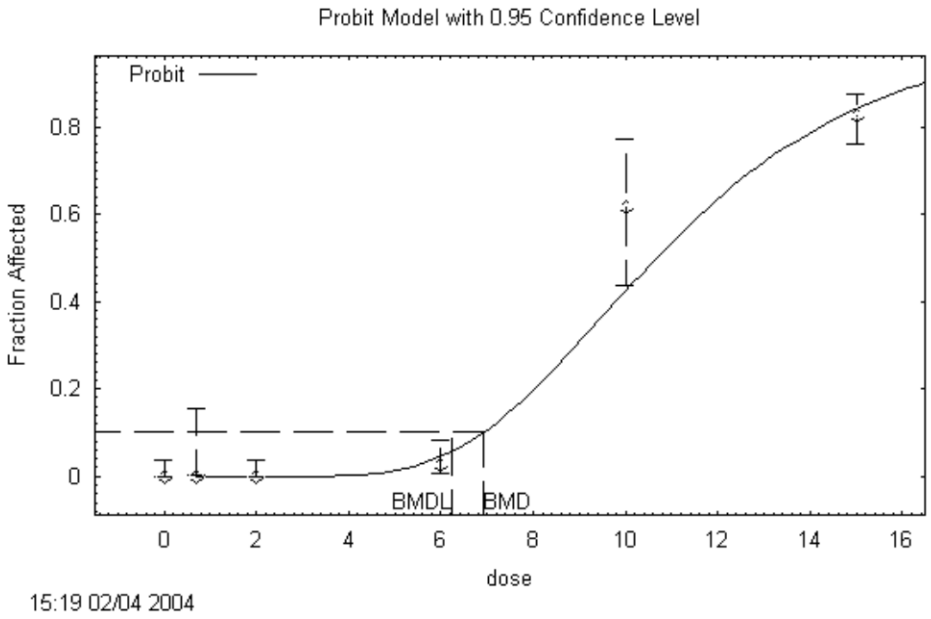


Figure 5-16. Replot of log-probit fit of the combined Kerns et al. (1983) and Monticello et al. (1996) data on tumor incidence showing BMC_{10} and $BMCL_{10}$.
Source: Adapted from Schlosser et al. (2003).

(2000). This expressed the local dose as pmol of formaldehyde equivalents covalently bound to DNA per unit volume of nasal tissue. Human CFD and PBPK models were exercised to determine the airborne concentration of formaldehyde that yields average DPX levels equal to those in the rat at the BMC. This airborne concentration was then the HEC. The human benchmark extrapolations in Schlosser et al. (2003) using flux and DPX are shown in Table 5-24, located at the end of Section 5.4.

The assumption in using DPX data was that lifetime exposure to the same DPX concentration for a given duration each day leads to equivalent risk across species. Table 5-24 shows their human benchmark calculations for a continuous environmental exposure. These were exposures that resulted in the same steady-state DPX concentrations as the weekly TWA DPX values in rats at the rat benchmark exposure concentrations.

5.3.4.2.3. ***Cell proliferation in CIIT benchmark modeling.***

Schlosser et al. (2003) also used cell proliferation as representing the adverse response. The BMDs and BMDLs calculated with these data did not differ appreciably from their other benchmark estimates. The use of cell proliferation as an end point is considered to have the

advantage that it represents an early step contributing to carcinogenesis. In this document, a BMD or BMDL is not calculated based solely on cell replication as a response. Instead, cell replication rates are used as input to the clonal growth model and a benchmark dose based on a fit to the tumor response using that model is considered a better choice since it integrates cell replication along with other relevant data, such as the number of cells at risk and DPXs.

5.3.4.3. *Kaplan-Meier Adjustment*

In the simplest consideration of the impact of competing risks on the nasal tumor incidence, tumor incidences were adjusted for early deaths according to Kaplan-Meier (KM) survival estimates (KS Crump Group, 2001). This procedure allows for the possibility that some tumors may otherwise have developed in the animals that died early due to other causes. All the animals in the study were considered except those that were kept past termination of exposure. A comparison of the adjusted incidence data is presented below in Table 5-25. While the adjustments have been provided in Table 5-25, it needs to be noted that the data allow for a full time-to-tumor analysis as presented below.

5.3.4.4. *EPA Time-to-Tumor Statistical Modeling*

Instead of using the KM adjustment, EPA has used the multistage Weibull time-to-tumor model (Portier et al., 1986; Krewski et al., 1983) in other assessments (e.g., ethylene oxide, 1,3-butadiene, chloroprene). This is a dose-response model that includes the exact time of observation of the tumors and therefore gives appropriate weight to the amount of time each animal was on study without a tumor and acknowledges earlier tumor incidence with increasing dose level. The data used in this analysis were obtained from the appendix in Conolly et al. (2003) with one crucial modification. These data combined the nasal squamous carcinoma data of Kerns et al. (1983) and Monticello et al. (1996) along with results from an additional 94 animals not previously examined in the Monticello et al. (1996) study. Animals in some exposure groups were held up to 6 months following the 24-month exposure period; these animals were deleted from the analysis for the following reason: there were no tumors among these animals, and inclusion of them would have required estimating an equivalent TWA exposure over the entire study period for these animals (40 in 2 ppm group, 39 in 6 ppm group, 3 in 15 ppm group), whereas the other animals would be represented by their actual exposure concentrations.

Table 5-24. Human benchmark extrapolations of nasal tumors in rats by using formaldehyde flux and DPX

Model	Source	Rat benchmark levels (ppm)				Extrapolated human benchmark levels (ppm)					Unit risk[a] $(ppm)^{-1}$		
			1%	5%	10%	Dose metric[b]		1%	5%	10%	1%	5%	10%
Weibull[c,d] (with threshold)	Schlosser et al. (2003)	ED	5.91	6.12	6.40	Flux[e]	ED	0.75	0.78	0.82			
		LED	5.58	5.94	6.22		LED	0.71	0.76	0.79	1.4×10^{-2}	6.6×10^{-2}	1.3×10^{-1}
						DPX[f]	ED	0.76	0.79	0.84			
							LED	0.71	0.76	0.81	1.4×10^{-2}	6.6×10^{-2}	1.2×10^{-1}
Multistage Weibull (time-to-tumor)[c,d,g]	EPA (this assessment)	ED	4.28	5.93	6.84	Flux[h]	ED	0.35	0.49	0.57			
		LED	3.57	5.52	6.41		LED	0.30	0.46	0.53	3.4×10^{-2}	1.1×10^{-1}	1.9×10^{-1}
											$q1^* = 2.2 \times 10^{-2}$		
BBDR models (see Table 5-23)	EPA (this assessment)	See Table 5-23 and associated text									at 1%: 2.2×10^{-2}		
											at 0.5%: 1.2×10^{-2}		

Note 1: Combined tumor incidence data from Kerns et al. (1983) and Monticello et al. (1996) were used for response.

[a]Slope of straight line extrapolation from the POD of the dose-response curve at the 1, 5, and 10% extra risk level.
[b]Flux: CFD modeling. DPX: CFD + PBPK modeling.
[c]*p* Value for Weibull model fit = 0.90. For the time-to-tumor modeling, goodness-of-fit *p* value was not provided by software package; therefore, fit was judged by comparing fitted curve to KM survival estimates (see Figure 5-19).
[d]For Weibull model, Schlosser et al. (2003) obtained best fit with a positive intercept on dose axis. For multistage Weibull model, curves pass through origin.
[e]Human benchmark levels extrapolated using flux were multiplied by $f_{HCHO\text{-}Rat}/f_{HCHO\text{-}Human}$ (= 0.71) for interspecies extrapolation and multiplied by (6/24) × (5/7) to adjust for continuous exposure.
[f]Human benchmark levels using DPX were continuous environmental exposures that would result in steady-state DPX levels in humans equal to the weekly TWA DPX levels in rats at the rat BMCs for 6 hours/day and 5 days/week.
[g]$P(d,t) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + ... + q_k d^k)^* t^z]$. q_0, q_1, q_2, q_3, q_4 were all taken to be zero. $q_5 = 2.9 \times 10^{-22}$, z = 8.1.
[h]Human benchmark levels extrapolated using flux were multiplied by $f_{HCHO\text{-}Rat}/f_{HCHO\text{-}Human} = 0.46$ for interspecies extrapolation and multiplied by (6/24) × (5/7) to adjust for continuous exposure (see Section 5.3.6.2).

Table 5-25. Formaldehyde-induced rat tumor incidences

Exposure level (ppm)	KM adjusted incidence	Observed tumor/ number at risk[a]
0.0	0.0	0/242
0.7	0.0	0/70
2.0	0.0	0/254
6.0	0.02	3/120[a]
10.0	0.61	22/36[a]
15.0	0.83	57/190[a]

[a]KM adjusted. Numbers not indicated by footnote were not amenable to KM adjustment because there were no tumors; these numbers at risk reflect all animals surviving 1 year on study.

Source: Monticello et al. (1996); Kerns et al. (1983).

Due to earlier tumor occurrence with increasing exposure level and increased mortality with increasing exposure level, methods that can reflect the influence of competing risks and intercurrent mortality on site-specific tumor incidence rates are preferred. EPA has generally used the multistage Weibull model because it incorporates the time at which death with tumor occurred, giving appropriate weight to the amount of time each animal was on study without a tumor; the model has the following form: $P(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + ... + q_k d^k) \times (t - t_0)^z]$, where *p(d)* represents the lifetime risk (probability) of cancer at dose *d* (i.e., human equivalent exposure in this case); parameters $q_i \geq 0$, for i = 0, 1, ..., k; *t* is the time at which the tumor was observed; and *z* is a parameter estimated in fitting the model, which characterizes the change in response with age. The parameter t_0 represents the time between when a potentially fatal tumor becomes observable and when it causes death.

A further consideration is the distinction between tumor types as being either fatal or incidental in order to adjust for competing risks. Incidental tumors are those tumors thought not to have caused the death of an animal (such as those observed during interim or terminal sacrifices), while fatal tumors are thought to have resulted in animal death. For these data, nasal tumors observed with early deaths were considered to be fatal.

The dose-response analyses (see Figures 5-17, 5-18, 5-19) were conducted by using the computer software program TOX_RISK, version 5.3 (ICF, Fairfax, VA), which is based on Weibull models drawn from Krewski et al. (1983). Parameters were estimated by using the

method of maximum likelihood. Specific multistage Weibull models were selected for the individual tumor types for each sex, based on the values of the log likelihoods according to the strategy used by EPA (2002b). If twice the difference in log-likelihoods was less than a χ^2 with degrees of freedom equal to the difference in the number of stages included in the models being compared, the models were considered comparable, and the most parsimonious model (i.e., the lowest-stage model) was selected contingent on visual fits of the data as follows. For incidental tumors, plots of model fits compared with Hoel-Walburg estimates of cumulative incidence were also examined for goodness of fit in the lower exposure region of the observed data (Gart et al., 1986) (see Figure 5-18). For fatal tumors, plots of model fits were compared with KM estimates of cumulative incidence. If a model with one more stage fitted the low-dose data better than the most parsimonious model, then the model with one higher stage was selected.

Due to the sharp increase in responses between 6 and 10 ppm, no adequate fit was achieved. Data for the highest dose were dropped in an effort to focus the fitting process for this empirical model on the low-dose region. The model that then provided the best overall fit included five stages but with coefficients for the lower stages estimated to be zero (see Figures 5-17, 5-18, 5-19). The parameter t_0 was estimated to be zero, consistent with rapidly fatal tumors. On the other hand, an alternate run treating all tumors as incidental to the death of the affected animals yielded BMCLs and BMCs within 10% of these estimates (see Figure 5-18); thus, tumor context is not a sensitive consideration for these data.

For the same reasons as discussed in Section 5.3.3 (the concluding discussion of the BBDR modeling), a linear low-dose extrapolation approach was used to estimate human carcinogenic risk associated with formaldehyde exposure. PODs for estimating low-dose risk were identified at doses at the lower end of the observed data, corresponding to 1% extra risk, defined as the extra risk over the background tumor rate $[P(d) - P(0)]/[1 - P(0)]$. PODs corresponding to 10% extra risk are also provided to facilitate comparison with other chemicals. Rat benchmark levels obtained by analysis of the tumor data are shown in Table 5-24. PODs were converted to continuous human-equivalent exposure levels by multiplying by (5 days/7 days) × (6 hours/24 hours), or 0.178, and by multiplying by the ratio of fluxes developed in Section 5.3.6.1.3. The lifetime continuous inhalation unit risk for humans is defined as the slope of the line from the lower 95% bound on the exposure at the POD, calculated by dividing the BMR level (1%) by the corresponding $BMCL_{01}$. This 95% UCL represents a plausible upper bound on the true risk.

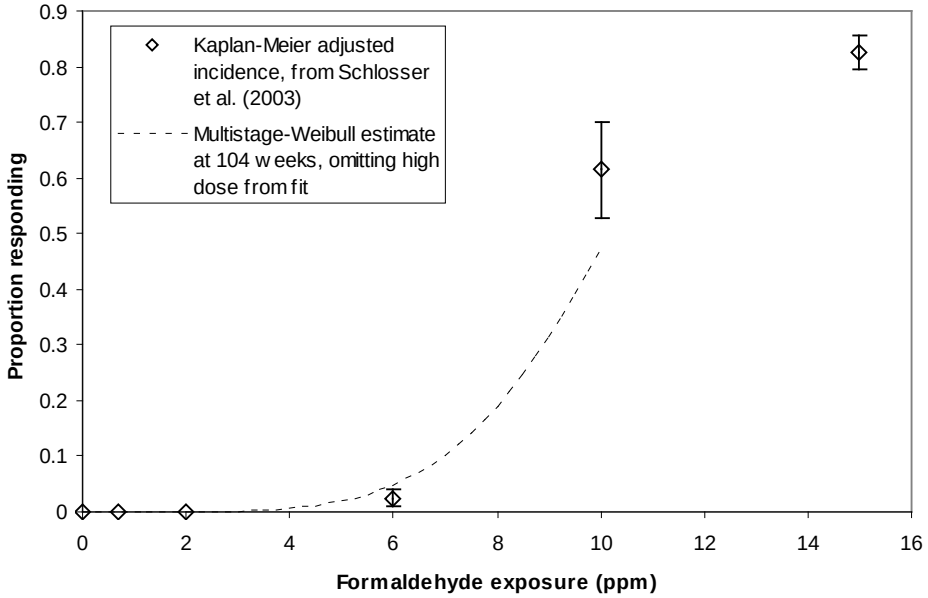


Figure 5-17. EPA Multistage Weibull modeling: nasal tumor dose response.

Note: Time-to-tumor modeling of Kerns et al. (1983) and Monticello et al. (1996) data compared with incidences adjusted by using KM estimates evaluated at 104 weeks.

Source: Adapted from Schlosser et al. (2003).

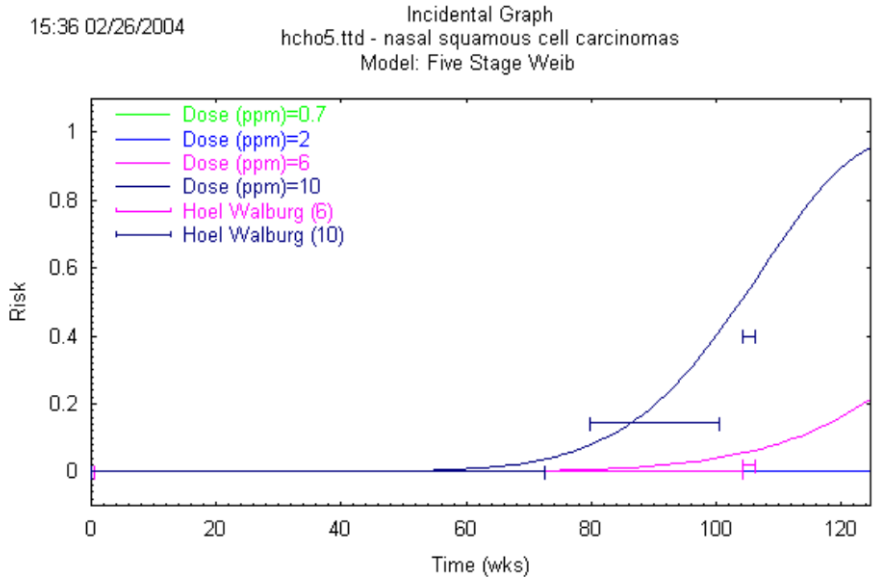


Figure 5-18. Multistage Weibull model fit.
Note: Data of Kerns et al. (1983) and Monticello et al. (1996) compared with Hoel-Walburg estimates of tumor incidences occurring at interim and terminal sacrifices.

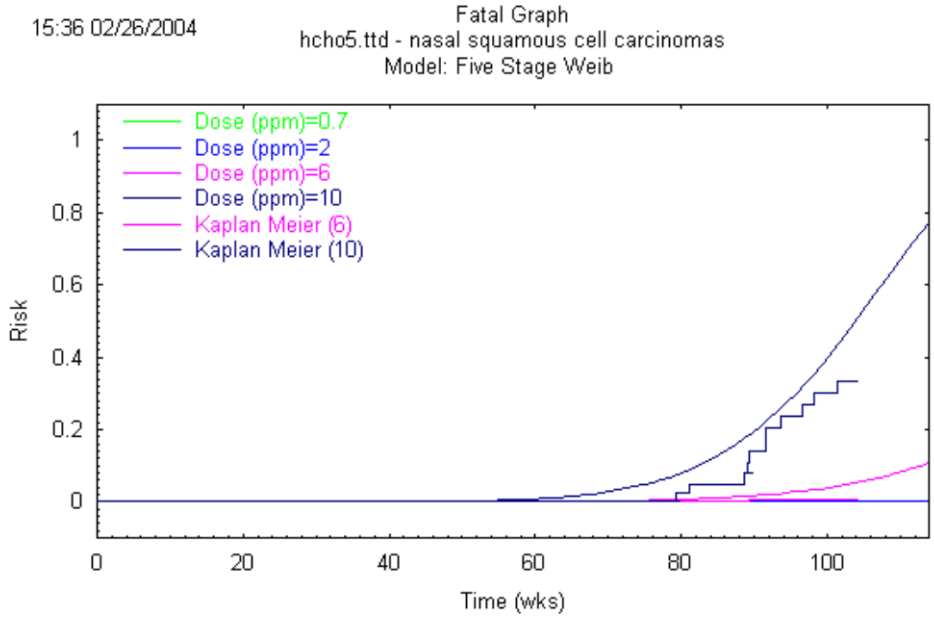


Figure 5-19. Multistage Weibull model fit of tumor incidence data compared with KM estimates of spontaneous tumor incidence.
Source: Developed from data reported in Kerns et al. (1983) and Monticello et al. (1996).

The extrapolation to humans in terms of using formaldehyde flux to tissue as the dose metric is shown in Table 5-24, where unit risk in terms of q1*, the statistical upper bound on the coefficient, q1, of the term linear in dose in the multistage model, is also presented. q1* is presented even though this is no longer done, as per current EPA practice (see Section 5.3.6 for discussion).

These results are to be compared with the preferred benchmark estimates obtained in Table 5-23 by using the results of biologically based models. In summary, the unit risks obtained by various methods, including the results in Schlosser et al. (2003), fall within a rather tight range. In particular, q1* was obtained to within a factor of two of other values even though q1 itself was zero. The general result may be noted here, that even in cases where q1 is zero, the upper bound q1* is linear with dose (Subramaniam et al., 2006; Guess et al., 1977). The large difference between q1 and q1* aptly reflects the large uncertainty in the low-dose response.

5.4. CONCLUSIONS FROM THE QUANTITATIVE ASSESSMENT OF CANCER RISK FROM FORMALDEHYDE EXPOSURE BY INHALATION

5.4.1. Inhalation Unit Risk Estimates Based on Human Data

As described in Section 5.2, a (plausible upper bound) lifetime extra cancer unit risk of 1.1×10^{-2} per ppm (8.8×10^{-6} per µg/m^3) of continuous formaldehyde exposure was estimated for NPC incidence using the log-linear modeling results (for NPC mortality from cumulative exposure) from a high-quality occupational epidemiologic study in a life-table analysis to obtain a POD and then applying linear low-dose extrapolation from the POD. Using similar methods and data from the same study for Hodgkin lymphoma and leukemia mortality from cumulative formaldehyde exposure, (plausible upper bound) lifetime extra cancer risk estimates of 1.7×10^{-2} per ppm (1.4×10^{-5} per µg/m^3) for Hodgkin lymphoma incidence and 5.7×10^{-2} per ppm (4.6×10^{-5} per µg/m^3) for leukemia incidence were derived. Sources of uncertainty in these estimates are discussed in sections 5.2.2.4 and 5.2.3.4. For the incidence risk for these three cancer types combined, a total (upper bound) cancer unit risk estimate of 8.1×10^{-2} per ppm (6.6×10^{-5} per µg/m^3) was obtained (see Section 5.2.4).

5.4.2. Inhalation Unit Risk Estimates Based on Rodent Data

As described in Section 5.3, the unit risk derived for SCC in the upper and lower respiratory tract (combined) based on linear extrapolation from PODs from several plausible models, including purely statistical modeling (nose only, quantal and time-to-tumor modeling) and biologically based modeling (entire respiratory tract), resulted in a narrow range of

1.2×10^{-2} to 2.2×10^{-2} per ppm. Risk to the lower respiratory tract was numerically insignificant compared to the nasal cancer risk.

5.4.3. Summary of Inhalation Unit Risk Estimates

The epidemiologic and rodent inhalation data indicate multiple sites of concern. Unit risk estimates calculated separately from these data are presented in Table 5-26.

As can be seen in the summary table (see Table 5-26), the unit risk estimate based on human data for NPC is in the range of the estimates calculated for respiratory tract cancer from the rodent nasal cancer data. The unit risk estimate for Hodgkin lymphoma is also in the same range, while the unit risk estimate for leukemia and the total cancer unit risk estimate are up to fourfold higher.

As noted in EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), when high-quality human data are available, they are generally preferred over laboratory animal data for quantitative risk assessment. Thus, the preferred (plausible upper bound) unit risk estimate in this assessment is the value of 8.1×10^{-2} per ppm (6.6×10^{-5} per $\mu g/m^3$) based on human data for NPC, Hodgkin lymphoma, and leukemia.

As documented in Section 4.5, formaldehyde is a mutagenic carcinogen and the weight of evidence supports the conclusion that formaldehyde carcinogenicity can be attributed, at least in part, to a mutagenic MOA. Therefore, since there are no adequate chemical-specific data to evaluate the susceptibilities of different life stages by the inhalation route of exposure, increased early-life susceptibility should be assumed, and, if there is early-life exposure, the ADAFs should be applied, in accordance with EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b). See Section 5.4.4 below for more details on the application of the ADAFs.

The inhalation unit risk estimates presented above, which are calculated based on a linear extrapolation from the POD (95% lower confidence bound on the EC), are expected to provide upper bounds on the risk of cancer incidence. However, for certain applications, such as benefit-cost analyses, estimates of "central tendency" for the risk below the POD are desired. Extra risk estimates per ppm based on linear extrapolation from the EC (e.g., $0.005/EC_{005}$) for the cancer responses based on the human data are reported in Table 5-27. Note that these extrapolated risk estimates are not central tendency estimates in any statistical sense because once risk is linearly extrapolated below the EC, it is no longer a function of the original (Cox regression) model which generated the ECs and the LECs. These estimates are dependent on the suitability of the EC estimates as well as on the applicability of the linear low-dose extrapolation. The assumption of low-dose linearity is supported by the mutagenicity of formaldehyde (see Section

4.5.3). [If

Table 5-26. Summary of inhalation unit risk estimates

Cancer type[a]	Dose metric	Unit risk estimate (ppm^{-1})
Based on epidemiologic data		
Nasopharyngeal	Cumulative exposure	0.011
Hodgkin lymphoma	Cumulative exposure	0.017
Leukemia	Cumulative exposure	0.057
Total cancer risk[b]	Cumulative exposure	0.081
Based on experimental animal data		
SCC of the respiratory tract	Local dose (flux) of formaldehyde in $pmol/mm^2$-hour	0.011–0.022

[a]The unit risk estimates are all for cancer incidence.
[b]The total cancer unit risk estimate is an estimate of the upper bound on the sum of risk estimates calculated for the 3 individual cancer types (nasopharyngeal cancer, Hodgkin lymphoma, and leukemia); it is not the sum of the individual (upper bound) unit risk estimates (see Section 5.2.4).

these estimates were to be used for benefit-cost analyses or some other purpose, ADAFs should be applied, as appropriate, in accordance with EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), as discussed above and in Section 5.4.4.]

5.4.4. Application of Age-Dependent Adjustment Factors (ADAFs)

When there is sufficient weight of evidence to conclude that a mutagenic MOA is operative in a chemical's carcinogenicity and there are inadequate chemical-specific data to assess age-specific susceptibility, as is the case for formaldehyde (by inhalation exposure; see Section 5.4.3), EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b) recommends the application of default ADAFs to adjust for potential increased susceptibility from early-life exposure (see U.S. EPA [2005b] for detailed information on the general application of these adjustment factors). In brief, EPA (2005b) establishes ADAFs for three specific age groups: 10 (for <2 years), 3 (for 2 to <16 years), and 1 (for 16 years and above). For risk assessments based on specific exposure assessments, the 10-fold and threefold adjustments to the unit risk estimates are to be

Table 5-27. Extra risk estimates per ppm based on ECs[a]

Cancer type	BMR[b]	Outcome	EC (ppm)[c]	BMR/EC_{BMR} (per ppm)[c]
nasopharyngeal cancer	0.0005	mortality	0.15	3.3×10^{-3}
		incidence	0.074	6.8×10^{-3}
Hodgkin lymphoma	0.0005	mortality	0.15	3.3×10^{-3}
		incidence	0.051	9.8×10^{-3}
leukemia	0.005	mortality	0.22	2.3×10^{-2}
		incidence	0.16	3.1×10^{-2}
Total cancer[d]		mortality		2.4×10^{-2} [d]
		incidence		4.7×10^{-2} [d]

[a]Based on all person-years. Values based on exposed person-years only would be virtually identical.
[b]BMR = benchmark response, i.e., extra cancer risk level used to calculate the ECs and LECs.
[c]To convert ppm to μg/m^3, multiply by 1,230; to convert ppm^{-1} to (μg/m^3)$^{-1}$, divide by 1,230.
[d]The extra risk estimates per ppm for total cancer are not derived from ECs but rather from the calculations of combined cancer risk at 0.1 ppm presented in Section 5.2.4 (see Table 5-20 for mortality and Table 5-21 for incidence). The sums of the MLEs of risk from Tables 5-20 and 5-21, multiplied by 10 to convert from per 0.1 ppm to per ppm, correspond to the extra risk estimates per ppm calculated from the ECs (in that they are based on MLEs and not bounds) but they are not equivalent to the sum of the EC-based values because those are calculated at different ECs and the MLEs of risk are all calculated at a common exposure level of 0.1 ppm.

combined with age-specific exposure estimates when estimating cancer risks from early-life (<16 years age) exposure. The ADAFs and their age groups may be revised over time. The most current information on the application of ADAFs for cancer risk assessment can be found at www.epa.gov/cancerguidelines.

For inhalation exposures, assuming ppm equivalence across age groups (i.e., equivalent risk from equivalent exposure levels, independent of body size) and using the preferred unit risk estimate of 6.6×10^{-5} per μg/m^3 from Section 5.4.3, the calculation is fairly straightforward. For example, the ADAF-adjusted total cancer unit risk estimate for a constant lifetime exposure level is calculated as shown in Table 5-28.

This 70-year risk estimate of 1.1×10^{-4} for a constant exposure of 1 μg/m^3 calculated in Table 5-28 is equivalent to a lifetime unit risk of 1.1×10^{-4} per μg/m^3 (0.13/ppm), adjusted for early-life susceptibility, assuming a 70-year lifetime and constant exposure across age groups. As mentioned above, for risk assessments based on specific exposure assessments, application of

Table 5-28. Total cancer risk from exposure to a constant formaldehyde

exposure level of 1 μg/m^3 from ages 0–70 years

Age group	ADAF	Unit risk (per μg/m^3)	Exposure concentration (μg/m^3)	Duration adjustment	Partial risk
0 to < 2 years	10	6.6×10^{-5}	1	2 years/70 years	1.9×10^{-5}
2 to < 16 years	3	6.6×10^{-5}	1	14 years/70 years	4.0×10^{-5}
≥ 16 years	1	6.6×10^{-5}	1	54 years/70 years	5.1×10^{-5}
				Total risk =	1.1×10^{-4}

(Note that the partial risk for each age group is the product of the values in columns 2–5 [e.g., $10 \times (6.6 \times 10^{-5}) \times 1 \times 2/70 = 1.9 \times 10^{-5}$], and the total risk is the sum of the partial risks.)

the ADAFs is to be combined with age-specific exposure estimates when estimating cancer risks from early-life (<16 years age) exposure. Further example calculations can be found in EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b).

In addition to the uncertainties discussed above for the inhalation unit risk estimate, there are uncertainties in the application of ADAFs to adjust for potential increased early-life susceptibility. The ADAFs are general default factors, and it is uncertain to what extent they reflect increased early-life susceptibility for exposure to formaldehyde, if, in fact, early-life susceptibility is increased as assumed. To some extent, the unit risk estimates for Hodgkin lymphoma and leukemia already reflect some partial increased risk from early-life exposure because the life-table programs include background rates for childhood cancers. However, the impact of this partial increased risk is negligible compared to the effect of the ADAFs on the final risk estimate. For example, eliminating the background rates up to age 16 from the life-table programs decreases the lifetime extra risks at the PODs by about 0.5% for leukemia and about 1.2% for Hodgkin lymphoma. The ADAFs, on the other hand, increased the lifetime unit risk estimate by about 66%.

5.4.5. Conclusions: Cancer Inhalation Unit Risk Estimates

As presented in Section 5.4.3, the preferred (plausible upper bound) cancer unit risk estimate for formaldehyde exposure in this assessment is the total cancer risk estimate of **8.1×10^{-2} per ppm (6.6×10^{-5} per μg/m^3) based on (adult) human data for NPC, Hodgkin lymphoma, and leukemia**.

In addition, as described in Section 5.4.4, because the weight of evidence supports the conclusion that formaldehyde carcinogenicity can be attributed, at least in part, to a mutagenic MOA and there are inadequate chemical-specific data to assess age-specific susceptibility, increased early-life susceptibility should be assumed and, if there is early-life exposure, ADAFs should be applied, in accordance with EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b). Consequently, applying the ADAFs to the preferred unit risk estimate to obtain a **full lifetime unit risk estimate** yields

$$0.081/\text{ppm} \times [(10 \times 2 \text{ years}/70 \text{ years}) + (3 \times 14/70) + (1 \times 54/70)]$$
$$= \mathbf{0.13/ppm = 1.1 \times 10^{-4}/(\mu g/m^3)}$$

Using the above full lifetime unit risk estimate of 0.13 per ppm, the lifetime chronic exposure level of formaldehyde corresponding to an increased cancer risk of 10^{-6} can be estimated as follows: $(10^{-6})/(0.13/\text{ppm}) = 7.7 \times 10^{-6}$ ppm = 0.008 ppb = 0.009 μg/m^3. Similarly, the lifetime chronic exposure level of formaldehyde corresponding to an increased cancer risk of 10^{-4} is 0.8 ppb, or 0.9 μg/m^3. (Note that for less-than-lifetime exposures scenarios [or for exposures that vary with age], the adult-based combined estimate of 0.081 per ppm should be used, but if there is early-life exposure, the ADAFs should be applied in accordance with EPA's *Supplemental Guidance* [see Section 5.4.4]).

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE-RESPONSE

6.1. SUMMARY OF HUMAN HAZARD POTENTIAL

6.1.1. Exposure

Formaldehyde (CH_2O) occurs as a gas at room temperature. It is highly reactive and dissolves readily in water. Formaldehyde is present in a wide variety of products including plywood adhesives, abrasive materials, insulation, insecticides and embalming fluids (IPCS, 2002a; Agency for Toxic Substances and Disease Registry [ATSDR], 1999). The major sources of anthropogenic emissions of formaldehyde are motor vehicle exhaust, power plants, manufacturing plants that produce or use formaldehydes or substances that contain formaldehyde (i.e., glues), petroleum refineries, coking operations, incineration, wood burning, and tobacco smoke (INEG, 2003). Reported outdoor air concentrations of formaldehyde in urban and suburban areas are near 3 $\mu g/m^3$ (~ 3 ppb) (U.S. EPA, 2008) and indoor residential levels are approximately 10 times higher (Health Canada and Environment Canada, 2001).

Limited United States data indicate that the upper end of the formaldehyde concentration range in drinking water is approximately 10 µg/L IPCS, 2002a). Formaldehyde is a natural component of a variety of foodstuffs (IARC, 1995; IPCS, 1989). In addition, foods may also be contaminated with formaldehyde as a result of fumigation (e.g., grain, seeds), cooking (as a combustion product), and release from formaldehyde resin-based tableware (IARC, 1995). Limited data measuring formaldehyde in food indicates that the concentration range is <0.03−14 mg/kg Health Canada and Environment Canada, 2001). Daily intake of formaldehyde has been estimated to be between 1.5 and 14 mg/day for an average adult (IPCS, 1989; Fishbein, 1992).

6.1.2. Absorption, Distribution, Metabolism, and Excretion

In water, less than 0.1% of formaldehyde exists unhydrated, with the majority reported to be in the hydrated form, methylene glycol ($CH_2(OH)_2$) (Priha et al., 1996). Formaldehyde is a reactive molecule that can react with both low molecular weight cellular components (e.g., GSH) as well as high molecular weight components. It is also a well-known cross-linking agent.

Further, formaldehyde is a product of normal cellular metabolic processes. Endogenous formaldehyde is a constituent of the one-carbon pool. It is thought that most endogenous formaldehyde exists in a form that is reversibly bound to nucleophiles (Heck et al., 1990; 1982) and that the formaldehyde hemithioacetal adduct formed with glutathione could account for 50−80% of the total formaldehyde normally present in cells (Heck et al., 1982).

Inhaled formaldehyde is efficiently absorbed ("scrubbed") in the upper respiratory tract. The fraction that is absorbed was determined to be approximately 97% in rats (Morgan et al., 1986), and 85% and 90% respectively in computer simulations of one rhesus monkey and human at rest (Kepler et al., 1998; Kimbell et al., 2001b). As the inspiratory rate increased, this fraction decreased to about 70% during light exercise and to 58% during heavy exercise conditions in the human (Kimbell et al. 2001). During heavy exercise, the absorption of formaldehyde in the first six to eight generations of the tracheobronchial airways is estimated to be comparable to that in the nasal region (Overton et al., 2001).

Airway geometry is an important determinant of inhaled-formaldehyde dosimetry in the respiratory tract. There are large differences across species in the anatomy of the upper respiratory tract and in airflow patterns. Using computer simulation, the regional uptake patterns of formaldehyde in the upper respiratory tract are observed to be spatially nonhomogeneous and to exhibit strong species differences. Airflow patterns are also significantly different as breathing patterns and activity profiles change, depending on whether breathing is oral or nasal.

The overall information on the disposition of inhaled formaldehyde comes from many studies using different experimental methods including: [^{14}C] radiolabeling, gas chromatography-mass spectroscopy (GC-MS), dual isotope labeling (^{3}H, ^{14}C) and high-performance liquid chromatography (HPLC) studies. In a study of rats following exposure to radiolabeled formaldehyde, the radioactivity was very high in the nasal mucosa but was also extensively distributed to various tissues including the bone marrow (Heck et al., 1983). The elevated ^{14}C in various tissues was thought unlikely to be due to free formaldehyde but instead to arise from either rapid metabolic incorporation or formation of covalent adducts or incorporation via carboxylation reactions of the $^{14}CO_2$ formed during metabolism (Heck et al., 1983; Casanova-Schmitz et al., 1984). Studies using the GC-MS method indicate that exposure to formaldehyde over a wide range of exposure concentrations and durations does not result in elevated levels in blood, above those of endogenous formaldehyde levels in rats, rhesus monkeys and humans (Heck et al., 1985; Casanova et al., 1998). These GC-MS measurements are consistent with the conclusions that formaldehyde does not appreciably reach the blood, is rapidly metabolized, interacts with macromolecules when it escapes metabolism, or is otherwise undetected.

In further studies on the disposition of inhaled formaldehyde, Casanova-Schmitz et al. (1984) and Casanova-Schmitz and Heck (1983) used dual-isotope labeling of inhaled formaldehyde as an approach to distinguish between formaldehyde adduct formation and metabolic incorporation. These were followed by more sensitive experiments using HPLC measurements in rats and rhesus monkeys exposed to radiolabeled formaldehyde (Casanova et

al. 1989, 1991). Results from this sets of experiments found that labeling in the nasal mucosa was due to both covalent binding and metabolic incorporation and labeling of bone marrow macromolecules was found to be entirely due to metabolic incorporation. Overall, Heck, Casanova-Schmitz, and their coworkers interpreted the results of these experiments to indicate that inhaled formaldehyde does not reach distant sites (beyond the portal of entry) at detectable levels.

Formaldehyde is primarily metabolized by glutathione-dependent formaldehyde dehydrogenase. In humans this enzyme is referred to using the protein code of ADH3. The major factor in the disposition of formaldehyde is metabolic clearance by oxidation to formate, which is either further metabolized to CO_2 and water, incorporated into the one-carbon pool, and/or eliminated in the urine as a sodium salt.

In radiolabeling studies, Heck et al. (1983) determined that the relative contributions of various excretion pathways in F344 rats following inhalation exposure to formaldehyde were independent of exposure concentration. Nearly 40% of inhaled [^{14}C] -formaldehyde appeared to be eliminated via expiration, presumably as CO_2, while about 17% and 5% was eliminated in the urine and feces, respectively. Nearly 40% of inhaled [^{14}C] -formaldehyde remained in the carcass, presumably due to metabolic incorporation. For exposure via the oral route, absorption of [^{14}C] -formaldehyde (7 mg/kg) in rats resulted in 40% exhaled (as $^{14}CO_2$), 10% excreted in urine, 1% excreted in feces, and much of the remaining 49% retained within the carcass, presumably due to metabolic incorporation (IARC, 1995; Buss et al., 1964).

Several human and animal studies have reported formaldehyde in exhaled breath (see Section 3.6.2). However, limitations of analytical techniques employed for breath analysis can only tentatively identify formaldehyde (Španěl and Smith, 2008, Wehinger et al., 2007). A recent study has illustrated that the use of proton transfer reaction in SIFT-MS may result in false positive results for formaldehyde as the characteristic analytical product ion for formaldehyde is also produced from methanol and ethanol (Španěl and Smith, 2008). Therefore, ethanol and methanol in exhaled breath will contribute to the analytical product tentatively identified as formaldehyde in the existing literature. Additionally, some studies do not have appropriate control samples to define formaldehyde levels for inhaled air prior to breath analysis. Therefore, the two major limitations of available studies of formaldehyde levels in human breath include the potential for false positives for formaldehyde from the primary analytical technique for breath analysis and the need for concurrent room air controls.

Although several studies of healthy subjects report levels of formaldehyde between the detection limit and 12 ppb (Wang et al., 2008; Cap et al., 2008 and Kushch et al., 2008), there was no adjustment for an artifact in the analytical method that makes it impossible to distinguish

between formaldehyde and reaction products for 1% of exhaled methanol and ethanol which are detected at the same mass to charge ratio as formaldehyde in these analytical techniques (Spanel and Smith, 2008). To date, there is no published study of formaldehyde in exhaled breath which makes this adjustment for reporting formaldehyde levels. Therefore, reports of formaldehyde in exhaled breath should be carefully interpreted as the mass reported as formaldehyde—is only tentatively identified as formaldehyde. A review of the data where methanol and ethanol levels are also provided, indicate that levels of formaldehyde (tentatively identified as m/z = 31) may reflect a significant contribution from reaction products of methanol and ethanol (see Section 3.6.2). In summary, there are insufficient data at this time to confidently establish a concentration of formaldehyde in exhaled breath that can be attributed to endogenous sources. This assessment identifies a critical research need for further studies on the measurement of exhaled formaldehyde.

6.1.3. Noncancer Health Effects in Humans and Laboratory Animals

A wide variety of human clinical and observational epidemiology and animal studies provide evidence for health effects in response to formaldehyde exposure. Some of these health effects are commonly noted at the portal of entry, as expected for exposure to a reactive gas. In addition, effects on the nervous and reproductive systems, developmental effects, and immunomodulation have been reported. The overall weight of evidence (WOE) of human and animal studies for the hazard potential of formaldehyde is discussed below, along with information on plausible modes of action (MOAs).

6.1.3.1. *Sensory Irritation*

Formaldehyde, a chemical irritant, binds to protein receptors of the trigeminal nerve, triggering a burning and painful sensation in humans. This process is distinct from taste and smell (Nielsen 1991; Cometto-Muniz and Cain, 1992). The trigeminal nerve, which has three branches (ophthalmic, maxillary and mandibular), not only acts as an afferent nerve relaying these sensations to the central nervous system, but also has efferent nerve activity (Stedman's Medical Dictionary: Meggs, 1993). Stimulation of the trigeminal nerve may result in reflex responses including lacrimation, coughing, and sneezing. Both the reflex responses as well as sensations such as burning, pain, and itching of the eyes, nose, and throat are considered adverse.

Formaldehyde-induced eye, nose, and throat irritation has been well documented in a wide range of epidemiologic studies. Common effects of chemically-induced sensory irritation include lacrimation, burning of the eyes and nose, rhinitis, burning of the throat, and cough

(Feron et al., 2001). Studies examining these endpoints were either controlled chamber studies with a defined population (e.g., healthy volunteers or sensitive individuals), worker/student studies, or general population studies (e.g., residential). Chamber studies, by design, are acute studies, although some researchers have investigated the outcomes after repeated exposures. Occupational, student, and residential exposures are generally of longer duration, although there is variability in exposure level and duration among subjects. The endpoints for assessing irritation include self-reporting of symptoms (e.g., pain, burning, itching) and objective measures of irritation (e.g., eye-blink counts, lacrimation).

Eye irritation is the most sensitive of reported effects in human studies. Two different short-term chamber studies provide similar 10% BMDLs for eye irritation of 560 ppb and 240 ppb for 3 and 5 hour exposures, respectively (Kulle, 1993; Andersen and Molhave, 1983, modeled by Arts et al., 2006b). Various occupational studies have noted increased eye irritation for average exposures ranging from 180 ppb to 690 ppb (Horvath et al., 1988, Alexandersson and Hedenstiera, 1998; Holmström and Wilhelmsson, 1988). The results of residential studies, where in-home formaldehyde levels are used to document exposure, indicate eye irritation may increase with increasing exposure from 70 to 200 ppb for these chronic exposure scenarios (Ritchie and Lehnen, 1987, Hanrahan et al., 1984; Liu et al., 1991.)

When a rodent is exposed to an irritant, the inhaled dose and pattern of deposition can be profoundly affected by reflex bradypnea, a protective reflex observed in rodents but not in humans. Reflex bradypnea is manifest as markedly decreased activity or prostration, reduced metabolism, hypothermia (as much as 5ºC), significantly reduced respiratory rate and minute volume, and altered blood and brain chemistry. Reflex bradypnea can occur when the trigeminal nerve is exposed to a sufficient concentration of an irritant, such as formaldehyde. Because of their small size, rodents are able to rapidly lower their metabolism and body temperature and therefore their oxygen demand. The consequence is that their inhaled dose of an irritating chemical is dramatically lowered. Reflex bradypnea is quantified as the RD_{50}, which is the concentration of a chemical that results in a 50% decrease in respiratory rate (see Tables 4-7 and 4-8). After the irritant exposure is removed, it can take up to two hours for rodents to fully recover from the effects of reflex bradypnea. Even though humans do not exhibit reflex bradypnea, involvement of trigeminal nerve stimulation, which is the mechanism for reflex bradypnea in rodents, may be relevant to MOAs for formaldehyde in other species, such as primates and humans. For example, trigeminal nerve stimulation has been associated with sensory irritation in humans, highlighting the relevance of this effect.

6.1.3.2. *Respiratory Tract Pathology*

Formaldehyde-induced respiratory tract pathology includes inflammation, rhinitis, goblet cell hyperplasia, metaplastic changes, squamous cell hyperplasia, and impaired mucociliary transport. Formaldehyde binding to the trigeminal nerve triggers the release of neurogenic mediators of inflammation resulting in tissue edema, lacrimation, mucus production, and leukocyte infiltration. Therefore, observed pathological changes may be directly related to neurogenic inflammation from activation of the trigeminal nerve or result, at least in part, from formaldehyde-induced cell damage to the mucosal tissue. A series of exposures has also been positively associated with reduced mucociliary clearance, and the induction of histopathologic lesions in the nose in both human and animal studies assessing formaldehyde-induced changes in the nasal mucosa suggest that these changes may be, at least in part, a protective or adaptive response and that increased mucus flow and metaplastic changes would progress in relation to the concentration and duration of exposure protecting the underlying tissue (Swenberg et al., 1983).

In rodent studies, formaldehyde-induced histopathological lesions ranging from inflammation to ulceration, necrosis, and metaplasia have been frequently reported in nasal turbinates, maxilloturbinates, and in goblet and microvilli cells (e.g., Bhalla et al., 1991; Monteiro-Riviere and Popp, 1986; Cassee and Feron, 1994; Ionescu et al., 1978; Schreibner et al.,1979; Monticello et al., 1989). These effects were observed after a variety of exposure scenarios (e.g., 10 ppm for 4 hrs (Bhalla et al., 1991), 0.5 or 2 ppm for 6 hrs/day for 1 or 4 days and 6 or 15 ppm for 6 hrs/day for 1 or 2 days (Monteiro-Riviere and Popp, 1986), 3.6 ppm intermittently for 3 days (Cassee and Feron, 1994), 3% aerosols of formaldehyde for 3 hrs/day for 50 days (Ionescu et al., 1978)). The progressive pathology of the nasal passages from formaldehyde inhalation exposure is dependent on increasing concentration and duration of exposure, as well as from proximal to distal regions of the nasal cavity. For example, some lesions may be transient (e.g., low-exposure cell proliferation), while others may have a maximum response and be irreversible (e.g., allergic rhinitis). The nasal epithelium responds with both adaptive and adverse epithelial changes. As respiratory epithelium transitions to squamous metaplasia, the effective tissue dose of formaldehyde increases posterior to these lesions. As epithelial barriers degrade (e.g., squamous metaplasia, keratinization), formaldehyde penetrates more deeply into the nasal passages. Therefore, the relationship between concentration and duration of exposure and health outcomes has been difficult to define and, in fact, may be different for various health effects. Formaldehyde-related histopathological lesions of the nasal mucosa have been observed at concentrations as low as 2 ppm for chronic exposure

and after a duration as short as 6 hrs at higher concentrations (e.g., 6 ppm) (**see** Table 4-32, Table 4-38).

Similar pathology has been reported for workers exposed to formaldehyde, including loss of cilia, goblet cell hyperplasia, and cuboidal and squamous cell metaplasia and dysplasia, and these pathology scores were significantly elevated in workers over controls (Holmström and Wilhelmsson, 1988; Edling et al., 1988; and Boysen et al., 1990). Holmström and Wilhelmsson (1988) reported associations between the mean daily exposure of 240 ppb (8hr TWA) and these changes. Edling et al. (1988) reported that workers experienced a range of exposures (80−900 ppb), with peak exposures of 4,000 ppb. Boysen et al. (1990) provided a range of estimated exposures from 500 ppb to more than 2,000 ppb for workers with elevated mean pathology scores. One controlled chamber study indicated formaldehyde-induced inflammatory changes which persisted for 18 hours in adults exposed at 400 ppb for only 2 hours (Pazdrak et al., 1993).

Short-term formaldehyde exposure also impairs the function of the mucociliary apparatus which is a critical defensive barrier for the upper respiratory tract. Numerous laboratory animal studies have reported impaired mucociliary clearance activity associated with formaldehyde exposures as low as 500 ppb (see Table 4-10). Low-concentration or short-term exposures first lead to an increased rate of ciliary beat, followed by impaired mucus flow, with slowed rate of ciliary beat and eventual mucostasis (lack of mucus flow) and ciliastasis (lack of ciliary beat) occurring at higher doses or longer exposure times. These effects have been shown to be both concentration- and duration-dependent and to occur within 15 minutes after the initial exposure. Morgan et al. (1983c) suggested that the initial stimulation of ciliary activity may be a defensive response to the irritant gas, at which time some penetration of formaldehyde to the underlying epithelial cells may occur. Later effects of mucostasis and ciliastasis may occur as a result of formaldehyde-induced glycoprotein cross-links, creating a rigid mucus that effectively stops mucus flow.

Formaldehyde-induced cell proliferation has been demonstrated in nasal epithelium in animal studies after a range of exposure conditions (e.g., Swenberg et al., 1986; Cassee and Feron, 1994; Reuzel et al., 1990; Woutersen et al., 1987) (see Table 4-43). Formaldehyde-induced histopathology and mitogenesis may occur as a direct effect of exposure (Tyihak et al., 2001) or as a secondary effect resulting from adaptive responses and/or compensatory tissue repair that can occur after formaldehyde exposure (Swenberg, 1983). In a study of Rhesus monkeys Monticello et al. (1996) noted that increased cell proliferation was seen in locations with minimal histological changes in the respiratory tract indicating that cell proliferation may be a more sensitive predictor of more severe health effects due to formaldehyde exposure.

Cellular proliferative responses may initiate lesion formation. A number of studies illustrate that the duration of repeated exposures may be an important determinant of cell proliferation rates (Wilmer et al., 1987; Swenberg et al., 1986). Reduced mucociliary clearance and the induction of histopathologic lesions in the nose effects have been noted in human formaldehyde studies.

Histopathological lesions and biochemical changes have been reported in the lung following formaldehyde inhalation exposure in experimental animal studies (Kamata et al., 1996a; Ionescu et al., 1978) following high exposure levels (128.4 or 294.5 ppm formaldehyde).

6.1.3.3. *Effects on Pulmonary Function*

The potential of formaldehyde exposure to cause pulmonary functional deficits in humans has been examined on several time scales. The epidemiologic literature includes studies of acute exposures among naïvely exposed anatomy graduate students (Kriebel et al., 1993; 2001), anatomy graduate students with several weeks of episodic exposure (Kriebel et al., 1993), and post-shift versus pre-shift worker pulmonary function among those with regular occupational exposure (Malaka and Kodama, 1990; Herbert et al., 1994; Alexandersson et al., 1982; Alexandersson and Hedenstierna, 1989). Depending on whether the exposures are naïve or not, the epidemiologic studies that assessed the pulmonary effects after acute exposures to formaldehyde are assessing different biological responses, namely, the acute effect alone or the acute effect(s) in people who may have already been sensitized to different and unknown degrees.

The observed effects in the previously unexposed anatomy students provide additional information on acute exposures in two naïve populations (Kriebel et al., 1993; 2001), as well as insight into the possible intermediate stages of sensitization (Kriebel et al., 1993). Kriebel and colleagues (1993) examined the prelaboratory and postlaboratory peak expiratory flow (PEF) in students attending anatomy classes once a week. They found the strongest pulmonary response when examining the average cross-laboratory decrement in peak expiratory flow in the first 2 weeks of the study when formaldehyde concentrations collected in the breathing zones had a geometric average concentration of 0.73 ppm. Overall, the students exhibited a 2% decrement in PEF, while the students with any history of asthma showed a 7.3% decrement in PEF. These findings of acute decreases in PEF following students' initial formaldehyde exposure were corroborated by the Kriebel et al. (2001) study, using a similar study design applied to a separate class of anatomy students. Similar findings have been reported for low-level residential formaldehyde exposure including decreased peak expiratory flow rates (PEFRs) (Krzyzanowski et al., 1990). Workers chronically exposed to formaldehyde have exhibited signs of reduced

lung function consistent with bronchial constriction, inflammation, or chronic obstructive lung disease. Lung function deficits have been reported both in preshift versus postshift measurements *and* as a result of chronic exposures (Malaka and Kodama, 1990; Herbert et al., 1994; Pourmahabadian et al., 2006, Alexandersson et al., 1982; Alexandersson and Hedenatiena 1989). Decreases in spirometric values, including vital capacity (VC), forced expiratory volume (FEV), forced vital capacity (FVC) and FEV/FVC have been reported in humans. Chronic studies also reported increased respiratory symptoms such as cough, increased phlegm, asthma, chest tightness and chest colds in exposed workers (Malaka et al., 1990; Herbert et al., 1994; Pourmahabadian et al., 2006, Alexandersson et al., 1982; Alexandersson and Hedenatiena 1989). Similar findings have been reported following low-level residential formaldehyde exposure including decreased PEFRs (Krzyzanowski et al., 1990).

Worker exposures associated with cross-shift differences in spirometric values are consistent with formaldehyde-induced sensory irritation. Concordance has also been reported between subjective irritant response and measured changes in pulmonary function further supporting the possibility that cross-shift and short-term evidence of bronchial constriction may be a reflexive response to sensory irritation.

A well-conducted residential epidemiology study by Krzyzanowski et al. (1990) was considered to be the strongest among the candidate studies on the adverse pulmonary function effects of formaldehyde for the purposes of deriving an RfC.

6.1.3.4. *Asthmatic Responses and Increased Atopic Symptoms*

The health effects of respiratory function, asthma and increased atopic response, have been shown to be clinically related. For example, asthma affects pulmonary function and may be triggered by an allergic response. These and other data suggest that there may be mechanistic links between these two health effects. Formaldehyde-induced sensitization (see Section 4.2.1.5) may enhance the asthmatic response or may enhance an individual's response to an allergen (see Section 4.4). In both cases, sensitization results in phenotypic switching—or an individual exhibiting clinical symptoms of a predisposition to asthma or atopy. Because of the connection between the two endpoints, they are considered together herein.

Several cross-sectional studies have described a positive association between formaldehyde concentration and asthma prevalence. A study on risk factors for the initial physician diagnosis of asthma has shown concentration-dependent associations between formaldehyde exposure and asthma (Rumchev et al., 2002). In a categorical analysis, Rumchev et al. (2002) observed statistically significant effects above in-home formaldehyde concentrations of 60 $\mu g/m^3$, with increased but nonsignificant effects at 50−59 $\mu g/m^3$ that were

consistent with a concentration-response relationship. No effect was apparent at concentrations in the next lower interval between 30−49 μg/m^3. Garrett et al. (1999 a,b) reported a borderline statistically significant association between bedroom formaldehyde concentrations and an increased risk of atopy. The authors computed a respiratory symptom score for each child based on the frequency of each of eight respiratory symptoms and this score was substantially and statistically significantly higher among the asthmatic children compared to nonasthmatic children. Health effects were reported at formaldehyde concentrations greater than 50 μg/m^3 but the lowest formaldehyde concentration interval at which health effects were observed was 20−50 μg/m^3. The findings of Garrett et al. (1999 a,b) are supported by the results of a chamber study reported by Casset et al. (2006) of 19 sensitized adult asthmatics exposed to formaldehyde at a concentration of 100 μg/m^3 for 30 minutes. Casset and colleagues observed an increased bronchial responsiveness to mite allergen exposure (p = 0.05) and noted the provocative dose (PD20 for FEV1) for mite allergen was 34.3 ng after formaldehyde exposure and 45.4 ng after air exposure. However, in study by Ezratty et al. (2007) exposure to 500 μg/m^3 formaldehyde did not affect an allergen-induced increase in responsiveness to methacholine (p = 0.42) and there was no formaldehyde-associated effect on the airway inflammatory response.

These observed health effects in humans are similar to the outcome of studies in laboratory animals that show that formaldehyde can exacerbate existing immunogenic hypersensitivity to known allergens (Sadakane et al., 2002; Tarkowski and Gorski, 1995; Riedel et al., 1996). While potentiation varied based on sensitization protocols and formaldehyde exposure regimens, the results support the finding that formaldehyde exposure can aggravate a Type-I hypersensitivity response and may do so via a neurogenically initiated response. Formaldehyde itself does not function as an allergen recognized by the immune system (Lee et al., 1984) and does not appear to trigger formation of formaldehyde-specific IgE. Although formaldehyde exposure has been reported to alter cytokine levels and immunoglobulins in some experimental systems (Fujimaki et al., 2004a; Ohtsuka et al., 2003), these effects do not support an immunogenically mediated type-I hypersensitivity. In studies in which either egg protein (ovalbumin, OVA)-sensitized or dust mite (DerF)-sensitized animals were exposed to formaldehyde, OVA-specific and DerF-specific antibody production was increased over sensitization alone, suggesting that formaldehyde may potentiate sensitization responses (Riedel et al., 1996; Sadakane et al., 2002). Formaldehyde-induced sensitivity responses may be neurogenic in origin based on findings that neurogenic factors such as nerve growth factor (NGF) and substance P were associated with formaldehyde exposure in sensitization protocols (Fujimaki et al., 2004b).

6.1.3.5.*Effects on the Immune System*

Formaldehyde-induced systemic immunomodulation in laboratory animals has been documented in the literature (Leach et al., 1983; Dean et al. 1984; Adams et al., 1987). A number of studies have evaluated the ability of formaldehyde to induce systemic immunotoxic effects in humans (Ohtani et al., 2004a, b; Erdei et al., 2003; Thrasher et al., 1990, 1987; Pross et al., 1987). Some studies have reported altered innate immune responses associated with formaldehyde exposure (Erdei et al., 2003), while others have noted adaptive immune response suppression associated with formaldehyde exposure (Thrasher et al., 1990, 1987) and changes associated with alterations to a predominant T-lymphocyte helper 2 (Th2) pattern (Ohtani et al., 2004a, b). In contrast, Pross et al. (1987) did not observe formaldehyde-associated changes in systemic immune function.

Diverse studies have investigated the possibility that formaldehyde exposure leads to increased respiratory tract infections (Lyapina et al., 2004; Krzyzanowski et al., 1990; Holness and Nethercott, 1989). Lyapina et al. (2004) reported increased respiratory tract infections and decreased neutrophil respiratory burst activity (NRBA) in formaldehyde-exposed workers (at 722 ppb TWA). Incidences of doctor-diagnosed chronic bronchitis were more prevalent in children under age 15 living in homes with higher formaldehyde (>60 ppb) readings in the kitchen ($p < 0.001$) (Krzyzanowski et al., 1990). Holness and Nethercott (1989) also report increased chronic bronchitis in formaldehyde-exposed funeral workers (380 ppb average exposure).

6.1.3.6.*Neurological Effects*

Formaldehyde exposure via inhalation has been shown to adversely impact nervous system function in laboratory animals and humans, although human data for formaldehyde-induced neurological effects are limited. Studies in formaldehyde-exposed histology technicians provide evidence of neurological impairment, including lack of concentration, impaired memory, disturbed sleep, impaired balance, variations in mood and irritability. These effects were significantly correlated with increasing duration of exposure to formaldehyde, but the findings are not conclusive due to confounding by concomitant exposures to other neurotoxic solvents (Kilburn et al., 1985, 1987). In a prospective study, Weisskopf et al. (2009) found a strong association between duration of formaldehyde exposure and death from amyotrophic lateral sclerosis (ALS), but information regarding exposure levels was not available. Short-term studies with controlled exposure to humans (chamber studies) also provide limited support for changes in cognitive function immediately following a single, controlled formaldehyde exposure (Bach et al., 1990; Lang et al. 2008).

Available animal data provide substantial evidence of behavioral changes in animals following single or short-term repeated inhalation exposures to relatively low levels of formaldehyde. Among the animal studies, none of the available studies examined effects on nervous system function following chronic formaldehyde inhalation, however.

Reported perturbations in nervous system function following formaldehyde exposure in animal studies include reductions in motor activity, lack of habituation, impairment in acquisition of a new learning task, deficits in retention of a previously learned task, increases in corticosterone levels, sensitization to cocaine-induced locomotor activity, and enhanced fear conditioning using an olfactory conditioned stimulus (CS) (see Table 4-57). Behavioral effects have been seen in multiple laboratories and in studies conducted by different investigators using a variety of testing paradigms. Many of these effects were observed at acute exposure levels at or below 1.0 ppm, and some persisted days to weeks after termination of exposure.

More limited data indicate possible effects on the development of the nervous system, including changes in brain structure and in the behavior of offspring (see Table 4-57). Similarly, there is very little information regarding the mechanism by which effects on the nervous system might be produced. The data regarding behavioral sensitization provide some support for a stress-related mechanism for those specific findings, but the applicability of this mechanism to the behavioral changes seen in the other studies, including the learning deficits and developmental findings, has not been evaluated. Although there are data supporting stimulation of the trigeminal nerve by formaldehyde (and documenting the relevance of this interaction to the sensory irritation caused by formaldehyde), there are no data supporting a causal relationship between irritant properties of formaldehyde and the behavioral and neurodevelopmental effects in humans that occur following formaldehyde exposure. In summary, none of the available data provide sufficient information to allow a determination of the mode of action for effects of formaldehyde on the adult or developing nervous system.

6.1.3.7. *Reproductive and Developmental Effects*

Formaldehyde inhalation exposure has been associated with adverse developmental and reproductive outcomes in both epidemiologic studies and experimental animal studies. Observed developmental outcomes include fetal loss, structural alterations, growth retardation, and delays in functional development.

Several occupational studies found an increased risk of spontaneous abortions among formaldehyde-exposed women (Taskinen et al., 1999, 1994; John et al., 1994; Seitz and Baron, 1990; Axelsson et al., 1984). The Taskinen et al. (1999) study examined several reproductive outcomes in women employed in the wood-processing industry, with a range of average daily

formaldehyde exposures. The authors found that formaldehyde was associated with a more than three-fold increased risk of spontaneous abortion, and with a nearly 50% decrease in a measure of delayed conception indicating reduced fertility, an increased time to pregnancy, and an increased risk for endometriosis in this study. In experimental animal studies, early fetal death was noted following maternal formaldehyde exposures (Kitaev et al., 1984; Sheveleva, 1972), supporting the epidemiologic findings that the spontaneous abortion is likely related to formaldehyde exposure. Kitaev et al. (l984) hypothesized that formaldehyde may affect reproductive function by stimulating the hypothalamus-pituitary-gonadal (HPG) axis, based on their observations of increased ovary weight, increased number of ovulating cells, and changes in blood levels of gonadotropins (LH and FSH) in female rats. Additionally, Maronpot et al. (l986) reported endometrial hypoplasia with a lack of ovarian luteal tissue in formaldehyde-exposed female rats. This finding may be relevant to the increased risk for endometriosis noted in the Taskinen et al. (1999) study. However, additional human and animal studies are needed to better understand the effects of inhalation exposure to formaldehyde on developmental outcomes after early gestational windows of exposure or on the female reproductive system.

The findings of some occupational studies have suggested formaldehyde-related associations with congenital malformations and low birth weight. In numerous experimental animal studies, developmental effects have been noted following inhalation exposures to formaldehyde (see Table 4-68). Exposure of rat dams to formaldehyde during pregnancy has been shown to result in significantly decreased fetal weight gain (Martin, 1990; Saillenfait et al., 1989; Kilburn and Moro, 1985). Other studies have noted changes in relative organ weight, undescended testes, biochemical changes (e.g., ascorbic acid), and blood acidosis (Senichenkova and Chebotar, 1996; Senichenkova, 1991; Kilburn and Moro, 1985; Gofmekler and Bonashevskaya, 1969; Gofmekler, 1968; Pushkina et al., 1968).

Studies designed to assess adult male reproductive system toxicity in rats following repeated inhalation exposures to formaldehyde have found concentration-dependent decreases in Leydig cell number and quality, degeneration of seminiferous tubules, decreases in testes weight, alterations in sperm measures, decreased testosterone levels, alterations in trace metals in the testes, and/or dominant lethal effects (Guseva, 1972; Özen et al., 2002, 2005; Sarsilmaz et al., 1999; Xing et al., 2007; Zhou et al., 2006) (see Table 4-71).

6.1.3.8. *Effects on General Systemic Toxicity*

Extrapulmonary effects such as changes in liver function enzymes and focal, chronic inflammation in the heart and kidney have been observed due to formaldehyde exposure in experimental animal studies. Most of these changes occurred at exposures of 20 ppm, and those

that occurred at lower formaldehyde exposures (3.7 ppm) were confounded by coexposures. The underlying modes of action of liver, kidney, and cardiac effects have not been elucidated, and the human relevance is unknown.

6.1.3.9.*Summary*

Formaldehyde-induced eye, nose and throat irritation, decreased pulmonary function, decreased mucociliary clearance and histopathological lesions have been extensively documented in human and laboratory animal studies. These health effects are commonly noted at the portal of entry as expected for exposure to a reactive gas. In addition, effects on immune system responses and on the nervous and reproductive systems, including developmental effects, have also been reported. An association between formaldehyde exposure and increased incidence and severity of response to allergens (i.e., asthma and atopy) has been noted in humans. This effect, which has also been studied in laboratory animals, might occur via a neurogenic mode of action. A limited database of information that evaluates neurological effects in humans following formaldehyde exposure demonstrates a potential for adverse outcomes, and studies in laboratory animals have reported a variety of formaldehyde-induced neurobehavioral and neurodevelopmental effects. Formaldehyde has also been associated with adverse reproductive outcomes. Epidemiology studies have reported an association between formaldehyde exposure and decreased fertility as well as an increased risk of spontaneous abortions. Other epidemiology studies have suggested formaldehyde-related associations with congenital malformations, low birth weight, and endometriosis. Animal studies have noted a variety of developmental effects, including fetal death, structural alterations, and growth retardation (e.g., delayed fetal skeletal ossification and decreased fetal body weight) following inhalation exposure to formaldehyde, and adverse reproductive effects have been observed in both males and females.

6.1.4. Carcinogenicity in Humans and Laboratory Animals

6.1.4.1.*Carcinogenicity in Humans*

Upper respiratory tract cancers:

Epidemiologic studies of formaldehyde-exposed workers provide sufficient evidence of a causal association between formaldehyde exposure and nasopharyngeal cancer (see Section 4.1.2.1.1) as well as nasal and paranasal cancers (see Section 4.1.2.1.2). The epidemiologic evidence of association between formaldehyde exposure and other upper

respiratory tract cancers (see Section 4.1.2.1.3) is consistent with, and supportive of, a causal association but insufficient on its own to reach a causal conclusion. However, taken together with the causal evidence of an association between formaldehyde and nasopharyngeal cancer and sinonasal cancer in neighboring tissues of the upper respiratory tract and sites of first contact with inhaled formaldehyde, along with the strongly supportive evidence of association in animals, the evidence is sufficient to conclude that formaldehyde is causally related to cancers of the upper respiratory tract as a group.

Based on the total weight of evidence, including the results from a large and well-followed longitudinal cohort study of 25,619 industrial workers and several case-control studies, the epidemiologic evidence is sufficient to characterize the association between formaldehyde nasopharyngeal cancer as causal in humans (Hauptmann et al., 2004; Hildesheim et al., 2001; Vaughan et al., 2000). As further evaluated below, the evidence supporting a positive association between formaldehyde exposure and NPC is unlikely due to chance, bias or confounding. However, it should be noted that other smaller studies of formaldehyde-exposed workers did not document increased NPC mortality (e.g., Coggon et al., 2003; Pinkerton et al., 2004). These smaller study sizes yielded effect estimates with wide confidence intervals that were not statistically inconsistent with the increased risk of mortality from nasopharyngeal cancer reported in Hauptmann et al. (2004).

Luce et al. (2002) evaluated pooled data from 12 case-control studies conducted in seven countries using a common job-exposure matrix and demonstrated a statistically significant increased risk between formaldehyde exposure and sinonasal cancer exhibiting a concentration-response relationship providing further causal evidence of carcinogenicity. This analysis was based on a very large dataset of 930 cases and 3,136 controls, enabling the investigators to control for multiple potential sources of bias and confounding and to conduct separate analyses by histological type. These results are particularly convincing, as the association was consistently seen for a rare subtype of sinonasal cancer which normally accounts for only 10% of the reported cases.

In addition to the evidence of formaldehyde carcinogenicity in the nasopharynx, nose and sinuses, other upper respiratory tract sites of direct contact with formaldehyde upon inhalation (i.e., larynx, mouth and salivary gland) also showed evidence of increasing relative risk with increasing average intensity and peak exposure in a large cohort study with exposure estimates for the individual workers, although these trends did not reach the level of statistical significance (Hauptmann et al., 2004). However, Hauptmann and colleagues (2004) concluded that in spite of the small numbers of deaths from these rare cancers of the upper respiratory tract, the positive associations of increased cancer risk with increased formaldehyde exposure were consistent with

the carcinogenicity of formaldehyde at these sites of first contact. Case-control studies also provide evidence of an association between formaldehyde exposure and oral squamous cell carcinoma (SCC), esophageal, and laryngeal cancers, and hypopharyngeal cancer (Gustavsson et al., 1998; Laforest et al., 2000.)

The finding that formaldehyde inhalation causes nasal squamous cell carcinoma in rodents (see Section 4.2.1.2) further supports the determination of a causal association of formaldehyde exposure and increased risk of upper respiratory tract cancer in humans. Both humans and animals developed tumors within the upper respiratory tract, the site expected to receive direct exposure to formaldehyde.

Several researchers have argued that the relationship between formaldehyde exposure and nasopharyngeal cancer based on existing studies has not been determined. Several limitations, such as the rarity of the cancer and the imprecise estimates of exposure, are often inherent in epidemiologic methods and exposure assessment. These constraints limit the ability of epidemiologic studies to statistically detect associations and can lead to false negatives. The results of the largest cohort study of nasopharyngeal cancer (Hauptmann et al., 2004) showed statistically significant concentration-response relationships with increased risk of cancer associated with increased formaldehyde exposure. However, even though this study was based on 25,619 workers, only 9 cases of nasopharyngeal cancer were observed, compared to an expected number of 5 cases, for a relative rate of 2.1 (with a confidence interval of 1.05−4.21) (Hauptmann et al., 2004).

The next largest cohort study of nasopharyngeal cancer was based on 14,014 workers (Coggon et al., 2003) and reported only 1 case compared to an expected number of 2 cases, for a relative risk of 0.5 (with an estimated 95% confidence interval of 0.07 − 3.55; see Bosetti et al., 2008). To put this finding into perspective, it is helpful to note not only the relative risk but also that this effect estimate is highly unstable due to a lack of statistical power. The large width of this interval (0.07 − 3.55) indicates that the range of possible true values includes both increased and decreased NPC mortality and therefore does not contradict the evidence of elevated risk of nasopharyngeal cancer mortality associated with formaldehyde exposure reported by Hauptmann et al. (2004). The even smaller study of 11,039 textile workers by Pinkerton et al. (2004) reported no cases of nasopharyngeal cancer compared to an expected number of one case—yielding an effective relative risk of zero with a highly unstable 95% confidence interval estimated at 0 − 3.00 (see Bosetti et al., 2008). While true that Pinkerton et al. (2004) did not report an increased risk of nasopharyngeal cancer, this study did not have sufficient statistical power to rule out a true association with less than a 3-fold increase in risk and therefore is likewise not inconsistent with the finding by Hauptmann et al. (2004). Thus, results from these

cohort studies, with limited power to detect the relatively rare upper respiratory tract cancers (e.g., NPC), are given less weight in the overall evaluation.

The largest occupational cohort study, conducted by the NCI (Hauptman et al., 2004), did report statistically significant associations of formaldehyde exposure with carcinogenicity at the sites of first contact with sufficient statistical power to rule out the null hypothesis of no association. The NCI investigations controlled for potential selection bias due to the healthy worker effect and for several potential confounders, including calendar year, age, sex, race, and pay category. However, other potential sources of bias or confounding have been suggested with respect to the strength of these data to support a causal conclusion.

Following reports of increased risk of NPC associated with formaldehyde exposure, a series of analyses of similar data were undertaken by Marsh and coworkers (Marsh et al., 2007a, b, 2002, 1996; Marsh and Youk, 2005). Briefly, these studies focused on the specific findings from a single plant in the NCI cohort (Wallingford, Connecticut) that generated the majority of the NPC cases. Marsh et al. (1996) confirm a significant adverse association of formaldehyde with nasopharyngeal cancer but note the effects are predominantly among workers at the Wallingford plant with less than one year employment. Marsh et al. (2002) report a five-fold excess in risk of nasopharyngeal cancer associated with formaldehyde in both short-term and long-term workers but note that the increase was concentrated among workers hired during 1947−1956. Marsh and Youk (2005) re-evaluated the same Wallingford workers and reported a regional rate-based standardized mortality ratio (SMR) of 10.32 (95% CI = 3.79 − 22.47) compared to 0.65 (95% CI = 0.08 − 2.33) for workers at the nine other plants combined. However, Marsh and Youk (2005) also show that rate-based mortality ratios standardized to both United States and local populations were elevated (nonsignificantly) not only at the Wallingford plant but individually at each of the four other plants at which a single case of nasopharyngeal cancer was reported: Plant 2 (SMR_{US} = 5.35), Plant 3 (SMR_{US} = 1.99), Plant 7 (SMR_{US} = 1.06), and Plant 10 (SMR_{US} = 1.44). It should be noted that Plant 1 (Wallingford) and Plant 2 had both the two highest median formaldehyde exposures and the two highest reported excess risks (Marsh and Youk, 2005).

In another reanalysis of the NCI cohort data on the workers at the Wallingford plant, Marsh and coworkers (2007a) suggested that an imprecise assessment of formaldehyde exposure and an inability of the study to separate formaldehyde exposure from other potential chemical or particulate exposures may have confounded the observed association between formaldehyde and cancer. However, there was no evidence of any differential measurement error that could have produced the observation of a spurious association. Any nondifferential exposure measurement error (i.e., random error in the exposure assessment) would likely have led to an attenuated

observed effect of formaldehyde that was less than that which would otherwise have been observed in the absence of measurement error.

The potential for confounding by particulates was explicitly examined by Hauptmann et al. (2004) and it was shown that there was an exposure-response relationship with formaldehyde among individuals with high particulate exposures—alleviating the potential for confounding and thereby strengthening the causal interpretation of the formaldehyde relationship with an increased risk of NPC. Marsh and coworkers (Marsh et al., 2007b) later suggested the reported formaldehyde association was confounded by an association between silversmithing and NPC. However, careful examination of that analysis (Marsh et al., 2007a) suggests that multiple comparisons may have led to the reported observation with silversmithing. Additionally, the reported effect was inconsistently reported between the results and the abstract sections using different confidence intervals, and both sets of confidence intervals around the reported association were extremely unstable spanning up to several hundred-fold. No prior studies identified an associated between silversmithing and NPC. Thus it may be that silversmithing is an artifactual potential confounder.

The increased NPC mortality observed in the NCI cohort (Hauptmann et al., 2004) has been thoroughly examined for sources of bias and confounding by both the primary researchers and Marsh and coworkers (Marsh et al., 2007a, b, 2002, 1996; Marsh and Youk, 2005). Despite the extensive scrutiny of these results, no convincing and consistent alternative hypothesis of causation has been identified. Taken together with the statistically significant association demonstrating an exposure-response relationship within exposed workers, these data support the conclusion that the association between formaldehyde exposure and increased risk of NPC is causal.

Therefore, after a thorough examination of potential confounders, the association between formaldehyde exposure and NPC mortality in the NCI cohort remains significant and provides a positive exposure-response relationship. Additionally, case-control studies, which have greater statistical power than cohort studies for rare diseases, provide strong additional evidence in support of a causal association between formaldehyde exposure and the incidence of NPC (Hildesheim et al., 2001; Vaughan et al., 2000). As these studies draw from different demographic groups, regions of the world, and evaluate various confounding factors, there is little potential for these consistently reported associations to be artifactual, confounded by common exposures, or a result of bias or chance.

Lymphohematopoietic cancers:

Numerous epidemiologic studies have also reported an association between formaldehyde-exposed workers, especially "professional" workers (e.g., pathologists, embalmers, and funeral directors), and increased risk of lymphohematopoietic cancers (see Table 4-90). Positive associations between formaldehyde exposure and lymphohematopoietic cancers have been reported for chemical workers (Wong et al., 1983; Bertazzi et al., 1986), embalmers (Walrath and Fraumeni, 1983, 1984; Hayes et al., 1990), anatomists and pathologists (Harrington and Shannon 1975; Hall et al., 1991; Levine at al., 1984; Stroup et al., 1986; Matanoski et al., 1989). However, clear associations (in terms of overall standardized mortality ratios (SMRs) or proportional mortality ratios (PMRs) were not reported in analyses for garment workers, iron-foundry workers, and a large US industrial cohort (Pinkerton et al., 2004; Andjelkovich et al., 1995; Beane Freeman et al., 2009; Marsh et al., 1996), although associations were observed in some of these studies when exposure-response relationships were considered. Several published meta-analyses are available which more formally assess the strength of association between formaldehyde exposure and mortality from all lymphohematopoietic cancers (see Section 4.1.2.2.1.3). Pooled SMRs indicate stronger associations for professional workers (embalmers, anatomists and pathologists) than industry workers (see Table 4-90). Bosetti et al. (2008) found similar relationships, with a pooled SMR of 1.31 (95% CI 1.16-1.47) for 'professionals' (i.e., embalmers, anatomists and pathologists) versus a pooled estimate of 0.85 (95% CI 0.74-0.96) for industrial workers. A recent metaanalysis by Zhang et al. (2009) reports a summary relative risk of 1.25 (95% CI 1.09−1.43) for both professional and industry workers for all lymphohematopoietic cancers (ICD 9 codes 200−209).

Two well-designed cohort studies found significant positive associations between formaldehyde-exposed professional workers and lymphohematopoietic cancer, particularly leukemia, using cumulative exposure measures not previously used and using internal comparison groups. The largest cohort study of industrial workers exposed to formaldehyde (N=25,619), with the most extensive exposure assessment (Blair et al., 1986; Stewart et al., 1986) and with the cohort followed for a median duration of 35 years (Hauptmann et al., 2003) demonstrated that formaldehyde was a risk factor for lymphohematopoietic cancers, independent of other risk factors, such as benzene exposure and smoking. This finding was reconfirmed with an additional 10 years of follow-up (Beane Freeman et al., 2009). Another industrial cohort study reported a significant increase in the risk of leukemia in garment workers 20 years after their initial exposure and in workers with 10 or more years of exposure to formaldehyde (Pinkerton et al. 2004). A third large occupational cohort study (Coggon et al., 2003) that did not evaluate their findings with regard to latency reported somewhat lower mortality from

leukemia and other lymphatic and hematopoietic cancers than expected compared to national rates.

The associations between myeloid leukemia and formaldehyde exposure are strong and consistent (see Table 4-92). Of the four studies which formally assess myeloid leukemia mortality, all are positive, including cohorts of both professional and industrial workers (Beane Freeman et al., 2009; Hayes et al., 1990; Pinkerton at al., 2003; Stroup et al., 1986). Although few cases exist for further subtype analysis, the available data indicate either no differences in SMRs for acute myeloid leukemia (AML) versus chronic myeloid leukemia (CML) (Hayes et al., 1990; Pinkerton et al., 2003) or suggest CML is more prominent (Blair et al., 2000; Stroup et al., 1986). The association between formaldehyde exposure and myeloid leukemia in embalmers has recently been confirmed in a large nested case control study by Hauptman et al (2009) which includes cases identified from the previous studies of Hayes et al. (1990) and Walrath and Fraumeni (1983 and 1984). Exposure estimates were based on interviews with next-of kin for duration of job actively embalming and total number of embalmings performed. Strong and statistically significant exposure-response relationships are demonstrated for duration of exposure, total number of embalmings performed and estimated cumulative exposure to formaldehyde with odds ratios of 13.6 (1.6−119.7), 12.7(1.4−112.8) and 13.2(1.5−115.4) respectively (Hauptmann et al., 2009).

The reported associations between formaldehyde exposure and lymphohematopoietic cancers in general, and leukemia (especially myeloid leukemia) in particular, were in workers exposed in very different environments (i.e., mortuary, chemical industry and garment industry). Since coexposures to other agents are considerably different between these work environments, it is unlikely that influence of confounding exposures plays a role in the observed associations. There is no evidence of bias in the published reports, and the consistency across numerous studies over time is sufficient to conclude that the results are not due to chance. Additionally, where data are available for analysis, increased myeloid leukemia is not the sole driver of increased leukemia and all lymphohematopoietic cancers (see Table 4-91). An evaluation of the epidemiologic evidence for solid tumors of lymphoid origin indicates an association between formaldehyde exposure and both Hodgkins lymphoma and multiple myeloma, but not non-Hodkins lymphoma in general (see Section 4.1.2.2.1.4 and Section 4.5.2.6).

It has been argued that it is biologically implausible for a highly reactive agent such as formaldehyde, whose primary action is expected to be at the portal of entry, to cause acute lymphoid or myeloid leukemias (ALL and AML, respectively), which are both commonly believed to arise from transformation of stem cells in the bone marrow. The modes of action (MOAs) by which formaldehyde may induce these observed cancers are unknown, although it

has been postulated that circulating stem cells (Hauptmann et al., 2003) (e.g., early progenitor cells in circulating blood or pluripotent cells in nasal/oral passages) may travel to bone marrow where they become leukemic stem cells (Zhang et al., 2010a, b). In contrast, the mechanism for the chronic lymphatic leukemia, lymphomas, multiple myelomas (from plasma B-cells) and unspecified lymphohematopoietic cancers may involve an etiology in peripheral tissues, such as cells, cell aggregates, germinal centers and lymph nodes. An association of these cancers to a reactive exogenous agent primarily acting at the point of entry is biologically plausible.

6.1.4.2.*Carcinogenicity in Laboratory Animals*

The carcinogenic potential of formaldehyde is well documented in numerous animal bioassays, especially for sites of first contact. Inhalation exposure of formaldehyde induced primarily squamous cell carcinomas (SCC) in nasal passages of rats (Feron et al., 1988; Holmström et al., 1989a; Woutersen et al., 1989; Tobe et al., 1985; Kamata et al., 1997; Albert et al., 1982; Sellakumar, 1985; Kerns et al., 1983; Monticello et al., 1996) and mice (Battelle Columbus Laboratories, 1981; Swenberg et al., 1980; Kerns et al., 1983; CIIT, 1982). Formaldehyde given as 0.5% formalin orally in drinking water to adult rats induced higher incidences of papillomas in the forestomach, adenomatous hyperplasia in the fundus, and adenocarcinomas in the pylorus in a 40-week study using an initiation-promotion protocol in rats (Takahashi et al., 1986). Formaldehyde is toxic at the portal of entry in rodents, causing increased cell proliferation, DPX formation, and focal lesions in the GI tract or upper respiratory tract (depending on the route of exposure). The portal of entry toxicity of formaldehyde further supports a finding of formaldehyde induced POE cancer in animal bioassays.

Direct support for lymphohematopoietic cancers in animal bioassays is less convincing. Although many of the available chronic studies did not examine lymphoma/leukemia incidence, four studies allow for some evaluation of the leukemic potential of formaldehyde. Inhalation exposure of formaldehyde increased lymphoma in female mice and leukemia in female F344 rats, but not male rats (Battelle Laboratories, 1981). No increases in leukemia or lymphoma were seen in male Wistar rats when exposed to formaldehyde in drinking water (Til et al., 1989) or male rats after chronic inhalation exposures (Sellakumar et al., 1985).

6.1.4.3.*Carcinogenic Mode(s) of Action*

Multiple plausible modes of action (MOAs) are presented in the document so as to explore ways in which a combination of factors may contribute to cancer incidence in a population exposed to formaldehyde. Multiple MOAs for formaldehyde-induced cancer can be reasonably supported based on various known biological actions of formaldehyde (e.g.,

mutation, cell proliferation, cytotoxicity and regenerative cell proliferation). Additionally, alternative actions, such as immunosuppression or viral reactivation, are possible, although few data exist to evaluate their potential relevance. Rather than a single MOA, it is plausible that a combination of these factors contribute to cancer incidence in an exposed population. Considering multiple factors may help to better understand the biological and mechanistic basis for the increases in cancer incidence observed in exposed human populations. Unlike animal bioassays, results in human epidemiological studies reflect not only the effects of the agent of concern but also numerous other risk factors (e.g., viral status, diet, smoking, etc.). Additionally, human studies may be impacted by biological human variability across individuals, cancer biology (subtypes) and wide variability in exposure regimens in human populations.

The overall weight of evidence supports a role of mutagenic activity in formaldehyde's carcinogenic MOA both for respiratory tract cancer and lymphohematopoietic cancers. As reviewed in Section 4.3 and summarized in Section 4.5.3.1, numerous studies provide evidence of formaldehyde's direct mutagenic activity and supports the relevance these data to formaldehyde's carcinogenicity. It can be shown that:

1) Formaldehyde directly interacts with DNA, generating DNA-protein cross-links and DNA adducts (in vitro, in vivo) in multiple species,
2) DNA-protein cross-links exhibit a dose-response relationship to formaldehyde exposure in respiratory tract of laboratory animals and are observed at exposure concentrations of relevance to some people (0.3 ppm, 0.7 ppm),
3) Formaldehyde-induced DNA-protein cross-links have been associated with formaldehyde-induced micronuclei and chromosomal aberrations (in vitro),
4) Mutations induced by formaldehyde due to small deletions and rearrangements in DNA in various experimental systems are consistent with formaldehyde's observed clastogenic effects (micronuclei and chromosomal aberrations) (in vitro, in vivo),
5) Formaldehyde-induced mutations and clastogenic effects occur at levels below where significant cytotoxicity is detected (in vitro),
6) Formaldehyde exposure has been correlated to similar increased micronuclei and chromosomal aberrations in human buccal and oral cells corresponding to sites where formaldehyde-induced tumors arise, and
7) Chromosomal damage in blood-borne immune cells, relevant to agent-induced lymphohematopoietic cancers has been documented in formaldehyde exposed workers including increased micronuclei and chromosomal aberrations, increased incidence and aneuploidy in hematopoietic stem cells.

In addition, mutations may arise indirectly from formaldehyde-induced DNA damage during cell proliferation or due to errors in DNA repair mechanisms. Therefore, formaldehyde's DNA reactivity on a population of proliferating cells strengthens the role of formaldehyde-induced mutagenicity in its carcinogenic MOA. The nasal and gut mucosa are tissues which are continually sloughing and regenerating cells (Junqueira et al., 1992). Mucosal cells proliferate in response to environmental challenges in order to repair cell damage, increase adaptive response and remodel tissue. Additionally, since the pseudostratified epithelium of the respiratory tract is only 1−2 cells in depth, cells with proliferative capacity would be directly impacted by formaldehyde during exposure. Formaldehyde-induced clastogenic effects have been demonstrated in these tissues (e.g., nasal) in humans, as well as in tissues which possess stratified epithelium (e.g., buccal). Therefore, formaldehyde would not need to transport beyond the portal of entry to directly impact and induce DNA mutations in routinely proliferating cells.

In regards to generating the observed clastogenic effects (micronuclei and chromosomal aberrations in peripheral blood lymphocytes, aneuploidy in circulating hematopoietic stem cells), it is less clear as to where formaldehyde is making contact with components of the immune system. Mature lymphocytes present in nasal and gut tissues, and would be vulnerable to the direct toxic actions of formaldehyde including genotoxicity. Since mature lymphocytes routinely traffic through the body and clonally respond in response to an immune challenge, the observed effects in peripheral blood lymphocytes (micronuclei and chromosomal aberrations) are consistent with direct action on these cells. Lymphohematopoietic cancers are known to arise from mature lymphocytes including: Hodgkin lymphoma, multiple myeloma some leukemia and non-Hodgkin lymphoma (Greaves 2004, Harris et al., 2000).

Formaldehyde may also be directly acting upon circulating stem cells or more mature progenitor cell in the peripheral blood (Zhang et al., 2010a). Any genetic damage sustained by circulating cells could contribute to a broad spectrum of lymphohematopoietic cancers if those cells returned to the bone marrow and contributed to hematopoiesis. Evidence of bone marrow toxicity and stem cell aneuploidy has been reported in formaldehyde exposed workers (Zhang et al., 2010b). Finally, formaldehyde is readily hydrated in aqueous systems, existing in equilibrium with its hydrated form methylene glycol, which is able to transport through the blood. It has been hypothesized that this hydration reaction may allow formaldehyde to act systemically and therefore on the bone marrow directly (Zhang et al., 2010a.) Formaldehyde-induced DNA damage, and resulting mutation in the bone marrow and circulating stem cells could contribute to any of the lymphohematopoietic cancers including leukemia (both lymphoid and myeloid) as well as myeloproliferative disorders.

Cell replication allows unrepaired DNA damage to be "fixed" into heritable changes to the genome. Therefore, increased cell proliferation could serve not only to increase the mutagenic effects of formaldehyde on a given tissue but also to enhance the mutagenic effects of other agents in the diet or in the environment. Since epidemiological studies include humans exposed to a range of agents in the environment, increased cell proliferation could contribute to increased cancer incidence. The promotion studies in animal bioassays, though limited in number, support the relevance of formaldehyde's ability to enhance the actions of other agents (initiators) on tumor formation.

Although the other biologic effects discussed above have not been explicitly tested in animal systems, the available data are consistent with these actions contributing to the carcinogenic potential of formaldehyde. For example, localized immunosuppression by formaldehyde may serve to increase viral reactivation (e.g., EBV, HPV etc.) or decrease tissue surveillance and immune activity against preneoplastic cells. Both these actions could contribute to increased cancer risk in a human population, which may not be evident in animal bioassays, where the animals are not subject to the many risk factors for human cancer. Even the simple action of the breakdown of the mucociliary apparatus could increase cancer incidence by increasing toxic insult to the URT and increasing URT infections. Again, these actions may be relevant to human populations, but they have not been adequately tested in animal bioassays.

Animal bioassays suggest a role for regenerative proliferation in contributing to formaldehyde's carcinogenicity. However, these data are not evidence against a role of direct mutagenic action either in the observed tumorigenicity or in the potential low-dose carcinogenicity of formaldehyde. As reviewed, a role for mutagenic action is also consistent with the results of the animal bioassays (Crump et al, 2008; Subramaniam et al., 2007, USEPA 2008). The mutagenic effects of formaldehyde are well-documented to occur below levels of significant cytotoxicity. This observation is important for the relevance of formaldehyde-induced mutagenicity to human health risk. Given the above sequence of evidence—from the nature of formaldehyde's DNA reactivity through clastogenic effects observed in human cells from the various tumor sites—there is an adequate weight of evidence (WOE) to consider formaldehyde-induced mutations relevant to human carcinogenic risk. Although occupational exposures may have resulted in high episodic exposures (especially historically), it is unlikely that any worker would have endured repeated exposures which resulted in gross focal lesions to the upper respiratory tract (URT) or oro-digestive tract as seen in the animal bioassays. It is noteworthy that even without these gross formaldehyde-induced lesions, cancer incidence is increased from occupational (and perhaps nonoccupational) exposures to formaldehyde.

Therefore, we believe formaldehyde carcinogenicity can be attributed, at least in part, to a mutagenic MOA.

6.1.5. Cancer Hazard Characterization

Formaldehyde is Carcinogenic to Humans by the Inhalation Route of Exposure. Human epidemiological evidence is sufficient to conclude a causal association between formaldehyde exposure and nasopharyngeal cancer, nasal and paranasal cancer, all leukemias, myeloid leukemia and lymphohematopoietic cancers as a group. Epidemiological evidence is also strongly supportive of, but in itself not sufficient for, a conclusion of causal association for other upper-respiratory tract cancers, Hodgkins lymphoma, or multiple myeloma. Animal bioassays consistently demonstrate formaldehyde-induced nasal cancers in rodents which provide strong support for the observed upper respiratory tract cancers in humans. Limited evidence from animal bioassays is available to support the conclusion from human epidemiologic data that formaldehyde causes some types of lymphohematopoietic cancers.

6.2. DOSE-RESPONSE CHARACTERIZATION

6.2.1. Noncancer Toxicity: Reference Concentration (RfC)

The portals of entry are major targets for formaldehyde, as can be seen in many studies, because formaldehyde is highly reactive and water soluble. Human and laboratory animal studies demonstrate that formaldehyde also causes systemic effects, including neurotoxicity, reproductive toxicity, developmental toxicity, and immunotoxicity, although the data are less extensive than those supporting the sensory irritation and respiratory tract effects. Critical data gaps have been identified and uncertainties associated with data deficiencies are more fully discussed in Chapter 5 and summarized below.

6.2.1.1. *Assessment Approach Employed*

RfC values for noncancer effects are derived using EPA's RfC methodologies (U.S. EPA, 1994, 1993, 2002b). EPA reviewed the existing literature and identified health effects associated with formaldehyde exposure, defining health effect categories where evidence was sufficient: sensory irritation, respiratory tract pathology, pulmonary effects, asthma, increased allergic sensitization, immune function, neurological and behavioral effects and reproductive and developmental effects. Specific key studies were identified within each health effects category which provided adequate exposure-response information to support RfC derivation (see Table 5-4). Although not all identified endpoints are represented by these studies, at least one study was identified for each category. A screening process (described in Section 5.1.3.1) was

used to identify key studies for a variety of health effects that would best inform the derivation of the RfC. For each selected key study, a candidate RfC (cRfC) was derived. In several cases more than one alternative was considered for application of the uncertainty factor (UF) addressing human variability (see Table 5-6).

6.2.1.2. *Derivation of Candidate Reference Concentrations*

Seven studies were selected as key studies for further consideration in RfC derivation (see Section 5.3.1, Table 5-4). Candidate RfCs from these studies address various health effects including: sensory irritation, respiratory effects, asthma, increased allergic sensitization, and decreased fecundity (see Table 5-6). From these studies three cocritical studies were selected which provide similar cRfCs for related health effects (Rumchev et al., 2002; Garrett et al., 1999 a,b; Krzyzanowski et al., 1999). These three studies identify serious health effects in residential populations including children: increased asthma incidence, decreased pulmonary function, increase in respiratory symptoms, and increased allergic sensitization (Rumchev et al., 2002; Garrett et al., 1999 a,b; Krzyzanowski et al., 1999). Asthma, allergic sensitization, altered pulmonary function, and symptoms of respiratory disease are not only clinically related, but etiologically related, and it is reasonable that they should be considered together. These health effects are observed below the exposure levels that result in sensory irritation, and the resulting cRfCs are correspondingly lower—ranging from 2.8 to11 ppb—depending on the study, endpoint considered, and the application of alternative uncertainty factors for human variability (see Table 6-1). Additionally, these cRfCs are considered protective of the decreased fecundability density ratio (FDR) reported by Taskinen et al. (1999) which yielded a cRfC of 8.6 ppb. One of the uncertainties in the cRfC for decreased FDR is the use of a time-weighted exposure metric which does not address possible contributions of peak exposure levels to the observed health effect thus; it is possible that a cRfC of 8.6 ppb is lower than is needed for protection against decreased FDR.

As discussed in Section 6.2.1.4, there are uncertainties in establishing an RfC which are not fully captured in the quantitative process or the standard uncertainty factors. The range of RfCs from the critical studies (even with various alternative considered for the human variability uncertainty factor are in close agreement spanning only ½ order of magnitude.) Therefore EPA is considering a simple mean of these cRfCs as adequately representative of the three cocritical studies. Alternatives are to take the median as a different way to represent the three studies together, or the lowest cRfC as most protective. There is little numerical difference in the result of these decisions.

6.2.1.3.*Adequacy of Overall Data Base for RfC Derivation*

The database of available laboratory animal studies, clinical and epidemiological studies, and supporting mechanistic information for formaldehyde is substantial. Many of the health effects are well studied in animals and humans, especially those endpoints related to sensory irritation and respiratory effects at the portal of entry, such as impacts on respiratory tract pathology, asthma and reduced pulmonary function. This is reflected in the number and high quality of human studies presented in Table 5-4 and supporting data summarized in Chapter 4.

The data also indicate effects in other health effect categories, specifically neurotoxic effects, reproductive toxicity, and developmental toxicity (see Section 5.1.2). These nonportal-of-entry effects are areas where additional research may be warranted to reduce uncertainty and better characterize the potential for health effects and the formaldehyde concentrations at which they might occur in humans.

EPA guidance indicates that an uncertainty factor for database deficiencies should be applied where there is an indication that the existing studies may not completely characterize the hazard of a specific agent. This may be the result of lacking studies to assess toxicity to key functional areas or organ systems, or where "… a review of existing data may also suggest that a lower reference value might result if additional data were available." (U.S. EPA 2002b)

Table 6-1. Summary of candidate reference concentrations (RfC) for cocritical studies

Endpoint	Study	Study size	Homes	Children	POD (ppb)	Application of study-specific UF			cRfC [1] (ppb)
						UF_L	UF_S	UF_H	
Respiratory effects/asthma and sensitization									
Reduction of PEFR in children (10%)	Krzyzanowski et al. (1990)	208	Yes	Yes	$BMCL_{10}$ = 17	1	1	[3]	**5.6**
Asthma prevalence	Rumchev et al. (2002)	192	Yes	Yes	NOAEL = 33	1	3	**Alternative A**	
								[3]	**3.3**
								Alternative B	
								[1]	**11**
Asthma, atopy and severity of allergic sensitization	Garrett et al. (1999 a,b)	148	Yes	Yes	LOAEL = 28	3	1	**Alternative A**	
								[3]	**2.8**
								Alternative B	
								[1]	**9.3**

Notes: 1: The final RfC will be rounded to one significant digit per EPA policy. Since the Candidate RfC is an interim calculation, two-significant digits are retained as is common practice in mathematics {i.e., one significant diget more that the final result, to avoid rounding errors compounding across multiple mathematical manipulations.

Application of an uncertainty factor of 3 was considered by EPA based on the lack of a satisfactory two-generation study to fully evaluate the effects of formaldehyde exposure on reproductive and developmental endpoints and limitations of the available studies evaluating neurotoxic effects. An uncertainty factor of 3 rather than 10 was considered given the relative completeness of the database across all major health effect categories such that it is believed all major health effects have been identified at least qualitatively. The observed adverse health effect levels (LOAELs) for those endpoints where the database is not adequate for alternative RfC derivation are above the range of candidate RfCs; however, it is unclear if the candidate RfCs would be protective of these other health effects (neurotoxic, reproductive and developmental) since NOAELs were not identified for several observed health effects.

Therefore EPA is considering several options to address database deficiencies in the final RfC.

Approaches to the application of a database uncertainty factor:
Options EPA is considering include:

(1) Provide an RfC derived from studies of respiratory and allergenic responses and protective of sensory irritation effects with a database uncertainty factor of one given significant data on formaldehyde, but noting that further research reproductive, developmental and neurotoxic effects would be valuable.

(2) Provide an RfC with a database uncertainty factor of one, with this RfC explicitly identified as being protective of the well-studied effects.

(3) Apply a database UF of 3 to the RfC derived from studies of respiratory and allergenic responses to reflect the potential that reproductive, developmental, or neurotoxic effects might occur at lower doses:

(4) Provide both an RfC identified as protective of the better-studied effects and an RfC with a database uncertainty factor of 3 incorporated to account for limits to the data on reproductive, developmental and neurotoxic effects.

It is unclear what uncertainty factors are appropriate to account for human variability and deficiencies in the overall database. For this reason, several alternatives have been presented.

6.2.1.4. *Uncertainties in the Reference Concentration (RfC)*

A number of uncertainties that underlie the RfC for formaldehyde are discussed in this section. A fundamental uncertainty in an RfC is that the critical study(ies) and endpoint(s)

selected reflect an actual hazard, i.e., a chemically related effect. As summarized in Section 6.1.3, there is strong and consistent evidence, from both human and laboratory animal studies, for the critical effects that form the basis of the RfC for formaldehyde. This section pertains to uncertainties in the quantitative derivation of the RfC.

6.2.1.4.1. ***Point of departure (POD).***

Most of the studies considered for RfC derivation did not provide enough data to support benchmark dose modeling. Rather, the PODs for most studies were LOAELs or NOAELs, which have a number of shortcomings relative to a POD obtained from benchmark dose-response modeling (i.e., a benchmark concentration or dose):

- LOAELs and NOAELs are a reflection of the particular exposure/dose levels used in a study, contributing some inaccuracy to the POD determination.
- LOAELs and NOAELs are often determined based on statistical significance and, thus, reflect the number of study subjects or test animals. Studies are typically dissimilar in detection ability and statistical power, with smaller studies tending to identify higher exposure levels as NOAELs relative to larger, but otherwise similarly designed, studies.
- Different LOAELs and NOAELs represent different response rates, so direct qualitative and quantitative comparisons are not possible.

PODs identified from benchmark dose models overcome some of the deficiencies associated with LOAELs and NOAELs. Benchmark models were used for two inhalation data sets—Hanrahan et al. (1984) and Krzyzanowski et al. (1990).

It should also be noted, however, that even for benchmark concentrations/doses there is often uncertainty, in particular for continuous responses, about what response level to select as the benchmark response, i.e., where to define the cut-point between a level of change that is not adverse and one that is adverse. In addition, benchmark dose models currently in use are purely mathematical models and are not intended to accurately reflect the biology of the effect being modeled.

Another source of uncertainty in the POD is the adjustment for continuous exposure. RfCs are meant to apply to continuous (24 hour/day) exposures. Exposure patterns in human and laboratory animal inhalation studies are typically not for continuous exposures, and assumptions must be made in converting reported exposure levels to equivalent continuous exposures. Similarly, there are uncertainties about potential dose rate effects, in particular the effect of peak exposures in occupational studies.

6.2.1.4.2. ***Extrapolation from laboratory animal data to humans.***

Because the inhalation database for formaldehyde contains many human studies for a variety of health effects, it was not necessary to rely on animal data for the endpoints from which the RfC was derived. Thus, unlike for most RfCs, this is *not* a source of uncertainty in the RfC for formaldehyde.

6.2.1.4.3. ***Human variation.***

Heterogeneity among humans is another uncertainty associated with extending results observed in a limited human study population or laboratory animal experiment to a larger, more diverse human population.

For three of the studies used to derive the RfC, a value of 3 was used for the human variability UF (rather than the default value of 10) because the studies had an apparent over-representation of populations expected to have increased susceptibility (see Section 5.5.3.1):

- The residential study by Ritchie and Lehnen (1987) evaluated eye, nose, and throat irritation in a large number of subjects, including children and the elderly. As a result of the study's participation criteria, individuals with greater sensitivity were potentially over-represented.
- Thirty percent of the subjects in the residential study by Krzyzanowski et al. (1990) are children, who are more sensitive to formaldehyde-associated decreases in peak expiratory flow rates (PEFR) than adults. The candidate RfC determination for this study focused on the results in the children, among which asthmatics were over-represented (roughly 3-times) compared to the national average.
- Garrett et al. (1999 a,b) conducted a cross-sectional survey of allergy and asthma-like symptoms in children with or without a doctor's diagnosis of asthma. The study was designed to include a high proportion of asthmatic children, a sensitive population for the effects being studied.

EPA notes, however, that, while a human variability UF of 3 rather than 10 was used to attempt to account for certain special attributes of these studies/effects, there is still uncertainty about how much of the overall population heterogeneity is actually reflected even in these relatively diverse residential studies.

6.2.1.4.4. ***Subchronic-to-chronic extrapolation.***

RfCs are intended to apply to chronic lifetime exposures. If a study is subchronic (typically less than 10% of lifetime), an UF for subchronic-to-chronic extrapolation is generally applied to the candidate RfC for that study. For the human residential and occupational studies comprising the key studies for the RfC in this assessment, the average durations of exposure in

the households or workplaces under study is unknown. In this assessment, these studies were considered chronic in nature and no subchronic-to-chronic UF was applied. However, there is uncertainty about whether or not the responses observed fully reflected the potential effects of chronic exposure, especially for effects in children, where effects on the developing respiratory and immune systems, for example, could be predisposing the children to further health effects later in life.

6.2.1.5.*Conclusions*

Seven different noncancer health effects were identified from formaldehyde inhalation exposure studies, including: 1) sensory irritation of the eyes, nose, and throat, 2) upper respiratory tract pathology, 3) pulmonary function, 4) asthma and atopy, 5) neurologic and behavioral toxicity, 6) reproductive and developmental toxicity, and 7) immunological toxicity. Of note, epidemiological evidence is available for most of these noncancer effects. EPA has derived candidate RfCs for critical effects based on seven key studies. Three cocritical studies were selected which provide similar cRfCs for related adverse health effects observed in residential populations including children i.e., increased asthma incidence, decreased pulmonary function, increase in respiratory symptoms, and increased allergic sensitization (Rumchev et al., 2002; Garrett et al., 1999 a,b; Krzyzanowski et al., 1999). The resulting cRfCs fall in a range between 2.8 and 11 ppb, depending on the study, or endpoints considered, and the application of alternative uncertainty factors for human variability (see Table 6-1). The RfC is taken as the average of the cRfCs from the three cocritical studies (See Section 6.2.1.2).

EPA has assessed the adequacy of the overall database for RfC derivation, and although the database is quite large, and provides significant information on well studied POE effects. There are remaining uncertainties in the database. Most notably, there is a need for additional exposure-response information for observed neurotoxic effects, reproductive and developmental effects as well as a two-generation study to evaluate the effects of formaldehyde exposure on reproductive and developmental endpoints. EPA is considering 4 options to address database uncertainties in the final RfC (see Section 6.2.1.3). It is unclear what uncertainty factors are appropriate to account for human variability and deficiencies in the overall database. For this reason, several alternatives have been presented. EPA is seeking advice from the NAS and the public on this matter.

6.2.2. Cancer Risk Estimates

6.2.2.1. *Choice of Data*

As explained above, the human epidemiologic data and the animal bioassay data indicate multiple sites of concern, remote as well as at the portal of entry. The quantitative cancer risk derivations in this document consider the risks of lymphohematopoietic cancers and solid cancers of the respiratory tract. When adequate human data are available, as is the case with formaldehyde, it is generally preferable to base cancer risk estimates on the human data rather than on data from experimental animals because of the inherent uncertainties associated with interspecies extrapolation. Sufficient exposure-response data from a large, high-quality epidemiologic study for the quantitative estimation of risk were available for some lymphohematopoietic cancers and for nasopharyngeal cancer.[15] Risk estimates based on nasal tumors in rats were also derived for comparison with the estimates based on human data. The data used for the quantitative risk assessment are as follows:

1. Nasopharyngeal cancer (NPC): The dose-response modeling of NPCs is based on results from a large NCI cohort study of over 25,000 workers in 10 U.S. plants producing or using formaldehyde (Hauptmann et al., 2004).
2. Lymphohematopoietic cancers: The dose-response modeling of select lymphohematopoietic cancers is based on results from a more recent follow-up study (of lymphohematopoietic malignancies only) of the same NCI cohort (Beane Freeman et al., 2009).
3. Squamous cell carcinoma (SCC) in the upper and lower respiratory tract: An increased incidence of nasal SCC was seen in two large long-term bioassays using F344 rats (Kerns et al., 1983; Monticello et al., 1996). Although other studies in laboratory animals exist, these two studies, when combined, provided the most robust data for analyses. The nasal tumor incidence data from these rat bioassays is used for extrapolating the risk of SCC to the entire human respiratory tract.[16]

[15] Only two other epidemiological studies were available with quantitative exposure estimates for the individual workers. One was a much smaller study (it focused on one of the ten plants covered in the selected study), and it evaluated only pharyngeal cancers. The second was a study of lymphohematopoietic and brain cancers in funeral industry workers which, as discussed in detail in Section 5.1.1, had serious limitations in the exposure assessment, precluding its use for quantitative risk assessment.

[16] That is, we do not assume site concordance between rat and human. This is reasonable because the respiratory and transitional cell types considered to be at risk of SCC in the upper respiratory tract are also prevalent in the lower human respiratory tract. Greater fractional penetration of formaldehyde is thought to occur posteriorly in the human respiratory tract compared to the rat (Kimbell et al. 2001, Overton et al. 2001). Furthermore, some epidemiological studies reported an increase in lung cancer with formaldehyde exposure (Gardner et al. 1993, Blair et al. 1990, 1986), and lesions were seen in the lower respiratory tract of rhesus monkeys exposed to formaldehyde (Monticello et al. 1989).

6.2.2.2.*Analysis of Epidemiologic Data*

The NCI cohort consisted of 25,619 workers employed in 10 plants prior to 1966. A follow-up through 1994 presented exposure-response analyses for 9 NPC deaths, as well as analyses of deaths from other solid cancers (Hauptmann et al., 2004). The most recent follow-up (through 2004; lymphohematopoietic cancers only) analyzed 319 deaths attributed to lymphohematopoietic malignancy from a total of 13,951 deaths (Beane Freeman et al., 2009). A detailed exposure assessment was conducted for each worker, based on exposure estimates for different jobs held and tasks performed (Stewart et al., 1986). Exposure estimates were made using several different metrics—peak exposure, average intensity, cumulative exposure, and duration of exposure. Respirator use and exposures to formaldehyde-containing particulates and other chemicals were also considered. Relative Risks (RRs) were estimated using log-linear Poisson regression models stratified by calendar year, age, sex, and race and adjusted for pay category (salary/wage/unknown). The NCI investigators used the low-exposure category as the reference category to "minimize the impact of any unmeasured confounding variables since nonexposed workers may differ from exposed workers with respect to socioeconomic characteristics."

Although other upper respiratory tract cancers were also identified as being causally associated with formaldehyde exposure in the weight-of-evidence analysis in Section 4.5, NPC was the only upper respiratory tract cancer with exposure-response data adequate for the derivation of unit risk estimates in the Hauptmann et al. (2004) follow-up study of solid tumors. Similarly, the weight-of evidence analysis in Section 4.5 concluded that there were causal relationships between formaldehyde exposure and all lymphohematopoietic cancers as a group as well as leukemias as a group (with the strongest evidence for myeloid leukemia). However, from the Beane Freeman et al. (2009) follow-up study of lymphohematopoietic malignancies, only all leukemias combined and Hodgkin lymphoma were judged to have exposure-response data adequate for the derivation of unit risk estimates.

For the NPCs, significant trends were observed for the cumulative and peak exposure metrics. The cumulative exposure metric provides a good fit to the data (p trend = 0.029 for all person-years). Since this is generally the preferred metric for quantitative risk assessment for environmental exposure to carcinogens, cumulative exposure is chosen as the exposure metric for the risk estimate calculations for NPC in this assessment. For the latency of solid cancers, including nasopharyngeal tumors, a 15-year lag interval was used by Hauptmann et al. (2004).

For the lymphohematopoietic cancers, using the peak exposure metric, statistically significant log-linear trends were observed for all lymphohematopoietic cancers, Hodgkin lymphoma, and leukemia (the latter only when the unexposed person-years were included)

(Beane Freeman et al., 2009). Using the average exposure metric, there was a significant trend for Hodgkin lymphoma. Similar results were seen with the cumulative exposure metric, although the trends were not statistically significant, with *p*-values slightly greater than 0.05 (Hodgkin lymphoma *p* trends = 0.06 and 0.08 with and without the unexposed person-years, respectively; leukemia *p* trends = 0.08 and 0.12 with and without the unexposed person-years, respectively). For the latency of lymphohematopoietic cancers, a 2-year lag interval was used by Beane Freeman et al. (2009).

Although the peak exposure metric provides the most statistically robust dose-response relationship, it is not clear how to extrapolate RR estimates based on the peak exposure estimates to meaningful estimates of lifetime extra risk of cancer from environmental exposures. The average exposure metric is also problematic because it suggests that duration of exposure is not important, i.e., exposure to a given exposure level for one year conveys the same amount of risk as exposure to the same level for 70 years.

Cumulative exposure is generally the preferred metric for quantitative risk assessment for environmental exposure to carcinogens. Given the consistency of increased mortality from Hodgkin lymphoma and leukemia overall (exposed versus unexposed) and for each exposure metric (see Table 5-12), indicating risk from these cancers is more than chance, a determination was made that the cumulative exposure results for these two cancer types constituted the best data sets from which to calculate unit risk estimates for lymphohematopoietic cancers from the NCI cohort.

Regression coefficients from the NCI log-linear trend test models for the NPCs (Hauptmann et al., 2004) and the various lymphohematopoietic cancers (Beane Freeman et al., 2009) were provided by Drs. Hauptmann and Beane Freeman, respectively. These trend tests were of the form $RR = e^{\beta * exposure}$. The coefficients (i.e., β) were used in lifetable analyses to calculate lifetime extra cancer risks from formaldehyde exposure (see Section 5.2). Extra risk estimates for cancer incidence for the three cancer types were approximated by assuming that cancer incidence and cancer mortality have the same dose-response relationships and then using background cause-specific incidence rates instead of mortality rates in the lifetable analysis.

Points of departure (PODs) based on the dose-response modeling of these cancers were calculated as the exposure concentration at which the 95% upper confidence bound on extra risk was 0.0005 (i.e., 0.05%) for NPC and for Hodgkin lymphoma and 0.005 (i.e., 0.5%) for leukemia (see Sections 5.2.2 and 5.2.3). These values approximate the lower confidence bounds on dose at these extra risk levels. The values for these extra risk levels, 0.0005 and 0.005, were chosen because they are near the lower end of the observable range of the data. Having such low response levels associated with the points of departure is warranted because of the low

background lifetime risks for these cancer types (e.g., 0.00022 for NPC mortality). Higher extra risk levels would entail extrapolation above the range of the bulk of the observable data to obtain PODs. The resulting effective concentration values for the selected extra risk values for cancer incidence are presented in Table 6-2.

Table 6-2. Effective concentrations (lifetime continuous exposure levels) predicted for specified extra cancer risk levels for selected formaldehyde-related cancers[a]

Cancer type	Extra risk level	EC[b](ppm)	LEC[c] (ppm)
NPC	0.0005	0.074	0.046
Hodgkin lymphoma	0.0005	0.052	0.030
Leukemias	0.005	0.16	0.088

[a]calculated including all person-years (see Section 5.2)
[b]effective concentration.
[s]95% lower confidence bound on the EC; this value is the POD.

Linear low-dose extrapolation from the PODs was used to derive unit risk estimates for NPC, Hodgkin lymphoma, and leukemia, as discussed in Section 6.2.2.4. To obtain an approximate (upper bound) unit risk estimate of the total cancer risk from formaldehyde exposure, risk estimates for these three cancer types (NPC, Hodgkin lymphoma, and leukemia) were combined assuming a normal distribution (see Section 5.2.4). This was considered the most reasonable approach for estimating total cancer risk from the available data; however, it should be noted that this estimate may not reflect all of the cancer types associated with formaldehyde exposure.

6.2.2.3.*Analysis of Laboratory Animal Data*

Various bioassays have been conducted studying the effects of formaldehyde on rats, mice, and rhesus monkeys and have been discussed at length earlier in this document. Of these, two inhalation bioassays of rats, when combined, allow for the most robust characterization of the long-term dose-response relationship in a laboratory species. These long-term bioassays found an increased incidence of nasal SCCs in rats exposed to formaldehyde by the inhalation route (Monticello et al., 1996; Kerns et al., 1983). In the combined data, rats were exposed to 0, 0.7, 2.0, 6.0, 9.93, and 14.96 ppm (0, 0.86, 2.5, 7.4, 12.2, and 18.4 mg/m^3) exposure

concentrations of formaldehyde (Monticello et al. 1996; Kerns et al. 1983). SCCs were observed only at 6 ppm and higher exposure concentrations.

A large amount of mechanistic information relevant to the dose-response relationship of formaldehyde in the respiratory tract has been generated either following or in conjunction with these two bioassays, as reviewed in Chapter 3, 4 and 5. This information includes the following:

1. Measurements of DNA-protein cross-links (DPXs) formed by formaldehyde in F344 rats and rhesus monkeys (Casanova et al., 1989, 1994). Several PBPK models have been developed in the literature based on these data. Some of these efforts integrated the data in both species (Casanova et al., 1991; Conolly et al., 2000; Klein et al., 2010).
2. Measurements of cell proliferation in F344 rats and rhesus monkeys (Monticello et al., 1989, 1990, 1991, 1996).
3. Simulations of airflow in anatomically realistic representations of the upper respiratory tract of the F344 rat, rhesus monkey and human, and in an idealized representation of the human lower respiratory tract, using computer and physical models (Kimbell et al., 1993, 1997a; Kepler et al., 1998; Subramaniam et al., 1998). These simulations were used to predict regional formaldehyde dosimetry in the corresponding sections of the respiratory tract of these three species (Kimbell et al., 2001a, b; Overton et al., 2001).

The combined nasal tumor incidence data in the two inhalation bioassays (Kerns et al. 1983, Monticello et al. 1996) were analyzed using a multistage-weibull time-to-tumor approach as well as models derived from the biologically based dose-response (BBDR) modeling approach in Conolly et al. (2003) [see Crump et al. (2005), Subramaniam et al. (2007), Section 5.3, and Appendix E for details]. The BBDR approach enabled integration of the mechanistic information and the time-to-tumor incidence data within a single conceptual framework.

6.2.2.4.*Extrapolation Approaches*

An EPA inhalation unit risk is developed to estimate cancer risk from environmental exposures or in order to determine exposure levels corresponding with cancer risks as low as 1 excess cancer in 10,000 or 1 excess cancer in 1 million. As neither data from animal studies, nor human epidemiological studies, provide direct observation of these low level risks, the observed exposure response relationship is extrapolated to estimate low dose risk. The model used to extrapolate below the range of exposures clearly associated with increased risk of health effects has a great influence on the inhalation unit risk, as there may be several orders of magnitude difference between the observed risk and the target risk range. In the absence of empirical data or a biologically-informed model, the EPA applies a simple straight line extrapolation from the point of departure to zero exposure (U.S. EPA, 2005a). The Mode of

Action evaluation reviews available data and determines if an MOA can be sufficiently established and whether it informs the shape of the exposure-response relationship.

6.2.2.4.1. ***Low-dose extrapolation for Lymphohematopoietic cancers.***

Formaldehyde is a mutagen, and known to act directly on cells at the site of first contact. Clastogenic effects have been documented in formaldehyde-exposed workers including peripheral blood lymphocytes and circulating stem cells (Zhang et al., 2010a). Thus a mutagenic MOA has been hypothesized for lymphohematopoeitic cancers, and supports a linear low-dose extrapolation of human cancer risk. Additionally, formaldehyde may also induce some form of bone marrow toxicity, as suggested by observed pancytopenia in exposed workers (Tang et al., 2008, Zhang et al., 2010b). However, as the mechanism of transport to the bone marrow, and biological activity leading to the observed toxicity are unknown, this information does not inform the low-dose extrapolation. Although the mechanisms underlying formaldehyde-induced leukemia and lymphoma are still largely speculative, there is little doubt of an association between formaldehyde exposures and lymphohematopoeitic cancer mortality, especially for myeloid leukemia. Therefore, without a known MOA which would justify an alternative approach, and with a hypothesized mutagenic MOA under consideration which supports a simple straight line extrapolation from the point of departure to zero risk at zero exposure, this is applied when estimating human cancer risk from both leukemia and Hodgkin lymphoma from the NCI cohort.

6.2.2.4.2. ***Low-dose extrapolation for cancer of the upper respiratory tract.***

There are multiple plausible MOAs for formaldehyde carcinogenesis regarding upper respiratory tract cancers (see Section 4.5.3), however they may not be necessarily relevant to describing the lower end of the exposure response curve. For example, although regenerative cell proliferation associated with focal and gross tissue lesions due to cell death may contribute to the high incidence of rat nasal tumor in F344 rats, these mechanisms may not be operative in the low exposure region expected for human environmental exposure (e.g., less than 1ppm) and therefore may not inform low-dose extrapolation. There are MOAs which are more appropriate to the low-dose region. Specifically, formaldehyde is a known mutagen, may inhibit DNA repair activity and may have additional activity as a tumor promoter. Finally, other effects such as formaldehyde-induced cell proliferation, immunosuppression and disruption of the mucociliary apparatus may influence both the level of tissue damage and ultimately cancer incidence.

EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) recommend using biologically based dose-response (BBDR) models for extrapolation when data permit. Conolly

et al. (2003, 2004) developed BBDR models to predict squamous cell carcinoma risk in the rat and human respiratory tract at exposures well below the range of the observed animal data. The primary conclusion from their modeling effort was that human exposure standards protective of effects of formaldehyde-induced cytotoxicity should be sufficient to protect from the potential carcinogenic effects of formaldehyde. The authors assessed that such a conclusion was conservative in the face of model uncertainties.[17] The current assessment evaluated the uncertainties and alternative parameterizations of the modeling in Conolly et al. (2003, 2004) extensively, and concluded that the human cancer risk values computed by Conolly et al. (2004) were not conservative estimates. Models resulting from alternative parametrizations were as consistent with the experimental data as the original model but resulted in maximum likelihood estimates of added human risk that ranged from negative to large positive values at environmental exposure concentrations. Model uncertainty far exceeded statistical uncertainty (see Table E-4 in Appendix E). Each of these models, including the modeling in Conolly et al.,

1. was judged to be just as biologically plausible given the available data,
2. described the rat tumor incidence data equally well,
3. was based on different characterizations of the same empirical cell kinetic data, and
4. was based on the same empirical data on DPX measurements.

This assessment's evaluation[18] (detailed in Section 5.3) of the above models concluded that these models, including alternative implementations of those in Conolly et al. (2003, 2004), were too uncertain to be useful for low-dose extrapolation of risk. In particular:

- When used for the purpose of extrapolating risk, the BBDR models did not appear to reasonably constrain either
  - risk estimates extrapolated from the F344 rat to the human, regardless of whether the extrapolation was carried out at low or comparable exposures, or
  - risk estimates for the F344 rat when extrapolated outside the range of observable data.

[17] Based on their modeling, Conolly et al. (2003, 2004) concluded that the directly mutagenic action of formaldehyde does not play a significant role in formaldehyde carcinogenicity. Respiratory cancer risks associated with inhaled formaldehyde were predicted to be *de minimis* (10^{-6} or less) at relevant human exposure levels when an upper bound on the model estimate for the directly mutagenic action of formaldehyde was used.

[18] The scope of this evaluation was informed by views provided by several experts convened by EPA in October 2004. The participants were Drs. Rory Conolly, Kenny Crump, Linda Hanna, Dale Hattis, Julia Kimbell, George Lucier, Christopher Portier and Fred Miller (guest participant). The meeting agenda and summary are provided in Appendix H.

- Human risk calculated from these BBDR models was numerically unstable when certain parameter conditions were realized (see Section 5.3.3 and Appendix F).

It may be noted that the sensitivity analyses on the basis of which these conclusions were reached have been criticized as resulting in implausible risk estimates (given the epidemiologic data) as a consequence of implementing model variations that are not biologically reasonable (Conolly et al. 2009). This criticism was rebutted by Crump et al. (2009) on biological and epidemiological grounds. These debates have been discussed fully in Appendix F.

However, using the BBDR model to characterize the dose-response in the range of the available data has the advantage of utilizing the available biological and dosimetry data on formaldehyde in an integrated manner as well as providing statistically sound descriptions of the empirical tumor incidence data. Therefore, this assessment uses the BBDR modeling of the rat data to derive multiple PODs (for SCC in the respiratory tract) in the range of the observed data and uses model-derived internal dose estimates. For the reasons detailed above, the BBDR modeling is not used to extrapolate far below the observed data.

The lowest observed incidence of SCC in the bioassays used in the dose-response assessment was equal to 0.0087 (at 6 ppm exposure). In addition, the BBDR modeling of the tumor data was informed by its use of data on cell proliferation and formation of DPXs at the lower exposure concentrations of 0.7 and 2.0 ppm. Thus, the available data supported estimation of response levels below the 10% response level commonly used in BMD analyses of tumor data. Therefore, points of departure corresponding to 95% statistical upper bound levels of extra risk of 0.005, 0.01 and 0.1 were estimated when the BBDR modeling was used.

6.2.2.4.3. ***Summary.***

As discussed earlier in the hazard characterization, formaldehyde is a direct-acting mutagen, and its genotoxic effects have been observed following human occupational exposures.[19] Furthermore, a low-dose nonlinear MOA for formaldehyde-induced lymphohematopoietic cancers, NPCs, or cancers in other regions of the respiratory tract has not been established. In particular, the formation of DPXs by formaldehyde, considered a dose surrogate for the molecular dose associated with formaldehyde's mutagenic action, has been observed at doses well below those considered cytotoxic. Therefore, linear low-dose extrapolation from the suitably chosen PODs was considered most appropriate for all the cancers

[19] While formaldehyde may also contribute to mutations indirectly, such an effect is likely to be relevant only at the higher doses.

(whether the PODs were based on epidemiological data or rodent bioassay data), which is also in accordance with EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a).

6.2.2.5. *Inhalation Unit Risk Estimates for Cancer*

The epidemiological and rodent inhalation data indicate multiple sites of concern. Unit risk estimates calculated separately from these data are summarized in Table 6-3.

As can be seen in the Table 6-3, the unit risk estimate based on human data for NPC is in the range of the estimates calculated for respiratory tract cancer from the rodent nasal cancer data. Experimental animal data were inadequate for estimating risk of lymphohematopoietic cancers. The unit risk estimate for Hodgkin lymphoma is also in the same range, while the unit risk estimate for leukemia and the total cancer unit risk estimate are up to 4-fold higher.

As documented in EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), when high-quality human data are available, they are generally preferred over laboratory animal data for quantitative risk assessment. Thus, the preferred (plausible upper bound) unit risk estimate in this assessment is the value of **8.1×10^{-2} per ppm (6.6×10^{-5} per µg/m^3)** based on (**adult**) human data for NPC, Hodgkin lymphoma, and leukemia. Note that, as discussed in Section 6.2.2.6 below, if there is early-life exposure, the age-dependent adjustment factors (ADAFs) should be applied, in accordance with EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b).

6.2.2.6.*Early-Life Susceptibility*

There are no chemical-specific data for quantitatively addressing the susceptibility of different life stages to carcinogenicity from inhalation exposure to formaldehyde. As documented in Section 4.5, formaldehyde is a mutagenic carcinogen and the weight of evidence supports the conclusion that formaldehyde carcinogenicity can be attributed, at least in part, to a mutagenic MOA. Therefore, increased early-life susceptibility should be assumed and, if there is early-life exposure, the ADAFs should be applied, in accordance with EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b). See Section 5.4.4 for details on the application of the ADAFs.

Accordingly, **for *full lifetime* exposures, the overall (plausible upper bound) unit risk estimate is 0.13 per ppm (1.1×10^{-4} per µg/m^3)** for the three cancer types (NPC, Hodgkin lymphoma, and leukemia) combined (see Table 5-26 for calculations).

Table 6-3. Inhalation unit risk estimates based on epidemiological and experimental animal data

Cancer type[a]	Dose metric	Unit Risk Estimate[b] (ppm^{-1})
Based on epidemiological data		
Nasopharyngeal	Cumulative exposure	0.011
Hodgkin lymphoma	Cumulative exposure	0.017
Leukemia	Cumulative exposure	0.057
All three cancer sites combined:		**0.081**[c]
Based on Experimental Animal Data		
Squamous cell carcinoma of the respiratory tract	Local dose (flux) of formaldehyde in $pmol/mm^2/hour$	0.011–0.022[d]

[a] the unit risk estimates are all for cancer incidence.
[b] these unit risk estimates do not include ADAFs (see Section 6.2.2.6 below).
[c] this total cancer unit risk estimate is an estimate of the upper bound on the sum of risk estimates calculated for the 3 individual cancer types (nasopharyngeal cancer, Hodgkin lymphoma, and leukemia); it is not the sum of the individual (upper bound) unit risk estimates (see Section 5.2.4).
[d] values are similar to estimates from Schlosser et al. (2003). These authors determined their PODs based on tumor and cell proliferation as endpoints, and extrapolated benchmark exposure concentrations to humans using formaldehyde flux to the tissue and DPX concentrations as internal dose metrics.

6.2.2.7. *Uncertainties in the Quantitative Risk Estimates*

Uncertainties in the risk estimates based on the human data are discussed in detail in Sections 5.2.2.4 and 5.2.3.4. Major uncertainties inherent in the NPC, Hodgkin lymphoma, and leukemia risk estimates are

- the retrospective exposure estimation,
- the appropriateness of the dose-response model and exposure metric, and
- the extrapolation from occupational exposures to lower environmental exposures.

In addition, the NPC and Hodgkin lymphoma estimates are limited by the sparse data for these cancers in the NCI cohort study (estimates are based on the exposure-response modeling of only 9 NPC deaths and 27 Hodgkin lymphoma deaths).

Of note, Marsh et al. (2002, 1996) independently studied one of the 10 plants that was in the NCI study, and there were large differences in the exposure estimates for that plant from the two different studies. If the exposure estimates of Marsh et al. (2002) are closer to the true

exposures, then the potency of formaldehyde could be greater than reflected in the risk estimates derived from the NCI data.

The linear low-dose extrapolation (see Section 6.2.2.4) from the 95% lower bound on the exposure level associated with the benchmark response is generally considered to provide a plausible upper bound on the risk at lower exposure levels. Although the linear low-dose extrapolation used here is supported by the mutagenicity of formaldehyde, nonlinearities in the exposure-response relationship may be present below, as well as above, the POD. The strong association with peak exposures for all 3 cancer types in the NCI study suggests that dose-rate effects may be operative (i.e., the risk from peak occupational exposures may be greater than the [linearly] proportional risks from lower exposures and, similarly, the risk from an occupational cumulative exposure may be greater than the proportional risk from a lower environmental cumulative exposure).[20] Any such dose-rate effects would not be reflected in the cumulative exposure metric used for the exposure-response modeling in the range of the occupational exposures nor in the linear low-dose extrapolation approach used in this assessment. Actual low-dose risks may be lower to an unknown extent.

Other significant uncertainties may also remain. For example, risk estimates could not be derived from the NCI cohort study for rare upper respiratory tract cancers other than NPC. In addition, although unit risk estimates were derived for Hodgkin lymphoma and leukemia because they exhibited the strongest trend results of the lymphohematopoietic cancers using the cumulative exposure metric, it is uncertain which specific lymphohematopoietic cancer subtypes are associated with formaldehyde exposure. Furthermore, the potential role of particulates in the NPC risk is unclear. Moreover, as for all occupational epidemiology studies, there is uncertainty in extrapolating risk from an adult worker population (in this case predominantly white males) to the more diverse general population.

Despite inevitable uncertainties, it is important not to lose sight of the strengths of the estimates, which are based on human data from a high-quality NCI study. In addition to the use of internal analyses and the extensive exposure assessment and consideration of potential confounding or modifying variables, the NCI study has a large cohort that has been followed for a long time. With the additional follow-up through 2004, reflected in the lymphohematopoietic cancer results of Beane Freeman et al. (2009), the median duration of follow-up was 42 years, and the 25,619 cohort members had accrued 998,106 person-years of follow-up.

[20] Dose-rate effects are also suggested by the very steep, nonlinear exposure-response relationships observed in the rodent cancer bioassays, although, in the rodents, this steep increase in tumor incidence at high exposures is thought to be due to the contribution of cytotoxicity and regenerative proliferation, which is not apparent with the human exposures (Section 4.5).

Significant uncertainties also exist in the risk estimates derived from the rodent bioassay data. In general, the difficulties in extrapolating from experimental animal bioassays are considerable, and the use of human data is preferred, while recognizing the different uncertainties that are present in risk estimates based on epidemiological data.

In the case of formaldehyde, this general uncertainty associated with extrapolation from rodent data is increased due to the highly curvilinear nature of the dose-response relationships associated with DPX formation, labeling index data, and tumor responses. The mechanistic interpretation of these observed data has provided grounds for arguments in the literature that formaldehyde tumorigenicity (at exposures ≥ 6 ppm) should be uncoupled from its potential carcinogenicity in the low-dose region.

Quantitative models have been used in the literature to further argue that the observed risk in animal experiments is entirely due to cell proliferation induced by regenerative hyperplasia in response to cell injury at cytotoxic doses, i.e., without a relevant role for the direct mutagenic action of formaldehyde. In the context of using these data for quantitative risk assessment, this document notes that such an inference of the data has been found to be extremely uncertain. A quantitative analysis of the uncertainties in interpreting the available data has shown that the directly mutagenic action of formaldehyde could be very important in explaining the high-dose effect (Subramaniam et al., 2007).

While acknowledging these substantial difficulties, the quantitative dose-response modeling of the rat data does allow inference about upper bound risks for respiratory cancer, consistent with the observed experimental tumorigenicity. These upper bound risk estimates are consistent with those estimated from the epidemiological data; however, such a consistency may be entirely artifactual. As noted earlier, the BBDR modeling helped characterize some of the uncertainty associated with extrapolating from the rodent data to the environmental risk in people. The actual risk may be substantially lower or higher than the reasonable upper bound risk estimated from the animal data.

6.2.2.8. *Conclusions*

Cancer unit risk estimates for formaldehyde inhalation exposure were derived from both human and laboratory animal data. As documented in EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), when high-quality human data are available, they are generally preferred over laboratory animal data for quantitative risk assessment. Thus, the preferred unit risk estimate in this assessment is based on human data for NPC, Hodgkin lymphoma, and leukemia from a high-quality NCI occupational cohort study (Hauptmann et al., 2004; Beane Freeman et al., 2009). (The qualitative hazard assessment suggests causal associations between

formaldehyde exposure and other cancer types as well [e.g., other upper respiratory tract cancers and possibly other lymphohematopoietic cancers; see Section 4.5], but quantitative data from the NCI cohort study were not amenable for deriving quantitative risk estimates for those cancer types. Because there were not clear exposure-response data for these cancer types in that cohort study [based on cumulative exposure], any contributions to the total cancer risk from environmental formaldehyde exposure for these cancers are not expected to be large; however, this is a source of uncertainty.)

The unit risk estimate for the total cancer incidence extra risk for these three cancer types combined based on the (adult) human data is 8.1×10^{-2} per ppm (6.6×10^{-5} per μg/m^3). As documented in Section 4.5, formaldehyde is a mutagenic carcinogen and the weight of evidence supports the conclusion that formaldehyde carcinogenicity can be attributed, at least in part, to a mutagenic MOA. Therefore, as there are no chemical-specific inhalation data on cancer susceptibility at different life-stages, increased early-life susceptibility is assumed and ADAFs should be applied in accordance with EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b). Applying the ADAFs, the overall (upper bound) unit risk estimate for *full lifetime* exposure is 0.13 per ppm (1.1×10^{-4} per μg/m^3) for the three cancer types (NPC, Hodgkin lymphoma, and leukemia) combined. Using this lifetime unit risk estimate, the upper bound estimate of the cancer risk at the RfC of 1 ppb is 1×10^{-4}.

6.3. SUMMARY AND CONCLUSIONS

Seven different noncancer health effects were identified from formaldehyde inhalation exposure studies, including: 1) sensory irritation of the eyes, nose, and throat, 2) upper respiratory tract pathology, 3) pulmonary function, 4) asthma and atopy, 5) neurologic and behavioral toxicity, 6) reproductive and developmental toxicity, and 7) immunological toxicity. Of note, epidemiological evidence is available for most of these noncancer effects. EPA has derived candidate RfCs for critical effects based on seven key studies. Three cocritical studies were selected which provide similar cRfCs for related adverse health effects observed in residential populations including children i.e., increased asthma incidence, decreased pulmonary function, increase in respiratory symptoms, and increased allergic sensitization (Rumchev et al., 2002; Garrett et al., 1999 a,b; Krzyzanowski et al., 1999). The resulting cRfCs fall in a range between 2.8 and 11 ppb, depending on the study, or endpoints considered, and the application of alternative uncertainty factors for human variability (see Table 6-1). The representative RfC for the cocritical studies is taken as the average of the cRfCs (see Section 6.2.1.2).

EPA has assessed the adequacy of the overall database for RfC derivation, and although the database is quite large, and provides significant information on well studied POE effects. There are remaining uncertainties in the database. Most notably, there is a need for additional exposure-response information for observed neurotoxic effects, reproductive and developmental effects as well as a two-generation study to evaluate the effects of formaldehyde exposure on reproductive and developmental endpoints. EPA is considering 4 options to address database uncertainties in the final RfC (see Section 6.2.1.3). It is unclear what uncertainty factors are appropriate to account for human variability and deficiencies in the overall database. For this reason, several alternatives have been presented. EPA is seeking advice from the NAS and the public on this matter.

Formaldehyde is Carcinogenic to Humans by the Inhalation Route of Exposure. Human epidemiological evidence is sufficient to conclude a causal association between formaldehyde exposure and nasopharyngeal cancer, nasal and paranasal cancer, all leukemias, myeloid leukemia and lymphohematopoietic cancers as a group. Epidemiological evidence is also strongly supportive of, but in itself not sufficient for, a conclusion of causal association for other upper-respiratory tract cancers, Hodgkins lymphoma, or multiple myeloma. Animal bioassays consistently demonstrate formaldehyde-induced nasal cancers in rodents which provide strong support for the observed upper respiratory tract cancers in humans. Limited evidence from animal bioassays is available to support the conclusion from human epidemiologic data that formaldehyde causes some types of lymphohematopoietic cancers.

The (upper bound) unit risk estimate for the total cancer incidence based on (adult) human data is 8.1×10^{-2} per ppm (6.6×10^{-5} per $\mu g/m^3$). Applying the age-dependent adjustment factors for increased early-life susceptibility, the overall combined cancer unit risk estimate for full lifetime exposure is 0.13 per ppm (1.1×10^{-4} per $\mu g/m^3$).

REFERENCES

Abbas, AK; Fausto, N; Kumar, V. (2004) Robbins and Cotran pathologic basis of disease. W.B. Saunders Co. Silver Spring, MD.

ACGIH (American Conference of Governmental Industrial Hygienists). (2002) Documentation of the TLVs® and BEIs® with other worldwide occupational exposure values CD-ROM—2002. American Conference of Governmental Industrial Hygienists, Cincinnati, OH.

Acheson, ED; Barnes, HR; Gardener, MJ; et al. (1984) Formaldehyde in the British chemical industry: an occupational cohort study. Lancet 1:611–616.

Achkor, H; Diaz, M; Fernandez, MR; et al. (2003) Enhanced formaldehyde detoxification by overexpression of glutathione-dependent formaldehyde dehydrogenase from Arabidopsis. Plant Physiol 132:2248–2255.

Adams, DO; Hamilton, TA; Lauer, LD; et al. (1987) The effect of formaldehyde exposure upon the mononuclear phagocyte system of mice. Toxicol Appl Pharmacol 88:165–174.

Ahmad, I; Whitson, TC. (1973) Formaldehyde: how much of a hazard? Ind Med Surg 42:26–27.

Ahmed, S; Tsukahara, S; Tin-Tin-Tin-Shwe; et al. (2007) Effects of low-level formaldehyde exposure on synaptic plasticity-related gene expression in the hippocampus of immunized mice. J. Neuroimmunol 186:104–111.

Akbar-Khanzadeh, F; Mlynek, JS. (1997) Changes in respiratory function after one and three hours of exposure to formaldehyde in non-smoking subjects. Occup Environ Med 54:296–300.

Akbar-Khanzadeh, F; Vaquerano, MU; Akbar-Khanzadeh, M; et al. (1994) Formaldehyde exposure, acute pulmonary response, and exposure control options in a gross anatomy laboratory. Am J Ind Med 26:61–75.

Alarie, Y. (1981) Toxicological evaluation of airborne chemical irritants and allergens using respiratory reflex reactions. In: Leong, B, ed. Proceedings of the inhalation toxicology and technology symposium. Ann Arbor, MI: Ann Arbor Science Publishers, Inc.; pp. 207–231.

Albert, RE; Pasternack, BS; Shore, RE; et al. (1975) Mortality patterns among workers exposed to chloromethyl ethers-a preliminary report. Environ Health Perspect 11:209–214.

Albert, RE; Sellakumar, AR; Laskin, S; et al. (1982) Gaseous formaldehyde and hydrogen chloride induction of nasal cancer in the rat. J Natl Cancer Inst 68:597–603.

Alderson, T. (1985) Formaldehyde-induced mutagenesis: a novel mechanism for its action. Mutat Res 154:101-10.

Alexandersson, R; Hedenstierna, G. (1988) Respiratory hazards associated with exposure to formaldehyde and solvents in acid-curing paints. Arch Environ Health 43:222–227.

Alexandersson, R; Hedenstierna, G. (1989) Pulmonary function in wood workers exposed to formaldehyde: A prospective study. Arch Environ Health 44:5–11.

Alexandersson, R; Hedenstierna, G; Kolmodin-Hedman, B. (1982) Exposure to formaldehyde effects on pulmonary function. Arch Environ Health 37:279–284.

Almaguer, D; Blade, LM. (1990) Health hazard evaluation report: Buckeye Hills Career Center, Rio Grande, Ohio. National Institute for Occupational Safety and Health, U.S. Department of Health and Human Services, Cincinnati, OH; HETA 88-153-2072

Almaguer, D; Klein, M. (1991) Health hazard evaluation report: Northwest Vocational School, Cincinnati, Ohio. National Institute for Occupational Safety and Health, U.S. Department of Health and Human Services, Cincinnati, OH; HETA 89-170-2100

Amdur, MO. (1960) The response of guinea pigs to inhalation of formaldehyde and formic acid alone and with a sodium chloride aerosol. Int J Air Pollut 3:201–220.

Ames, BN; Shigenaga, MK; Gold, LS. (1993) DNA lesions, inducible DNA repair, and cell division: three key factors in mutagenesis and carcinogenesis. Environ Health Perspect 101(Suppl 5):35–44.

Andersen, I; Lundqvist, GR. (1974) Design and performance of an environmental chamber. Int J Biometeor 14:402–405.

Andersen, I; Mølhave, L. (1983) Controlled human studies with formaldehyde. In: Gibson, JE; ed. Formaldehyde toxicity. Washington, DC: Hemisphere Publishing Corporation; pp. 154–165.

Andersen, ME; Jarabek, AM. (2001) Nasal tissue dosimetry. Issues and approaches for "Category 1" gases: a report on a meeting held in Research Triangle Park, NC, February 11-12, 1998. Inhal Toxicol 13:415–435.

Andersen, ME; Clewell III, HJ, Bermudez, E; et al. (2008) Genomic signatures and dose dependent transitions in nasal epithelial responses to inhaled formaldehyde in the rat. Toxicol Sci 105:368–383.

Andersen, I (1979) Indoor climate: Effects on human comfort, performance, and health in residential, commercial, and light-industry buildings. In: Fanger, P.O. and Valbjørn; ed. Proceedings of the first international indoor climate symposium in Copenhagen Aguust 30-September 1, 1978. Danish Building Research Institute; Copenhagen; pp. 65-87.

Anderson, H; Dally, KA; Hanrahan, L; et al. (1983) The epidemiology of mobile home formaldehyde vapor concentration and residents' health status. Chicago, IL: Region V. Available from NTIS, PB84-123504. EPA/905/1-83-001.

Andersson, M; Agurell, E; Vaghef, H, et al. (2003) Extended-term cultures of human T-lymphocytes and the comet assay: a useful combination when testing for genotoxicity in vitro? Mutat Res 540:43-55.

Anderson, S; Jensen, O; Oliva, D. (1982) Formaldehyde exposure and lung cancer in Danish doctors. Ugeskr Laeg 144:1571–1573.

Andjelkovich, DA; Janszen, DB; Brown, MH; et al. (1995) Mortality of iron foundry workers: IV. Analysis of a subcohort exposed to formaldehyde. J Occup Environ Med 37:826–837.

Ang, HL; Deltour, L; Zgombic-Knight, M; et al. (1996a) Expression patterns of class I and class IV alcohol dehydrogenase genes in developing epithelia suggest a role for alcohol dehydrogenase in local retinoic acid synthesis. Alcohol Clin Exp Res 20:1050–1064.

Ang, HL; Deltour, L; Hayamizu, TF; et al. (1996b) Retinoic acid synthesis in mouse embryos during gastrulation and craniofacial development inked to class IV alcohol dehydrogenase gene expression. J Biol Chem 271:9526-9534.

Apfelbach, R; Reibenspies, M. (1991) Olfactory learning is inhibited after low-level formaldehyde gas exposure in the ferret. Chem Senses 16:498

Apfelbach, R; Weiler, E. (1991) Sensitivity to odors in Wistar rats is reduced after low-level formaldehyde-gas exposure. Naturwissenschaften 78:221–223.

Apfelbach, R; Reibenspies, M; Schmidt, R. (1992) The effect of long-lasting formaldehyde gas exposure on the olfactory epithelium and on the olfactory discrimination ability in the ferret. Chem Senses 17:590–591.

Appelman, LM; Woutersen, RA; Zwart, A; et al. (1988) One-year inhalation toxicity study of formaldehyde in male rats with a damaged or undamaged nasal mucosa. J Appl Toxicol 8:85–90.

Armstrong, RW; Imrey, PB; Lye, MS; et al. (2000) Nasopharyngeal carcinoma in Malaysian Chinese: occupational exposures to particles, formaldehyde and heat. Int J Epidemiol 29:991–8.

Arts, JH; Dröge, SC; Spanhaak, S; et al. (1997) Local lymph node activation and IgE responses in brown Norway and Wistar rats after dermal application of sensitizing and non-sensitizing chemicals. Toxicology 117:229–234.

Ashby, J; Callander, RD; and Rose, FL. (1985) Weak mutagenicity to Salmonella of the formaldehyde-releasing anti-tumour agent hexamethylmelamine. Mutat Res 142:121-5.

Aslan, H; Songur, A; Tunc, AT; et al. (2006) Effects of formaldehyde exposure on granule cell number and volume of dentate gyrus: A histopathological and stereological study. Brain Res 1122:191–200.

ASTM (American Society for Testing and Materials). (2000) Standard test method for estimating sensory irritancy of airborne chemicals. Annual book of ASTM standards. Designation E981 (reapproved 2000). Philadelphia, PA: American Society for Testing of Materials.

ATC (American Thoracic Society). (2000) What constitutes an adverse health effect of air pollution? Am J Respir Crit Care Med 161:665–673.

ATSDR (Agency for Toxic Substances and Disease Registry). (1999) Toxicological profile for formaldehyde. Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA. Available online at http://www.atsdr.cdc.gov/toxprofiles/tp111.html.

ATSDR, 2007. An update and revision of ATSDR's February 2007 health consultation: Formaldehyde sampling of FEMA temporary-housing trailers; Baton Rouge, Louisiana September-October, 2006. Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA. Available online at www.atsdr.cdc.gov/substances/formaldehyde/public_assessment.html.

Auerbach, C; Moutschen-Dahmen, M; Moutschen, J. (1977) Genetic and cytogenetical effects of formaldehyde and related compounds. Mutat Res 39:317–361.

Axelsson, G; Lűtz, C; Rylander, R. (1984) Exposure to solvents and outcome of pregnancy in university laboratory employees. Br J Ind Med 41:305–312.

Baba, K; Yagi, T; Niwa, S; et al. (2000) Measurement of formaldehyde-specific IgE antibodies in adult asthmatics. Arerugi 49(5):404–411.

Babiuk, C; Steinhagen, WH; Barrow, CS. (1985) Sensory irritation response to inhaled aldehydes after formaldehyde pretreatment. Toxicol Appl Pharmacol 79:143–149.

Bach, B; Pedersen, OF; Mølhave, L. (1990) Human performance during experimental formaldehyde exposure. Environ Int 16:105–114.

Baird, DD; Wilcox, AJ. (1985) Cigarette smoking associated with delayed conception. J Am Med Assoc 253:2979-2983.

Baker, H; Kawano, T; Margolis, FL; et al. (1983) Transneuronal regulation of tyrosine hydroxylase expression in olfactory bulb of mouse and rat. J Neurosci 3:69–78.

Ballarin, C; Sarto, F; Giacomelli, L; et al. (1992) Micronucleated cells in nasal mucosa of formaldehyde-exposed workers. Mutat Res 280:1–7.

Barker, S; Weinfeld, M; Murray, D. (2005) DNA-protein crosslinks: Their induction, repair, and biological consequences. Mutat Res 589:111–135.

Barrow, CS; Steinhagen, WH; Chang, JCF. (1983) Formaldehyde sensory irritation. In: Gibson, JE; ed. Formaldehyde toxicity. Washington, DC: Hemisphere Publishing Corporation; pp. 16–25.

Bartnik, FG; Gloxhuber, C; Zimmermann, V. (1985) Percutaneous absorption of formaldehyde in rats. Toxicol Lett 25:167–172.

Basecke, J; Griesinger, F; Trumper, L; et al. (2002) Leukemia- and lymphoma-associated genetic aberrations in healthy individuals. Ann Hematol 81:64–75.

Basler, A; v d Hude, W; and Scheutwinkel-Reich, M. (1985) Formaldehyde-induced sister chromatid exchanges in vitro and the influence of the exogenous metabolizing systems S9 mix and primary rat hepatocytes. Arch Toxicol 58:10-3.

Bateson, TF; Schwartz, J. (2008) Children's response to air pollutants. J Toxicol Environ Health A 71:238-243.

Battelle Columbus Laboratories. (1981) Final report on a chronic inhalation toxicology study in rats and mice exposed to formaldehyde. Prepared by Battelle Columbus Laboratories, Columbus, OH for the Chemical Industry Institute of Toxicology (CIIT), Research Triangle Park, NC; CIIT Docket # 10922.

Bauchinger, M; Schmid, E. (1985) Cytogenetic effects in lymphocytes of formaldehyde workers of a paper factory. Mutat Res 158:195–199.

Beane-Freeman, LE; Blair, A; Lubin, JH; et al. (2009) Mortality from lymphohematopoietic malignancies among workers in formaldehyde industries: the National Cancer Institute cohort. J Natl Cancer Inst 101:751–761.

Beland, FA; Fullerton, NF; Heflich, RH. (1984) Rapid isolation, hydrolysis and chromatography of formaldehyde-modified DNA. J Chromatogr 308:121–131.

Bender, JR; Mullin, LS; Graepel, GJ; et al. (1983) Eye irritation response of humans to formaldehyde. Am Ind Hyg Assoc J 44:463–465.

Benedetti, MS; Whomsley, R; Canning, M. (2007) Drug metabolism in the paediatric population and in the elderly. Drug Discov Today 12:599–610.

Berke, JH. (1987) Cytologic examination of the nasal mucosa in formaldehyde-exposed workers. J Occup Med 29:681–684.

Bermudez, E; Allen, PF. (1984) The assessment of DNA damage and repair in rat nasal epithelial cells. Carcinogenesis 5:1453–1458.

Bermudez, E; Chen, Z; Gross, EA, et al. (1994) Characterization of cell lines derived from formaldehyde-induced nasal tumors in rats. Mol Carcinog 9:193-199.

Bermudez, E; Delehanty, LL. (1986) The effects of in vitro formaldehyde treatment on the cells of the rat nasal epithelium. Environ Mutagen 8 (Suppl.6):11.

Bertazzi, PA; Pesatori, AC; Radice, L; et al. (1986) Exposure to formaldehyde and cancer mortality in a cohort of workers producing resins. Scand J Work Environ Health 12:461–468.

Bertazzi, PA; Pesatori, A; Guercilena, S; et al. (1989) Carcinogenic risk for resin producers exposed to formaldehyde: extension of follow-up. Med Lav 80:111–122.

Bhalla, DK; Mahavni, V; Nguyen, T; et al. (1991) Effects of acute exposure to formaldehyde on surface morphology of nasal epithelia in rats. J Toxicol Environ Health 33:171–188.

Biagini, RE; Moorman, WJ; Knecht, EA; et al. (1989) Acute airway narrowing in monkeys from challenge with 2.5 ppm formaldehyde generated from formalin. Arch Environ Health 44:12–17.

Bickis, M; Krewski, D. (1989) Statistical issues in the analysis of the long-term carcinogenicity bioassay in small rodents: An empirical evaluation of statistical decision rules. Fundam Appl Toxicol 12:202–221.

Binzak, BA; Vockley, JG; Jenkins, RB; et al. (2000) Structure and analysis of the human dimethylglycine dehydrogenase gene. Mol Genet Metab 69:181-187.

Black, KA; Eells, JT; Noker, PE; et al. (1985) Role of hepatic tetrahydrofolate in the species difference in methanol toxicity. Proc Natl Acad Sci USA 82:3854–3858.

Blackburn, GR; Dooley, JFI; Schreiner, CA; et al. (1991) Specific identification of formaldehyde-mediated mutagenicity using the mouse lymphoma L5178Y TK +/- assay supplemented with formaldehyde dehydrogenase. In Vitro Toxicol 4:121–132.

Blair, A; Stewart, P; O'Berg, M; et al. (1986) Mortality among industrial workers exposed to formaldehyde. J Natl Cancer Inst 76:1071–1084.

Blair, A; Stewart, PA; Hoover, RN; et al. (1987) Cancers of the nasopharynx and oropharynx and formaldehyde exposure. J Natl Cancer Inst 78:191–193.

Blair, A; Stewart, PA. (1990) Correlation between different measures of occupational exposure to formaldehyde, Am J Epidemiol 131:510–516.

Blair, A; Stewart, PA; Hoover, RN. (1990) Mortality from lung cancer among workers employed in formaldehyde industries. Am J Ind Med 17:683–699.

Blair, A; Linos, A; Stewart, A; et al. (1993) Evaluation of risks for Non-Hodgkin's Lymphoma by occupation and industry exposures from a case-control study. Am J Ind Med 23:301–312.

Blasiak, J; Trzeciak, A; Malecka-Panas, E, et al. (2000) *In vitro* genotoxicity of ethanol and acetaldehyde in human lymphocytes and the gastrointestinal tract mucosa cells. Toxicol I*n Vitro* 14:287-295.

Blejer, HP; Miller, BH. (1966) Occupational health report of formaldehyde concentrations and effects on workers at the Bayly Manufacturing Company, Visalia, California. Bureau of Occupational Health, California Department of Public Health, Los Angeles, CA; Study No. S-1806.

Bloom, B; Cohen, RA; Freeman, G. (2009) Summary health statistics for U.S. children: National Health Interview Survey, 2008. National Center for Health Statistics. Vital Health Stat 10(244).

Boeniger, MF. (1987) Formate in urine as a biological indicator of formaldehyde exposure: A review. Am Ind Hyg Assoc J 48:900–908.

Boffetta, P; Stellman, SD; Garfinkel, L. (1989) A case-control study of multiple myeloma nested in the American Cancer Society prospective study. Int J Cancer 43:554–559.

Bogdanffy, MS; Morgan, PH; Starr, TB; et al. (1987) Binding of formaldehyde to human and rat nasal mucus and bovine serum albumin. Toxicol Lett 38:145–154.

Bogdanffy, MS; Sarangapani, R; Plowchalk, DR; et al. (1999) A biologically based risk assessment for vinyl acetate-induced cancer and noncancer inhalation toxicity. Toxicol Sci 51:19–35.

Bogdanffy, MS; Plowchalk, DR; Sarangapani, R, et al. (2001) Mode of action-based dosimeters for interspecies extrapolation of vinyl acetate inhalation risk. Inhal Toxicol 13:377–396.

Boja, JW; Nielsen, JA; Foldvary, E; et al. (1985) Acute low-level formaldehyde behavioural and neurochemical toxicity in the rat. Prog Neuro-Psychopharm 9:671–674.

Bolumar, F; Olsen, J; Boldsen, J. (1996) Smoking reduces fecundity: A European multicenter study on fertility and subfecundity. The European study group on infertility and subfecundity. Am J Epidemiol 143:578–587.

Bolumar, F; Olsen, J; Boldsen, J. (1997) Smoking reduces fecundity: A European multicenter study on infertility and subfecundity. The European study group on infertility and subfecundity. Am J Epidemiol 145:81–82.

Bond, G; Flores, G; Shellenberger, R. (1986) Nested case-control study of lung cancer among chemical workers. Am J Epidemiol 124:53–66.

Boreiko, CJ; and Ragan, DL (1983). Formaldehyde effects in the C3H/10T½ cell transformation assay. In: Gibson, JE,ed, *Formaldehyde toxicity*, Washington, DC: Hemisphere Publishing Corporation, 63-71.

Bos, PMJ; Zwart, A; Reuzel, PGJ; et al. (1992) Evaluation of the sensory irritation test for the assessment of occupational health risk. Crit Rev Toxicol 21:423–450.

Bosetti, C; McLaughlin, JK; Tarone, RE; et al. (2008) Formaldehyde and cancer risk: a quantitative review of cohort studies through 2006. Annal Oncol 19:29–43.

Bosworth, D; Crofton-Sleigh, C; Venitt, S. (1987) A forward mutation assay using ampicillin-resistance in Escherichia coli designed for investigating the mutagenicity of biological samples. Mutagenesis 2:455–467.

Boysen, M; Zadig, E; Digernes, V; et al. (1990) Nasal mucosa in workers exposed to formaldehyde: a pilot study. Br J Ind Med 47:116–121.

Brenneman, KA; James, RA; Gross, EA; et al. (2000) Olfactory neuron loss in adult male CD rats following subchronic inhalation exposure to hydrogen sulfide. Toxicol Pathol 28:326–333.

Brinton, LA; Blot, W; Becker, J; et al. (1984) A case-control study of cancers of the nasal cavity and paranasal sinuses. Am J Epidemiol 119:896–906.

Brooks, SM. (1985) Occupational asthma. In Bronchial Asthma; Mechanism and Therapeutics. Second edition. Eds Weiss, EB; Segal, MS; Stein, M. Little, Brown and Co, Boston/Toronto.

Brutlag, D; Schlehuber, C; Bonner, J. (1969) Properties of formaldehyde-treated nucleohistone. Biochemistry 8:3214–3218.

Buckley, LA; Jiang, XZ; James, RA; et al. (1984) Respiratory tract lesions induced by sensory irritants at the RD_{50} concentration. Toxicol Appl Pharmacol 74:417–429.

Bull, RJ. (2000) Mode of action of liver tumor induction by trichloroethylene and its metabolites, trichloroacetate and dichloroacetate. Environ Health Perspect 108(Suppl 2):241–259.

Burgaz, S; Çakmak, G; Erdem, O; et al. (2001) Micronuclei frequencies in exfoliated nasal mucosa cells from pathology and anatomy laboratory workers exposed to formaldehyde. Neoplasma 48:144–147.

Burgaz, S; Erdem, O; Çakmak, G; et al. (2002) Cytogenetic analysis of buccal cells from shoe-workers and pathology and anatomy laboratory workers exposed to n-hexane, toluene, methyl ethyl ketone and formaldehyde. Biomarkers 7:151–161.

Burge, PS; Harries, MG; Lam, WK; et al. (1985) Occupational asthma due to formaldehyde. Thorax 40:255–260.

Burt, RD; Vaughan, TL; McKnight, B. (1992) Descriptive epidemiology and survival analysis of nasopharyngeal carcinoma in the United States. Int J Cancer 52:549–556.

Buss, J; Kuschinsky, K; Kewitz, H; et al. (1964) Enteric resorption of formaldehyde. Naunyn Schmiedebergs Arch Pharmacol 247:380–381.

Cain, H. (2001) Bronchoprovocation testing. Clin Chest Med 22:651–659.

Cain, W; See, L; Tosun, T. (1986) Irritation and odor from formaldehyde: chamber studies. In: IAQ '86: Managing indoor air for health and energy conservation: proceedings of the American Society of Heating, Air Conditioning Engineers Conference, Atlanta, GA; pp. 126–137.

CalEPA (2008) California OEHHA. Hot Spots Guidelines. Air Toxicology and Epidemiology. Acute, 8-hour and Chronic Reference Exposure Levels. Appendix D. http://oehha.ca.gov/air/hot_spots/2008/AppendixD1_final.pdf

Callas, PW; Pastides, H; Hosmer, DW, Jr. (1996) Lung cancer mortality among workers in formaldehyde industries. J Occup Environ Med 38:747–748.

Cáp, P; Dryahina, K; Pehal, F; et al. (2008) Selected ion flow tube mass spectrometry of exhaled breath condensate headspace. Rapid Commun Mass Spectrom. 22(18):2844–2850.

Carraro, E; Gasparini, S; Petrini, T; et al. (1997) Immune response prevalence to formaldehyde-human serum albumin molecular adduct in a healthy population. J Environ Pathol Toxicol Oncol 16(2-3):215–218.

Carson, S; Goldhamer, R; Weinberg, MS. (1966) Characterization of physical, chemical and biological properties of mucus in the intact animal. Ann NY Acad Sci 130:935–943.

Casanova, M; Heck, HD. (1987) Further studies of the metabolic incorporation and covalent binding of inhaled [^{3}H]- and [^{14}C]formaldehyde in Fischer-344 rats: effects of glutathione depletion. Toxicol Appl Pharmacol 89:105-121.

Casanova, M; Heck, HD; Everitt, JI; et al. (1988) Formaldehyde concentrations in the blood of rhesus monkeys after inhalation exposure. Food Chem Toxicol 26:715–716.

Casanova, M; Devo, DF; Heck, HD. (1989) Covalent binding of inhaled formaldehyde to DNA in the nasal mucus of Fischer 344 rats: analysis of formaldehyde and DNA by high performance liquid chromatography and provisional pharmacokinetic interpretation. Fundam Appl Toxicol 12:397–417.

Casanova, M; Morgan, KT; Steinhagen, WH; et al. (1991) Covalent binding of inhaled formaldehyde to DNA in the respiratory tract of rhesus monkeys: pharmacokinetics, rat-to-monkey interspecies scaling, and extrapolation to man. Fundam Appl Toxicol 17:409–428.

Casanova, M; Morgan, KT; Gross, EA; et al. (1994) DNA-protein cross-links and cell replication at specific sites in the nose of F344 rats exposed subchronically to formaldehyde. Fundam Appl Toxicol 23:525–536.

Casanova, M; Bell, DA; and Heck, HD. (1997) Dichloromethane metabolism to formaldehyde and reaction of formaldehyde with nucleic acids in hepatocytes of rodents and humans with and without glutathione S-transferase T1 and M1 genes. Fundam Appl Toxicol 37:168-180.

Casanova, M; and Heck, HA. (1997) Lack of evidence for the involvement of formaldehyde in the hepatocarcinogenicity of methyl tertiary-butyl ether in CD-1 mice. Chem Biol Interact 105:131-143.

Casanova, M; Cole, P; Collins, JJ; et al. (2004) Re: mortality from lymphohematopoietic malignancies among workers in formaldehyde industries. J Natl Cancer Inst 96:966–967.

Casanova-Schmitz, M; Heck, HD. (1983) Effects of formaldehyde exposure on the extractability of DNA from proteins in the rat nasal mucosa. Toxicol Appl Pharmacol 70:121–132.

Casanova-Schmitz, M; David, RM; Heck, HD. (1984a) Oxidation of formaldehyde and acetaldehyde by NAD+-dependent dehydrogenases in rat nasal mucosal homogenates. Biochem Pharmacol 33:1137–1142.

Casanova-Schmitz, M; Starr, TB; Heck, HD. (1984b) Differentiation between metabolic incorporation and covalent binding in the labeling of macromolecules in the rat nasal mucosa and bone marrow by inhaled [14C]- and [3H]-formaldehyde. Toxicol Appl Pharmacol 76:26–44.

Cascieri, T; Clary, JJ. (1992) Formaldehyde--oral toxicity assessment. Comments Toxicol 4:295–304.

Cassee, FR; Arts, JH; Groten, JP; et al. (1996) Sensory irritation to mixtures of formaldehyde, acrolein, and acetaldehyde in rats. Arch Toxicol 70:329–337.

Cassee, FR; Feron, VJ. (1994) Biochemical and histopathological changes in nasal epithelium of rats after 3-day intermittent exposure to formaldehyde and ozone alone or in combination. Toxicol Lett 72:257–268.

Cassee, FR; Groten, JP; Feron, VJ. (1996) Changes in the nasal epithelium of rats exposed by inhalation to mixtures of formaldehyde, acetaldehyde, and acrolein. Fundam Appl Toxicol 29:208–218.

Casset, A; Purohit, A; Marchand, C; et al. (2006) The bronchial response to inhaled formaldehyde. Rev Mal Respir 23(1 Suppl):3S25–3S34.

Cassidy, SL; Dix, KM; Jenkins, T. (1983) Evaluation of a testicular sperm head counting technique using rats exposed to dimethoxyethyl phthalate (DMEP), glycerol alpha-monochlorohydrin (GMCH), epichlorohydrin (ECH), formaldehyde (FA), or methyl methanesulphonate (MMS). Arch Toxicol 53:71–78.

Castonguay, A; Pepin, P; Briere, N. (1991) Modulation of 4-(methylnitrosamino)-1-(3-pyridyl)-1 butanone demethylation and denitrosation by rat liver microsomes. Cancer Lett 59:67-74.

CDC (U.S. Centers for Disease Control and Prevention). (2000) Measuring childhood asthma prevalence before and after the 1997 redesign of the National Health Interview Survey -- United States. MMWR 49:908–911.

CDC. (2008) Final report on formaldehyde levels in FEMA-supplied travel trailers, park models, and mobile homes. Available from http://www.cdc.gov/nceh/ehhe/trailerstudy/residents.htm#final.

Ceder, R; Merne, M; Staab, C; et al. (2007) The application of normal, SV40 T-antigen-immortalized and tumour-derived oral keratinocytes, under serum-free conditions, to the study of the probability of cancer progression as a result of environmental exposure to chemicals. Alternatives to Laboratory Animals 35:621–639.

Cederbaum, AI; Qureshi, A. (1982) Role of catalase and hydroxyl radicals in the oxidation of methanol by rat liver microsomes. Biochem Pharmacol 31:329-335.

Chang, JC; Barrow, CS. (1984) Sensory irritation tolerance and cross-tolerance in F-344 rats exposed to chlorine or formaldehyde gas. Toxicol Appl Pharmacol 76:319–327.

Chang, JC; Steinhagen, WH; Barrow, CS. (1981) Effect of single or repeated formaldehyde exposure on minute volume of B6C3F1 mice and F-344 rats. Toxicol Appl Pharmacol 61:451–459.

Chang, JC; Gross, EA; Swenberg, JA; et al. (1983) Nasal cavity deposition, histopathology, and cell proliferation after single or repeated formaldehyde exposures in B6C3F1 mice and F-344 rats. Toxicol Appl Pharmacol 68:161-176.

Chebotarev, AN; Titenko, NV; Selezneva, TG, et al. (1986) [Comparison of chromosome aberrations, sister chromatid exchanges and unscheduled DNA synthesis in the evaluation of the mutagenicity of environmental factors]. Tsitol Genet 20:109-115.

Cheng, YS; Hansen, GK; Su, FY; et al. (1990) Deposition of ultrafine aerosols in rat nasal molds. Toxicol Appl Pharmacol 106:222–233.

Cheng, G; Shi, Y; Sturla, SJ; et al. (2003) Reactions of formaldehyde plus acetaldehyde with deoxyguanosine and DNA: formation of cyclic deoxyguanosine adducts and formaldehyde cross-links. Chem Res Toxicol 16:145–152.

Cheng, XM; Zhao, J; Feng, B; et al. (2008) Relationship between susceptibility of formaldehyde metabolism and genetic polymorphisms of ALD2 and cytochrome P4502E1. Zhonghua Yu Fang Yi Xue Za Zhi 42:582–587.

Cheng, G; Wang, M; Upadhyaya, P, et al. (2008) Formation of formaldehyde adducts in the reactions of DNA and deoxyribonucleosides with alpha-acetates of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), and N-nitrosodimethylamine (NDMA). Chem Res Toxicol 21:746-751.

Chernoff, N; Kavlock, RJ. (1982) An in vivo teratology screen utilizing pregnant mice. J Toxicol Environ Health 10:541–550.

Chiu, WA, Euling SY, Scott CS, and Subramaniam RP. (2010) Approaches to advancing quantitative human health risk assessment of environmental chemicals in the post-genomic era. Toxicology and Applied Pharmacology (Epub ahead of print March 29). Doi:10.1016/j.taap.2010.03.019.

Cho, JY; Min, N; Franzen, L; et al. (1996) Rapid down-regulation of tyrosine hydroxylase expression in the olfactory bulb of naris-occluded adult rats. J Comp Neurol 369:264–276.

Chowdhury, AR; Gautam, AK; Patel, KG; et al. (1992) Steroidogenic inhibition in testicular tissue of formaldehyde exposed rats. Ind J Physiol Pharmacol 36:162–168.

CIIT (Chemical Industry Institute of Toxicology). (1982) Summary of final report on formaldehyde study. CIIT Activities 2:1, 9–10.

CIIT. (1999) Formaldehyde: Hazard characterization and dose-response assessment for carcinogenicity by the route of inhalation (Revised Edition). Research Triangle Park, NC: Chemical Industry Institute of Toxicology.

Claeys, S; Cuvelier, C; Quatacker, J; et al. (1996a) Ultrastructural investigation of M-cells and lymphoepithelial contacts in naso-pharangeal associated lymphoid tissue (NALT). Acta Otolaryngeal Supple 523:40–42.

Claeys, S; Cuvelier, C; Van Cauwenberge, P. (1996b) Immunohistochemical analyses of the lymphoepithelium in human nasopharyngeal associated lymphoid tissue (NALT) Acta Otolaryngol Suppl 523:38–39.

Coggon, D; Pannett, B; Acheson, ED. (1984) Use of job-exposure matrix in an occupational analysis of lung and bladder cancers on the basis of death certificates. J Natl Cancer Inst 72(1):61–65.

Coggon, D; Harris, EC; Poole, J; et al. (2003) Extended follow-up of a cohort of British chemical workers exposed to formaldehyde. J Natl Cancer Inst 95:1608–1615.

Cohen-Hubal, EA; Schlosser, PM; Conolly, RB; et al. (1997) Comparison of inhaled formaldehyde dosimetry predictions with DNA-protein cross-link measurements in the rat nasal passages. Toxicol Appl Pharmacol 143:47-55.

Cole, P; Axten, C. (2004) Formaldehyde and leukemia: an improbable causal relationship. Regul Toxicol Pharmacol 40:107–112.

Collins, JJ. (2004) Formaldehyde exposure and leukemia. Occup Environ Med 61:875–876.

Collins, JJ; Lineker, GA. (2004) A review and meta-analysis of formaldehyde exposure and leukemia. Regul Toxicol Pharmacol 40:81–91.

Collins, JJ; Acquavella, JF; Esmen, NA. (1997) An updated analysis of formaldehyde exposure and upper respiratory tract cancers. J Occup Environ Med 39: 639–651.

Collins, JJ; Esmen, NA; Hall, TA. (2001a) A review and meta-analysis of formaldehyde exposure and pancreatic cancer. Am J Ind Med 39:336–345.

Collins, JJ; Ness, R; Tyl, RW; et al. (2001b) A review of adverse pregnancy outcomes and formaldehyde exposure in human and animal studies. Regul Toxicol Pharmacol 34:17–34.

Cometto-Muñiz, JE; Cain, WS. (1992) Sensory irritation. Relation to indoor air pollution. Ann NY Acad Sci 641:137–151.

Conaway, CC; Whysner, J; Verna, LK, et al. (1996) Formaldehyde mechanistic data and risk assessment: endogenous protection from DNA adduct formation. Pharmacol Ther 71:29-55.

Connor, TH; Barrie, MD; Theiss, JC; et al. (1983) Mutagenicity of formalin in the Ames assay. Mutat Res 119:145-149.

Connor, TH; Theiss, JC; Hanna, HA; et al. (1985) Genotoxicity of organic chemicals frequently found in the air of mobile homes. Toxicol Lett 25:33–40.

Conolly, RB, (1995) Cancer and non-cancer risk assessment: Not so different if you consider mechanisms. Toxicology 102:179–188.

Conolly, RB. (2002) The use of biologically based modeling in risk assessment. Toxicology 181–182:275–279.

Conolly, RB. (2009) Commentary on "Toxicity testing in the 21st century: Implications for human health risk assessment" by Krewski et al. Risk Anal 29:480–481.

Conolly, RB; Lutz, WK. (2004) Nonmonotonic dose-response relationships: Mechanistic basis, kinetic modeling, and implications for risk assessment. Toxicol Sci 77:151–157.

Conolly, RB; Lilly, PD; Kimbell, JS. (2000) Simulation modeling of the tissue disposition of formaldehyde to predict nasal DNA-protein cross-links in Fischer 344 rats, rhesus monkeys, and humans. Environ Health Perspect 108 Suppl 5:919–924.

Conolly, RB; Kimbell, JS; Janszen, D; et al. (2003) Biologically motivated computational modeling of formaldehyde carcinogenicity in the F344 rat. Toxicol Sci 75:432–447.

Conolly, RB; Kimbell, JS; Janszen, D; et al. (2004) Human respiratory tract cancer risks of inhaled formaldehyde: dose-response predictions derived from biologically-motivated computational modeling of a combined rodent and human dataset. Toxicol Sci 82:279–296.

Conolly, RB; Miller, FJ; Kimbell, JS; et al. (2009) Formaldehyde risk assessment. Ann Hyg 53:181–184.

Cook, RJ; Champion, KM; Giometti, CS. (2001) Methanol toxicity and formate oxidation in NEUT2 mice. Arch Biochem Biophys 393:192–198.

Coon, RA; Jones, RA; Jenkins, LJ, Jr; et al. (1970) Animal inhalation studies on ammonia, ethylene glycol, formaldehyde, dimethylamine, and ethanol. Toxicol Appl Pharmacol 16:646–655.

Correa, A; Gray, RH; Cohen, R; et al. (1996) Ethylene glycol ethers and risks of spontaneous abortion and subfertility. Am J Epidemiol 143:707–717.

Cosma, GN; Marchok, AC. (1988) Benzo[a]pyrene- and formaldehyde-induced DNA damage and repair in rat tracheal epithelial cells. Toxicology 51:309–320.

Cosma, GN; Jamasbi, R; Marchok, AC. (1988) Growth inhibition and DNA damage induced by benzo[a]pyrene and formaldehyde in primary cultures of rat tracheal epithelial cells. Mutat Res 201:161–168.

Cosma, GN; Wilhite, AS; and Marchok, AC. (1988) The detection of DNA-protein cross-links in rat tracheal implants exposed in vivo to benzo[a]pyrene and formaldehyde. Cancer Lett 42:13-21.

Costa, M; Zhitkovich, A; Harris, M; et al. (1997) DNA-protein cross-links produced by various chemicals in cultured human lymphoma cells. J Toxicol Environ Health 50:433–449.

Costa, S; Coelho, P; Costa, C, et al. (2008) Genotoxic damage in pathology anatomy laboratory workers exposed to formaldehyde. Toxicology 252:40–48.

Coste, A; Rateau, JG; Roudot-Thoraval, F; et al. (1996) Increased epithelial cell proliferation in nasal polyps. Arch Otol Head Neck Surg 122:432–436.

Couch, DB; Allen, PF; and Eales, HC. (1982) The mutagenicity of formaldehyde to Salmonella typhimurium [Abstract]. Environ Mutagen 4:336-337.

Cox, DR. (1972) Regression models and life tables (with discussion). J Roy Stat Soc (Series B) 34:187–220. (as cited in Battelle Columbus Laboratories [1981]).

Cox, D; Hinkley, D. (1974) Theoretical Statistics. Chapman and Hall, London, UK.

Cox, D; Oakes, D. (1984) Analysis of Survival Data. Chapman and Hall, London, UK.

Craft, TR; Bermudez, E; Skopek, TR. (1987) Formaldehyde mutagenesis and formation of DNA-protein crosslinks in human lymphoblasts in vitro. Mutat Res 176:147–155.

Cralley, LV. (1942) The effect of irritant gases upon the rate of ciliary activity. J Ind Hyg Toxicol 24:193–198.

Crosby, RM; Richardson, KK; Craft, TR; et al. (1988) Molecular analysis of formaldehyde-induced mutations in human lymphoblasts and E. coli. Environ Mol Mutagen 12:155–166.

Crump, KS. (1994a) Limitations of biological models of carcinogenesis for low-dose extrapolation. Risk Anal 14:883–886.

Crump, KS. (1994b) Use of mechanistic models to estimate low-dose cancer risks. Risk Anal 14:1033–1038.

Crump, K. (2002) Benchmark analysis. In: Encyclopedia of Environmetrics. Eds: El-Shaarawi, A; Piegorsch, WW. Wiley, West Sussex, UK.

Crump, KS; Subramaniam, RP; Van Landingham, C. (2005) A numerical solution to the non-homogenous two-stage MVK model of cancer. Risk Anal 25:921–926.

Crump, K; Subramanian, RP; Van Landingham, C. (2006) Acknowledgment of prior solution to MVK cancer model. Risk Anal 26:3–4.

Crump, KS; Chen, C; Fox, JF; et al. (2008) Sensitivity analysis of biologically motivated model for formaldehyde-induced respiratory cancer in humans. Ann Occup Hyg 52:481–495.

Crump, KS; Chen, C; Fox, JF; et al. (2009) Reply to Conolly et al. (2009) Ann Hyg 53:184–189.

Dahl, AR; Hadley, WM. (1983) Formaldehyde production promoted by rat nasal cytochrome P-450-dependent monooxygenases with nasal decongestants, essences, solvents, air pollutants, nicotine, and cocaine as substrates. Toxicol Appl Pharmacol 67:200-205.

Dalbey, WE. (1982) Formaldehyde and tumors in hamster respiratory tract. Toxicology 24:9–14.

Dalhamn, T. (1956) Mucus flow and ciliary activity in the trachea of healthy rats and rats exposed to respiratory irritant gases (SO_2, H_3N, HCHO): a functional and morphologic (light microscopy and electron microscopy) study, with special reference to technique. Acta Physiol Scand 36(Suppl):1–161.

Dallas, CE; Badeaux, P; Theiss, JC; et al. (1989) The influence of inhaled formaldehyde on rat lung cytochrome P450. Environ Res 49:50–59.

Dallas, CE; Scott, MJ; Ward, JB, Jr.; et al. (1992) Cytogenetic analysis of pulmonary lavage and bone marrow cells of rats after repeated formaldehyde inhalation. J Appl Toxicol 12:199–203.

Dally, K; Hanrahan, L; Woodbury, M; et al. (1981) Formaldehyde exposure in nonoccupational environments. Arch Environ Health 36(6):277–284.

Dalton, P. (2003) Upper airway irritation, odor perception and health risk due to airborne chemicals. Toxicol Lett 140–141:239–248.

Dasgupta PK, J Li, G Zhang, WT Luke, WA McClenny, J Stutz and A Fried. Summertime ambient formaldyde in five U.S. metropolitan areas: Nashville, Atlanta, Houston, Philadelphia and Tampa. Environ Sci Technol 39:4767-4783.

Daston, GP. (2008) Gene expression, dose-response, and phenotypic anchoring: Applications for toxicogenomics in risk assessment. Toxicol Sci 105:233–234.

Day, JH; Lees, RE; Clark, RH; et al. (1984) Respiratory response to formaldehyde and off-gas of urea formaldehyde foam insulation. Can Med Assoc J 131:1061–1065.

De Ceaurriz, JC; Micillino, JC; Bonnet, P; et al. (1981) Sensory irritation caused by various industrial airborne chemicals. Toxicol Lett 9:137–143.

De Flora, S. (1981) Study of 106 organic and inorganic compounds in the Salmonella/microsome test. Carcinogenesis 2:283-98.

de Flora, S; Camoirano, A; Zanacchi, P, et al. (1984) Mutagenicity testing with TA97 and TA102 of 30 DNA-damaging compounds, negative with other Salmonella strains. Mutat Res 134:159-165.

De Stefani, E; Boffetta, P; Brennan, P; et al. (2005) Occupational exposures and risk of adenocarcinoma of the lung in Uruguay. Cancer Causes and Control 16:851–856.

Dean, JH; Lauer, LD; House, RV; et al. (1984) Studies of immune function and host resistance in B6C3F1 mice exposed to formaldehyde. Toxicol Appl Pharmacol 72:519–529.

Dearman, RJ; Humphreys, N; Skinner, RA; et al. (2005) Allergen-induced cytokine phenotypes in mice: role of CD4 and CD8 T cell populations. Clin Exp Allergy 35:498–505.

Degitz, SJ; Zucker, RM; Kawanishi, CY; et al. (2004a) Pathogenesis of methanol-induced cranofacial defects in C57BL/6J mice. Birth Defects A Clin Mol Teratol 70:172–178.

Degitz, SJ; Rogers, JM; Zucker, RM; et al. (2004b) Developmental toxicity of methanol: Pathogenesis in CD-1 and C57BL/6J mice exposed in whole embryo culture. Birth Defects Res A Clin Mol Teratol. 70(4):179–184.

Delfino, RJ. (2002) Epidemiologic evidence for asthma and exposure to air toxics: linkages between occupational, indoor, and community air pollution research. Environ Health Perspect 110 Suppl 4:573–589.

Delfino, RJ; Gong, H Jr; Linn, WS; et al. (2003) Asthma symptoms in Hispanic children and daily ambient exposures to toxic and criteria air pollutants. Environ Health Perspect 111:647–656.

Dell, L; Teta, MJ. (1995) Mortality among workers at a plastics manufacturing and research and development facility: 1946-1988. Am J Ind Med 28:373–384.

Deltour, L; Foglio, MH; Duester, G. (1999) Metabolic deficiencies in alcohol dehydrogenase Adh1, Adh3, and Adh4 null mutant mice. Overlapping roles of Adh1 and Adh4 in ethanol clearance and metabolism of retinol to retinoic acid. J Biol Chem 274:16796–16801.

Demkowicz-Dobrzanski, K; and Castonguay, A. (1992) Modulation by glutathione of DNA strand breaks induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and its aldehyde metabolites in rat hepatocytes. Carcinogenesis 13:1447-1454.

Dicker, E; Cederbaum, AI. (1986) Inhibition of the low-Km mitochondrial aldehyde dehydrogenase by diethyl maleate and phorone in vivo and in vitro. Implications for formaldehyde metabolism. Biochem J 240:821–827.

Dikalova, AE; Kadiiska, MB; Mason, RP. (2001) An in vivo ESR spin-trapping study: free radical generation in rats from formate intoxication--role of the Fenton reaction. Proc Natl Acad Sci USA 98:13549–13553.

Dillon, D; Combes, R; Zeiger, E. (1998) The effectiveness of Salmonella strains TA100, TA102 and TA104 for detecting mutagenicity of some aldehydes and peroxides. Mutagenesis 13:19–26.

Dinsdale, D; Riley, RA; Verschoyle, RD. (1993) Pulmonary cytochrome P450 in rats exposed to formaldehyde vapor. Environ Res 62:19–27.

Doenecke, D. (1978) Digestion of chromosomal proteins in formaldehyde treated chromatin. Hoppe Seylers Z Physiol Chem 359:1343–1352.

Doi, S; Suzuki, S; Morishita, M; et al. (2003) The prevalence of IgE sensitization to formaldehyde in asthmatic children. Allergy 58(7):668–671.

Doll, R; Peto, R. (1978) Cigarette smoking and bronchial carcinoma: Dose and time relationships among regular smoker and lifetime nonsmokers. J Epidem Commun Health 32:303–313.

Donovan, SM; Krahn, DF; Stewart, JA; et al. (1983) Mutagenic activities of formaldehyde (HCHO) and hexamethylphosphoramide (HMPA) in reverse and forward Salmonella typhimurium mutation assays. Environ Mutagen 5:476.

Doolittle, DJ; Furlong, JW; and Butterworth, BE. (1985) Assessment of chemically induced DNA repair in primary cultures of human bronchial epithelial cells. Toxicol Appl Pharmacol 79:28-38.

Doty, RL; Cometto-Muñiz, JE; Jalowayski, AA; et al. (2004) Assessment of upper respiratory tract and ocular irritative effects of volatile chemicals in humans. Crit Rev Toxicol 34:85–142.

Dragan, YP; Hully, J; Baker, K; et al. (1995) Comparison of experimental and theoretical parameters of the Moolgavkar-Venzon-Knudson incidence function for the stages of initiation and promotion in rat hepatocarcinogenesis. Toxicology 102:161–175.

Dresp, J; and Bauchinger, M. (1988) Direct analysis of the clastogenic effect of formaldehyde in unstimulated human lymphocytes by means of the premature chromosome condensation technique. Mutat Res 204:349-352.

Dubreuil, A; Bouley, G; Godin, J; et al. (1976) Continuous inhalation of small amounts of formaldehyde: experimental study in the rat. Eur J Toxicol Environ Hyg 9:245–250.

Dulskienė, V; Gražulevičienė, R. (2005) Environmental risk factors and outdoor formaldehyde and risk of congenital heart malformations. Medicina (Kaunas) 41:787–795.

Dykewicz, M; Patterson, R; Cugell, DW; et al. (1991) Serum IgE and IgG to formaldehyde-human serum albumin lack of relation to gaseous formaldehyde exposure and symptoms. J Allergy Clin Immunol 87:48–57.

Edling, C; Jarvholm, B; Andersson, L; et al. (1987) Mortality and cancer incidence among workers in an abrasive manufacturing industry. Br J Ind Med 44:57–59.

Edling, C; Hellquist, H; Ödkvist, L. (1988) Occupational exposure to formaldehyde and histopathological changes in the nasal mucosa. Br J Ind Med 45:761–765.

Egle, JL, Jr. (1972) Retention of inhaled formaldehyde, propionaldehyde, and acrolein in the dog. Arch Environ Health 25:119–124.

Ehlers, CL. (2007) Variations in ADH and ALDH in Southwest California Indians. Alcohol Res Health 30:14–17.

Emri, G; Schaefer, D; Held, B; et al. (2004) Low concentrations of formaldehyde induce DNA damage and delay DNA repair after UV irradiation in human skin cells. Exp Dermatol 13:305–315.

Environment California. (2008) New Report: Formaldehyde from baby nursery furniture contaminates indoor air Californis's green chemistry initative can protect consumers. Available at http://www.environmentcalifornia.org/newsroom/environmental-health/environmental-health/new-report-formaldehyde-from-baby-nursery-furniture-contaminates-indoor-air.

Epstein, S.S.; Shafner, H. (1968) Chemical mutagens in human environment. Nature 219: 385.

Epstein, S.S. et al (1972) Detection of chemical mutagens by the dominant lethal assay in the mosue. Toxicol. Appl. Pharmacol. 23:288.

Erdei, E; Bobvos, J; Brózik, M; et al. (2003) Indoor air pollutants and immune biomarkers among Hungarian asthmatic children. Arch Environ Health 58:337–347.

Estonius, M; Svensson, S; Höög, JO. (1996) Alcohol dehydrogenase in human tissues: localization of transcript coding for five classes of the enzyme. FEBS Lett 397:338–342.

Ezratty, V; Bonay, M; Neukirch, C; et al. (2007) Effect of formaldehyde on asthmatic response to inhaled allergen challenge. Environ Health Perspect 115(2):210–214.

Fanucchi, MV; Buckpitt, AR; Murphy, ME; et al. (1997) Naphthalene cytotoxicity of differentiating Clara cells in neonatal mice. Toxicol Appl Pharmacol 144:96–104.

Fanucchi, MV; Buckpitt, AR; Murphy, ME; et al. (2000) Development of phase II xenobiotic metabolizing enzymes in differentiating murine Clara cells. Toxicol Appl Pharmacol 168:253–267.

Fanucchi, MV; Day, KC; Clay, CC; et al. (2004) Increased vulnerability of neonatal rats and mice to 1-nitronaphthalene-induced pulmonary injury. Toxicol Appl Pharmacol 201:53–65.

Farooqui, MY; Upreti, RK; Ahmed, AE; et al. (1986) Influence of intraperitoneally administered formaldehyde on bile production and tissue glutathione levels in rats. Res Commun Chem Pathol Pharmacol 53:233–236.

Fayerweather, WE; Pell, S; Bender, J. (1983) Case-control study of cancer deaths in DuPont workers with potential exposure to formaldehyde. In: Clary, J; Gibson, J; Waritz, RS; eds. Formaldehyde: toxicology, epidemiology, and mechanisms. New York: Marcel Dekker; pp. 47–121.

Fennell, TR. (1994) Development of methods for measuring biological markers of formaldehyde exposure. Res Rep Health Eff Inst:1–20; discussion 21–26.

Fernandez, MR; Biosca, JA; Pares, X. (2003) S-nitrosoglutathione reductase activity of human and yeast glutathione-dependent formaldehyde dehydrogenase and its nuclear and cytoplasmic localization. Cell Mol Life Sci 60:1013–1018.

Feron, VJ; Woutersen, RA; Appelman, LM. (1984) Epithelial damage and tumours of the nose after exposure to four different aldehydes by inhalation. In: Grosdanoff, P; Bass, R; Hackenberg, U; et al.; eds. Problems of inhalation toxicity studies. Munich: MMV Medizin Verlag; pp. 587–610.

Feron, VJ; Bruyntjes, JP; Woutersen, RA; et al. (1988) Nasal tumours in rats after short-term exposure to a cytotoxic concentration of formaldehyde. Cancer Lett 39:101–111.

Feron, VJ; Wilmer, JW; Woutersen, RA; et al. (1989) Inhalation toxicity and carcinogenicity of formaldehyde in animals: significance for assessment of human health risk. In: Bates, D; Dungworth, DL; Lee, P; et al.; eds. Assessment of inhalation hazards: integration and extrapolation using diverse data. Berlin: Springer-Verlag; pp. 131–138.

Feron, VJ; Til, HP; de Vrijer, F; et al. (1991) Aldehydes: occurrence, carcinogenic potential, mechanism of action and risk assessment. Mutat Res 259:363–385.

Feron, VJ; Arts, JH; Kuper, CF; et al. (2001) Health risks associated with inhaled nasal toxicants. Crit Rev Toxicol 31:313–347.

Fiddler, W; Miller, AJ; Pensabene, JW; et al. (1984) Investigation on the mutagenicity of N-nitrosothiazolidine using the Ames Salmonella test. IARC Sci Publ:95–100.

Figuero, WG; Raszkowski, R; Weiss, W. (1973) Lung cancer in chloromethyl ether workers. N Engl J Med 288:1096–1097.

Fireman, P. (2003) Understanding asthma pathophysiology. Allergy Asthma Proc 24:79–83.

Fishbein, L. (1992) Exposure from occupational versus other sources. Scand J Work Environ Health 18(Suppl 1):5-16.

Fleig, I; Petri, N; Stocker, WG; et al. (1982) Cytogenetic analyses of blood lymphocytes of workers exposed to formaldehyde in formaldehyde manufacturing and processing. J Occup Med 24:1009–1012.

Fleisher, J. (1987) Medical students' exposure to formaldehyde in gross anatomy laboratories. N Y State J Med 87(7):385–388.

Fló-Neyret, C; Lorenzl-Filho, G; Macchione, M; et al. (2001) Effects of formaldehyde on the frog's mucociliary epithelium as a surrogate to evaluate air pollution effects on the respiratory epithelium. J Med Bil Res 34:639–643.

Fontignie-Houbrechts, N. (1981) Genetic effects of formaldehyde in the mouse. Mutat Res 88:109–114.

Fontignie-Houbrechts, N; Moutschen-Dahmen, M; Degraeve, N; et al. (1982) Genetic effects in the mouse of formaldehyde in combination with adenosine and hydrogen peroxide. Mutat Res 104:371–376.

Fornace, AJ, Jr. (1982) Detection of DNA single-strand breaks produced during the repair of damage by DNA-protein cross-linking agents. Cancer Res 42:145-149.

Franklin, P; Dingle, P; Stick, S. (2000) Raised exhaled nitric oxide in healthy children is associated with domestic formaldehyde levels. Am J Respir Crit Care Med 161:1757–1759.

Franks, SJ. (2005) A mathematical model for the absorption and metabolism of formaldehyde vapour in humans. Toxicol Appl Pharmacol 206:309–320.

Fransman, W; McLean, D; Douwes, J; et al. (2003) Respiratory symptoms and occupational exposures in New Zealand plywood mill workers. Ann Occup Hyg 47(4):287–295.

Frazelle, JH; Abernethy, DJ; and Boreiko, CJ. (1983) Weak promotion of C3H/10T1/2 cell transformation by repeated treatments with formaldehyde. Cancer Res 43:3236-3239.

Frei, E; Pool, BL; Plesch, W; et al. (1984) Biochemical and biological properties of prospective N-nitrodialkylamine metabolites and their derivatives. IARC Sci Publ:491–497.

Friedman, GD; Ury, HK. (1983) Screening for possible drug carcinogenicity: second report of findings. J Natl Cancer Inst 71:1165–1175.

Frigas, E; Filley, WV; Reed, CE. (1984) Bronchial challenge with formaldehyde gas: lack of bronchoconstriction in 13 patients suspected of having formaldehyde-induced asthma. Mayo Clin Proc 59:295–299.

Fujimaki, H; Kurokawa, Y; Kunugita, N; et al. (2004a) Differential immunogenic and neurogenic inflammatory responses in an allergic mouse model exposed to low levels of formaldehyde. Toxicology 197:1–13.

Fujimaki, H; Kurokawa, Y; Kakeyama, M; et al. (2004b) Inhalation of low-level formaldehyde enhances nerve growth factor production in the hippocampus of mice. Neuroimmunomodulation 11:373–375.

Fujimaki, H; Kurokawa, Y; Kunugita, N; et al. (2005) Erratum to "Differential immunogenic and neurogenic inflammatory responses in an allergic mouse model exposed to low levels of formaldehyde" [Toxicology 197 2004 (1-13)]. Toxicology 209:305.

Fujimura, Y. (2000) Evidence of M cells as portals of entry for antigens in the nasopharyngeal lymphoid tissue of humans. Virchows Arch 436:560–566.

Galloway, SM; Bloom, AD; Resnick, M, et al. (1985) Development of a standard protocol for in vitro cytogenetic testing with Chinese hamster ovary cells: comparison of results for 22 compounds in two laboratories. Environ Mutagen 7:1-51.

Galter, D; Carmine, A; Buervenich, S; et al. (2003) Distribution of class I, III and IV alcohol dehydrogenase mRNAs in the adult rat, mouse and human brain. Eur J Biochem 270:1316−1326.

Gandhi, M; Aweeka, F; Greenblatt, RM; et al. (2004) Sex differences in pharmacokinetics and pharmaco-dynamics Ann Rev Pharmacol Toxicol 44:499–523.

Garcia, CL; Mechilli, M; De Santis, LP, et al. (2009) Relationship between DNA lesions, DNA repair and chromosomal damage induced by acetaldehyde. Mutat Res 662:3-9.

Garcia, GJ; Schroeter, JD; Segal, RA; et al. (2009) Dosimetry of nasal uptake of water-soluble and reactive gases: a first study of interhuman variability. Inhal Toxicol. 21(7):607-18.

Gardner, MJ; Pannett, B; Winter, PD; et al. (1993) A cohort study of workers exposed to formaldehyde in the British chemical industry: an update. Br J Ind Med 50:827–834.

Gardner, RJ; Burgess, BA; Kennedy, GL, Jr. (1985) Sensory irritation potential of selected nasal tumorigens in the rat. Food Chem Toxicol 23:87–92.

Garrett, MH; Hooper, MA; Hooper, BM; et al. (1999a) Increased risk of allergy in children due to formaldehyde exposure in homes. Allergy 34:330–337.

Garrett, MH; Hooper, MA; Hooper, BM; et al. (1999b) Errata. Allergy 54(12):1327.

Garry, VF; Kreiger, RA; and Wiencke, JK. (1981) The mutagenic and cytotoxic effects of formaldehyde in cultured human lymphocytes [Abstract]. Environ Mutagen 3:341.

Gart, JJ; Krewski, D; Lee, PN; et al. (1986) Statistical methods in cancer research. Volume III--The design and analysis of long-term animal experiments. IARC Sci Publ (79):1–219.

Gaylor, DW; Zheng, O. (1996) Risk assessment of nongenotoxic carcinogens based upon cell proliferation/death rates in rodents. Risk Anal 16:221–225.

Gaylor, DW; Lutz, WK; Conolly, RB. (2004) Statistical analysis of nonmonotonic dose-response relationships: Research design and analysis of nasal cell proliferation in rats exposed to formaldehyde. Toxicol Sci 77:158–164.

Gee, IL; Watson, AFR; Tavernier, G; et al. (2005) Indoor air quality, environmental tobacco smoke and asthma: A case control study of asthma in a community population. Indoor Built Environ 14(3-4):215–219.

Georgieva, AV; Kimbell, JS; Schlosser, PM. (2003) A distributed-parameter model for formaldehyde uptake and disposition in the rat nasal lining. Inhal Toxicol 15:1435–1463.

Gerard, C. (2005) Biomedicine. Asthmatics breathe easier when its SNO-ing. Science 308:1560–1561.

Gerberich, HR; Seaman, GC. (1994) Formaldehyde. In: Kroshwitz, JI; Howe-Grant, M; eds. Kirk-Othmer encyclopedia of chemical technology. Vol. 11. 4th edition. New York, NY: John Wiley and Sons; pp. 929–951.

Gerde, P; Cheng, YS; Medinsky, MA. (1991) In vitro deposition of ultrafine aerosols in the nasal airways of the rat. Fundam Appl Toxicol 16:330–336.

Gerin, M; Siemiatycki, J; Nadon, L; et al. (1989) Cancer risks due to occupational exposure to formaldehyde: Results of a multi-site case-control study in Montreal Canada. Int J Cancer 44:53–58.

Ginsberg, G; Smolenski, S; Hattis, D; et al. (2002) Population distribution of aldehyde dehydrogenase-2 genetic polymorphism: implications for risk assessment. Regul Toxicol Pharmacol 36:297–309.

Ginsberg, G; Hattis, D; Sonawane, B. (2004) Incorporating pharmacokinetic differences between children and adults in assessing children's risks to environmental toxicants. Toxicol Appl Pharmacol 198:164–183.

Ginsberg, G; Foos, B; Firestone, M. (2005) Review and Analysis of Inhalation Dosimetry Methods for Application to Children's Risk Assessment. J. Tox. & Env. Health 68:573-615.

Ginsberg,G; Asgharian, B; Kimbell, J; et al. (2008) Modeling approaches for estimating the dosimetry of inhaled toxicants in children. J. Tox. & Env. Health 71:166-195.

Global Insight, (2006) Economic primer on formaldehyde. Prepared for Formaldehyde Council, Inc. http://www.formaldehyde.org/pdfs/GIEconomicPrimer-04-20-06.pdf.

Gochfeld, M. (2007) Framework for gender differences in human and animal toxicology. Environ Res 104:4–21.

Gofmekler, V. (1968) Embryotropic action of benzene and formaldehyde inhalation. Hyg Sanit 33:327–332.

Gofmekler, V; Bonashevskaya, T. (1969) Experimental studies of teratogenic properties of formaldehyde, based on pathological investigations. Hyg Sanit 34:266–268.

Golaliour MJ; Azarhoush R; Ghafari S; Gharravi AM; Fazeli SA; Davarian A. 2007. Formaldehyde exposure induces histopathological and morphometric changes in the rat testis. Folia Morphol. 66(3): 167-171.

Golden, R; Pyatt, D; Shields, PG. (2006) Formaldehyde as a potential human leukemogen; An assessment of biological plausibility. Crit Rev Toxicol 36:135–153.

Goldmacher, VS; and Thilly, WG. (1983) Formaldehyde is mutagenic for cultured human cells. Mutat Res 116:417-422.

Goldstein, BD. (1973) Textiles and the chemical industry: A marriage. J Am Assoc Textile Chemists and Colorists 10:209–214.

Gordon, MY; Marley, SB; Apperley, JF; et al. (2003) Clinical heterogeneity in chronic myeloid leukemia reflecting biological diversity in normal persons. Brit J Haematol 122:424–429.

Gordon, CJ; Spencer, PJ; Hotchkiss, J; et al. (2008) Thermoregulation and its influence on risk assessment. Toxicology 244:87–97.

Gorski, P; Tarkowski, M; Krakowiak, A; et al. (1992) Neutrophil chemiluminescence following exposure to formaldehyde in healthy subjects and in patients with contact dermatitis. Immunologia Clinica 11:53–58.

Gottschling, LM; Beaulieu, HJ; Melvin, WW. (1984) Monitoring of formic acid in urine of humans exposed to low levels of formaldehyde. Am Ind Hyg Assoc J 45:19–23.

Grafström, RC; Fornace, AJ, Jr.; Autrup, H; et al. (1983) Formaldehyde damage to DNA and inhibition of DNA repair in human bronchial cells. Science 220:216–218.

Grafström, RC; Fornace, A, Jr.; Harris, CC. (1984) Repair of DNA damage caused by formaldehyde in human cells. Cancer Res 44:4323–4327.

Grafström, RC; Curren, RD; Yang, LL; et al. (1985) Genotoxicity of formaldehyde in cultured human bronchial fibroblasts. Science 228:89–91.

Grafström, RC. (1990) In vitro studies of aldehyde effects related to human respiratory carcinogenesis. Mutat Res 238:175-84.

Grafström, RC; Hsu, IC; and Harris, CC. (1993) Mutagenicity of formaldehyde in Chinese hamster lung fibroblasts: synergy with ionizing radiation and N-nitroso-N-methylurea. Chem Biol Interact 86:41-49.

Grammer, LC; Harris, KE; Shaughnessy, M; et al. (1990) Clinical and immunologic evaluation of 37 workers exposed to gaseous formaldehyde. J Allergy Clin Immunol 86:177–181.

Grammer, LC; Harris, KE; Cugell, DW; et al. (1993) Evaluation of a worker with possible formaldehyde-induced asthma. J Allergy Clin Immunol 92:29–33.

Grasl-Kraupp, B; Luebeck, G; Wagner, A; et al. (2000) Quantitative analysis of tumor initiation in rat liver: Role of cell replication and cell death (apoptosis). Carcinogenesis 21:1411–1421.

Graves, RJ; Callander, RD; and Green, T. (1994) The role of formaldehyde and S-chloromethylglutathione in the bacterial mutagenicity of methylene chloride. Mutat Res 320:235-243.

Graves, RJ; Trueman, P; Jones, S; et al. (1996) DNA sequence analysis of methylene chloride-induced HPRT mutations in Chinese hamster ovary cells: comparison with the mutation spectrum obtained for 1,2-dibromoethane and formaldehyde. Mutagenesis 11:229–233.

Gray, RH; Wu, LY. (2000) Subfertility and risk of spontaneous abortion. Am J Public Health 90:1452–1454.

Gražulevičienė, R; Dulskienė, V; Venclovienė, J. (1998) Formaldehyde exposure and low birth weight incidence. J Occup Health 40:61–67.

Greaves, MA. (2004) Biological models for leukemia and lymphoma. IARC Sci Publ (157):351–372.

Green, DJ; Sauder, LR; Kulle, TJ; et al. (1987) Acute response to 3.0 ppm formaldehyde in exercising healthy nonsmokers and asthmatics. Am Rev Respir Dis 135:1261–1266.

Green, DJ; Bascom, R; Healey, EM; et al. (1989) Acute pulmonary response in healthy, nonsmoking adults to inhalation of formaldehyde and carbon. Toxicol Environ Health 28:261–275.

Griesemer, RA; Boreiko, CJ; de Serres, FJ; et al. (1985) Report on the consensus workshop on formaldehyde: Carcinogenicity/Histopathology/Genotoxicity Panel report. Environ Health Perspect 58:340–343.

Grosjean D, Swanson RD, Ellis C. (1983) Carbonyls in Los Angeles air: Contribution of direct emissions and photochemistry. Sci Total Environ 29:65-85.

Guess, H; Crump, K; Peto, R. (1977) Uncertainty estimates for low dose-rate extrapolations of animal carcinogenicity data. Cancer Res 37:3475–3483.

Guseva, VA. (1972) Study of the gonadotropic effect in male rats under the effect of formaldehyde administered simultaneously with air and water [translation]. Gig Sanit 37:101–102.

Gustavsson, P; Jakobsson, R; Johannson, H; et al. (1998) Occupational exposures and squamous cell carcinoma of the oral cavity, pharynx, larynx, and oesophagus: a case-control study in Sweden. Occup Environ Med 55:393–400.

Hagiwara, M; Watanabe, E; Barrett, JC, et al. (2006) Assessment of genotoxicity of 14 chemical agents used in dental practice: ability to induce chromosome aberrations in Syrian hamster embryo cells. Mutat Res 603:111-120.

Hakim, RB; Gray, RH; Zacur, H. (1995) Infertility and early pregnancy loss. Am J Obstet Gynecol 172:1510–1517.

Hall, A; Harrington, J; Aw, TC. (1991) Mortality study of British pathologists. Am J Ind Med 20:83–89.

Hamaguchi, F; and Tsutsui, T. (2000) Assessment of genotoxicity of dental antiseptics: ability of phenol, guaiacol, p-phenolsulfonic acid, sodium hypochlorite, p-chlorophenol, m-cresol or formaldehyde to induce unscheduled DNA synthesis in cultured Syrian hamster embryo cells. Jpn J Pharmacol 83:273-276.

Hanahan, D; Weinberg, RA. (2000) The hallmarks of cancer. Cell 100:57–70.

Hanrahan, LP; Dally, KA; Anderson, HA; et al. (1984) Formaldehyde vapor in mobile homes: a cross sectional survey of concentrations and irritant effects. Am J Public Health 74(9):1026–1027.

Hansel, A; Jordan, A; Holzinger, R; et al. (1995) Proton transfer reaction-mass spectrometry: on-line trace gas analysis at the ppb level. Int. J. Mass Spectrom. Ion Process 149–150:609–619.

Hansen, J; Olsen, JH. (1995) Formaldehyde and cancer morbidity among male employees in Denmark. Cancer Causes Control 6:354–360.

Hanzlik, RP; Fowler, SC; Eells, JT. (2005) Absorption and elimination of formate following oral administration of calcium formate in female human subjects. Drug Metab Dispos 33:282–286.

Harrington, JM; Shannon, H. (1975) Mortality study of pathologists and medical laboratory technicians. Br Med J 4:329–332.

Harrington, JM; Oakes, D. (1984) Mortality study of British pathologists 1974-80. Br J Ind Med 41:188–191.

Harris, NL; Jaffe, ES; Diebold, J; et al. (2000a) The World Health Organization classification of neoplasms of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee meeting—Airlie House, Virginia, November, 1997. Hematol J 1:53–66.

Harris, NL; Jaffe, ES; Diebold, J; et al. (2000b) The World Health Organization classification of hematological malignancies report of the Clinical Advisory Committee meeting, Airlie House, Virginia, November 1997. Mod Pathol 13:193–207.

Harris, C; Wand, S-W; Lauchu, JJ; et al. (2003) Methanol metabolism and embryotoxicity in rat and mouse conceptuses: comparison of alcohol dehydrogenase (ADH1), formaldehyde dehydrogenase (ADH3), and catalase. Reprod Toxicol 17:349–357.

Harving, H; Korsgaard, J; Pedersen, OF; et al. (1990) Pulmonary function and bronchial reactivity in asthmatics during low-level formaldehyde exposure. Lung 168:15–21.

Haseman, JK. (1995) Data analysis: Statistical analysis and use of historical control data. Regul Toxicol Pharmacol 21:52–59.

Hauptmann, M; Lubin, JH; Stewart, PA; et al. (2003) Mortality from lymphohematopoietic malignancies among workers in formaldehyde industries. J Natl Cancer Inst 95:1615–1623.

Hauptmann, M; Lubin, JH; Stewart, PA; et al. (2004a) Mortality from solid cancers among workers in formaldehyde industries. Am J Epidemiol 159:1117–1130.

Hauptmann, M; Lubin, JH; Stewart, PA; et al. (2004b) Response. J Natl Cancer Inst 96:967−968.

Hauptmann, M; Stewart, P; Lubin, J; et al. (2009) Mortality from lymphohematopoietic malignancies and brain cancer among embalmers exposed to formaldehyde. JNCI 101:1-13

Haworth, S; Lawlor, T; Mortelmans, K; et al. (1983) Salmonella mutagenicity test results for 250 chemicals. Environ Mutagen 5 Suppl 1:1–142.

Hayashi, H; Kunugita, N; Arashidani, K; et al. (2004) Long-term exposure to low levels of formaldehyde increases the number of tyrosine hydroxylase-immunopositive periglomerular cells in mouse main olfactory bulb. Brain Res 1007:192–197.

Hayes, RB; Raatgever, JW; de Bruyn, A; et al. (1986) Cancer of the nasal cavity and paranasal sinuses, and formaldehyde exposure. Int J Cancer 37:487–492.

Hayes, RB; Blair, A; Stewart, PA; et al. (1990) Mortality of U.S. embalmers and funeral directors. Am J Ind Med 18:641–652.

Hayes, RB; Klein, S; Suruda, A, et al. (1997) O6-alkylguanine DNA alkyltransferase activity in student embalmers. Am J Ind Med 31:361-365.

He, JL; Jin, LF; Jin, HY. (1998) Detection of cytogenetic effects in peripheral lymphocytes of students exposed to formaldehyde with cytokinesis-blocked micronucleus assay. Biomed Environ Sci 11:87–92.

Health Canada and Environment Canada. (2001) Priority substances list assessment report: formaldehyde. Hull, Quebec, Canada: Environment Canada and Health Canada.

Heck, HD, Casanova-Schmitz, M. (1984) The relevance of disposition studies to the toxicology of formaldehyde. CIIT Activities 4:1–5.

Heck HD; Casanova, M. (1987) Isotope effects and their implications for the covalent binding of inhaled [3H]- and [14C]formaldehyde in the rat nasal mucosa. Toxicol Appl Pharmacol 89:122−134.

Heck, HD; Casanova, M. (1999) Pharmacodynamics of formaldehyde: applications of a model for the arrest of DNA replication by DNA-protein cross-links. Toxicol Appl Pharmacol 160:86−100.

Heck, HD, Casanova, M. (2004) The implausibility of leukemia induction by formaldehyde: A critical review of the biological evidence on distant-site toxicity. Regul Toxicol Pharmacol 40:92–106.

Heck, HD; White, EL; Casanova-Schmitz, M. (1982) Determination of formaldehyde in biological tissues by gas chromatography/mass spectrometry. Biomed Mass Spectrom 9:347–353.

Heck, HD; Chin, TY; Schmitz, MC. (1983) Distribution of [14C] formaldehyde in rats after inhalation exposure. In: Gibson, J E , eds. Formaldehyde Toxicity. Washington, DC: Hemisphere Publishing Corporation; pp. 26–37.

Heck Hd, A; Casanova, M; L., C-W, et al. (1986). The formation of DNA-protein crosslinks by aldehydes present in tobacco smoke. In: Hoffman, D and CC Harris,ed, Mechanisms in Tobacco Carcinogenesis: Cold Spring Harbor, New York: CSH Press, 215−230.

Heck, HD; Casanova-Schmitz, M; Dodd, PB; et al. (1985) Formaldehyde (CH2O) concentrations in the blood of humans and Fischer-344 rats exposed to CH2O under controlled conditions. Am Ind Hyg Assoc J 46:1–3.

Heck, HD; Casanova, M; Starr, TB. (1990) Formaldehyde toxicity--new understanding. Crit Rev Toxicol 20:397-426.

Hedberg, JJ; Høøg, JO; Nilsson, JA; et al. (2000) Expression of alcohol dehydrogenase 3 in tissue and cultured cells from human oral mucosa. Am J Pathol 157:1745–1755.

Hedberg, JJ; Grafström, RC; Vondracek, M; et al. (2001a) Micro-array chip analysis of carbonyl-metabolizing enzymes in normal, immortalized and malignant human oral keratinocytes. Cell Mol Life Sci 58:1719–1726.

Hedberg, JJ; Backlund, M; Stromberg, P; et al. (2001b) Functional polymorphism in the alcohol dehydrogenase 3 (ADH3) promoter. Pharmacogenetics 11:815–824.

Hedberg, JJ; Griffiths, WJ; Nilsson, SJ; et al. (2003) Reduction of S-nitrosoglutathione by human alcohol dehydrogenase 3 is an irreversible reaction as analyzed by electrospray mass spectrometry. Eur J Biochem 270:1249–1256.

Heikkilä, P; Priha, E; Savola, A. (1991) Exposure at work. 14. Formaldehyde. Institute of Occupational Health. Finnish Work Environmental Fund.

Heineman, EF; Olsen, JH; Pottern, LM; et al. (1992) Occupational risk factors for multiple myeloma among Danish men. Cancer Causes Control 3:555–568.

Hemminki, K; Mutanen, P; Salonienmi, I; et al. (1982) Spontaneous abortions in hospital staff engaged in sterilizing instruments with chemical agents. Br Med J 285:1461–1463.

Hemminki, K; Kyyrönen, P; Lindbohm, ML. (1985) Spontaneous abortions and malformations in the offspring of nurses exposed to anaesthetic gases, cytostatic drugs, and other potential hazards in hospitals, based on registered information of outcome. J Epidemiol Community Health 39:141–147.

Hendrick, DJ; Lane, DJ. (1975) Formalin asthma in hospital staff. Br Med J 1(5958):607–608.

Hendrick, DJ; Lane, DJ. (1977) Occupational formalin asthma. Br J Ind Med 34:11–18.

Hendrick, DJ; Rando, RJ; Lane, DJ; et al. (1982) Formaldehyde asthma: challenge exposure levels and fate after five years. J Occup Med 24:893–897.

Herbert, FA; Hessel, PA; Melenka, LS; et al. (1994) Respiratory consequences of exposure to wood dust and formaldehyde of workers manufacturing oriented strand board. Arch Environ Health 49(6):465–470.

Hernandez, O; Rhomberg, L; Hogan, K; et al. (1994) Risk assessment of formaldehyde. J Hazard Mater 39:161-172.

Hess, DT; Matsumoto, A; Kim, SO; et al. (2005) Protein S-nitrosylation: purview and parameters. Nat Rev Mol Cell Biol 6:150–166.

Hester, SD; Benavides, GB; Yoon, L; et al. (2003) Formaldehyde-induced gene expression in F344 rat nasal respiratory epithelium. Toxicology 187:13–24.

Hester, SD; Barry, WT; Zou, F; et al. (2005) Transcriptomic analysis of F344 rat nasal epithelium suggests that the lack of carcinogenic response to glutaraldehyde is due to its greater toxicity compared to formaldehyde. Toxicol Pathol 33:415–424.

Hikiba, H; Watanabe, E; Barrett, JC; et al. (2005) Ability of fourteen chemical agents used in dental practice to induce chromosome aberrations in Syrian hamster embryo cells. J Pharmacol Sci 97:146–152.

Hildesheim, A; Dosemeci, M; Chan, CC; et al. (2001) Occupational exposure to wood, formaldehyde, and solvents and risk of nasopharyngeal carcinoma. Cancer Epidemiol Biomarkers Prev 10:1145–1153.

Hileman, B. (1984) Formaldehyde: assessing the risk. Environ Sci Technol 18:216A–221A.

Hilton, J; Dearman, RJ; Harvey, P; et al. (1998) Estimation of relative skin sensitizing potency using the local lymph node assay: a comparison of formaldehyde with glutaraldehyde. Am J Contact Dermat 9:29–33.

Hines, RN; McCarver, DG. (2002) The ontogeny of human drug-metabolizing enzymes: phase I oxidative enzymes. J Pharmacol Exp Ther 300:361–366.

Holly, EA; Aston, DA; Ahn, DK; et al. (1996) Intraocular melanoma linked to occupations and chemical exposures. Epidemiology 7:55–61.

Holmström, M; Wilhelmsson, B. (1988) Respiratory symptoms and pathophysiological effects of occupational exposure to formaldehyde and wood dust. Scand J Work Environ Health 14:306−311.

Holmström, M; Wilhelmsson, B; Hellquist, H. (1989a) Histological changes in the nasal mucosa in rats after long-term exposure to formaldehyde and wood dust. Acta Otolaryngol 108:274−283.

Holmström, M; Rynnel-Dagöö, B; Wilhelmsson, B. (1989b) Antibody production in rats after long-term exposure to formaldehyde. Toxicol Appl Pharmacol 100:328–333.

Holmström, M; Wilhelmsson, B; Hellquist, H; et al. (1989c) Histological changes in the nasal mucosa in persons occupationally exposed to formaldehyde alone and in combination with wood dust. Acta Otolaryngol 107:120–129.

Holness, DL; Nethercott, JR. (1989) Health status of funeral service workers exposed to formaldehyde. Arch Environ Health 44:222–228.

Höög, JO; Hedberg, JJ; Strömberg, P; et al. (2001) Mammalian alcohol dehydrogenase -functional and structural implications. J Biomed Sci 8:71–76.

Höög, JO; Strömberg, P; Hedberg, JJ; et al. (2003) The mammalian alcohol dehydrogenases interact in several metabolic pathways. Chem Biol Interact 143–144:175–181.

Hoogenveen, R; Clewell, H; Andersen, M; et al. (1999) An alternative exact solution of the two-stage clonal growth model of cancer. Risk Anal 19:9–14.

Horton, A; Tye, R; Stemmer, K. (1963) Experimental carcinogenesis of the lung. Inhalation of gaseous formaldehyde or an aerosol of coal tar by C3H mice. J Natl Cancer Inst 30:31–43.

Horvath, E, Jr; Anderson, J; Pierce, WE; et al. (1988) Effects of formaldehyde on the mucous membranes and lungs: A study of an industrial population. JAMA 259:701–707.

Hou, Y; Hu, WG; Hirano, T; et al. (2002) A new intra-NALT route elicits mucosal and systemic immunity against Moraxella catarrhalis in a mouse challenge model. Vaccine 20:2375–2381.

HSDB (Hazardous Substances Data Bank). (2001) Formaldehyde. National Library of Medicine, Bethesda, MD. Available from <http://toxnet.nlm.nih.gov>.

Hurni, H; Ohder, H. (1973) Reproduction study with formaldehyde and hexamethylenetetramine in beagle dogs. Food Cosmet Toxicol 11:459–462.

IARC (International Agency for Research on Cancer). (1995) IARC monographs on the evaluation of carcinogenic risks to humans. Vol. 62. Wood dust and formaldehyde. Lyon, France: International Agency for Research on Cancer. Available from <http://www.inchem.org/documents/iarc/vol62/wood.html> and <http://www.inchem.org/documents/iarc/vol62/formal.html>.

IARC (2006) IARC Monographs on the evaluation of carcinogenic risks to humans. Formaldehyde, 2-Butoxyethanol and 1-tert-Butoxy-2-propanol, vol. 88. IARC, Lyon, France. http://monographs.iarc.fr/ENG/Monographs/vol88/mono88-6A.pdf

Iarmarcovai, G; Botta, A; Orsière, T. (2006a) Number of centromeric signals in micronuclei and mechanisms of aneuploidy. Toxicol Lett 166:1–10.

Iarmarcovai, G; Orsière, T; Sari-Minodier, I; et al. (2006b) Different pathways of centromeric micronuclei formation in peripheral blood lymphocytes of cancer patients and workers occupationally exposed to mutagens/carcinogens? Advances in Molecular and Clinical Aspects of Cancer Prevention,4th International Conference on Cancer Prevention 2006, 4:54–55, Switzerland.

Iarmarcovai, G; Bonassi, S; Sari-Minodier, I; et al. (2007) Exposure to genotoxic agents, host factors, and lifestyle influence the number of centromeric signals in micronuclei: a pooled re-analysis. Mutat Res 615:18–27.

Ibanes, JD; Leininger, JR; Jarabek, AM; et al. (1996) Reexamination of respiratory tract responses in rats, mice, and rhesus monkeys chronically exposed to inhaled chlorine. Inhal Tox 8:859–876.

ICRP (International Commission on Radiological Protection). (1994) Human respiratory tract model for radiological protection. Publication 66 Annals of the ICRP Vol 24; Pergamon Press, Oxford, UK.

Ihrig, A; Hoffmann, J; Triebig, G. (2006) Examination of the influence of personal traits and habituation on the reporting of complaints at experimental exposure to ammonia. Int Arch Occup Environ Health 79:332–338.

ILSI (International Life Sciences Institute). (2003) Final report of a workshop to develop a framework for assessing risks to children from exposures to environmental agents. ILSI Risk Science Institute, Washington, DC.

Im, H; Oh, E; Mun, J; et al. (2006) Evaluation of toxicological monitoring markers using proteomic analysis in rats exposed to formaldehyde. J Proteome Res 5:1354–1366.

Ionescu, J; Marinescu, D; Tapu, V; et al. (1978) Experimental chronic obstructive lung disease. I. Bronchopulmonary changes induced in rabbits by prolonged exposure to formaldehyde. Morphol Embryol 24:233-242.

IPCS (International Programme on Chemical Safety). (1989) Environmental Health Criteria. Vol. 89. Formaldehyde. Geneva, Switzerland: World Health Organization. Available from <http://www.inchem.org/documents/ehc/ehc/ehc89.htm>.

IPCS (International Programme on Chemical Safety). (1997) Environmental Health Criteria 196 -- Methanol 180.

IPCS. (2002) Formaldehyde. Concise international chemical assessment document. Vol. 40. World Health Organization, Geneva, Switzerland. Available online at http://www.inchem.org/documents/cicads/cicads/cicad40.htm.

Iravani, J. (1974) Clearance function of the respiratory ciliated epithelium in normal and bronchitic rats. Inhaled Part 1:143–148.

Iravani, J; Melville, GN. (1974) Long-term effect of cigarette smoke on mucociliary function in animals. Respiration 31:358–366.

Ishidate, JM; Sofuni, T; and Yoshikawa, K. (1981) Chromosomal aberration tests in vitro as a primary screening tool for environmental mutagens and/or carcinogens. GANN Monogr Cancer Res 27:95-108.

Ito, K; Sakamoto, T; Hayashi, Y; et al. (1996) Role of tachykinin and bradykinin receptors and mast cells in gaseous formaldehyde-induced airway microvascular leakage in rats. Eur J Pharmacol 307:291–298.

Jaeger, RJ; Gearhart, JM. (1982) Respiratory and metabolic response of rats and mice to formalin vapor. Toxicology 25:299–309.

Jaiswal, S; Traver, D; Miyamoto, T; et al. (2003) Expression of BCR/ABL and BCL-2 in myeloid progenitors leads to myeloid leukemias. Proc Natl Acad Sci USA 100:10002–10007.

Jakab, GJ. (1992) Relationship between carbon black particulate-bound formaldehyde, pulmonary antibacterial defenses, and alveolar macrophage phagocytosis. Inhal Toxicol 4:325−342.

James, JT; Limero, TF; Beck, SW; et al. (2002) Environmental Monitoring Air Quality. Report of the Lunar-Mars Support Test Project (LMLSTP4.1). In: Isolation: NASA Experiments in Closed Environment Living. Eds: Lane, HW; Sauer, RL; Feeback, DL. Univeit, Inc, San Diego, CA. 104:177–191. Data also available at National Aeronautics and Space Administration Life Sciences Data Archive, Houston, TX. Available at http://lsda.jsc.nasa.gov/docs/research/research_detail.cfm?experiment_type_code=24&researchtype=.

Jarabek, AM; Fisher, JW; Rubenstein, R; et al. (1994) Mechanistic insights aid the search for CFC substitutes: risk assessment of HCHC-123 as an example. Risk Anal 14:231–250.

Javdan, M; Taher, TE. (2000) The short and long term effects of formaldehyde vapour (2 ppm and 5 ppm) on rat nasal epithelium. Pathol Int 50:A77.

Jeffcoat, AR; Chasalow, F; Feldman, DB; et al. (1983) Disposition of [14C] formaldehyde after topical exposure to rats, guinea pigs, and monkeys. In: Gibson, J E , eds. Formaldehyde Toxicity. Washington, DC: Hemisphere; pp. 38-50.

Jensen, DE; Belka, GK; Du Bois, GC. (1998) S-Nitrosoglutathione is a substrate for rat alcohol dehydrogenase class III isoenzyme. Biochem J 331 (Pt 2):659–668.

Johannsen, FR; Levinskas, GJ; Tegeris, AS. (1986) Effects of formaldehyde in the rat and dog following oral exposure. Toxicol Lett 30:1–6.

John, EM; Savitz, DA; Shy, CM. (1994) Spontaneous abortions among cosmetologists. Epidemiology 5:147–155.

Joos, GF. (2003) Bronchial hyperresponsiveness: too complex to be useful? Curr Opin Pharmacol 3:233–238.

Jörnvall, H; Höög, JO; Persson, B; et al. (2000) Pharmacogenetics of the alcohol dehydrogenase system. Pharmacology 61:184–191.

Junqueira, LC; Carneiro, J; Kelley, RO. (1992) Basic Histology. Appleton and Lange, Norwalk, CN.

Jung, R; Engelhart, G; Herbolt, B; et al. (1992) Collaborative study of mutagenicity with Salmonella typhimurium TA102. Mutat Res 278:265–270.

Jurvelin, J; Vartiainen, M; Jantunen, M; et al. (2001) Personal exposure levels and microenvironmental concentrations of formaldehyde and acetaldehyde in the Helsinki metropolitan area. J Air Waste Manag Assoc 51:17–24.

K S Crump Group. (2001) TOX_RISK dose-response software, version 5.2 (2000–2001). KS Crump Group.

Kakeyama, M; Kurokawa, Y; Kunugita, N; et al. (2004) Low dose exposure to formaldehyde alters glutamate NMDA receptor mRNA expressions in the rat neocortex. Neurotoxicology 25:717.

Kalasz, H. (2003) Biological role of formaldehyde, and cycles related to methylation, demethylation, and formaldehyde production. Mini Rev Med Chem 3:175-192.

Kallen, RG; Jencks, WP. (1966a) The dissociation constants of tetrahydrofolic acid. J Biol Chem 241:5845-5850.

Kallen, RG; Jencks, WP. (1966b) The mechanism of the condensation of formaldehyde with tetrahydrofolic acid. J Biol Chem 241:5851-5863.

Kamata, E; Nakadate, M; Ogawa, Y; et al. (1996a) Acute inhalation toxicity study of formaldehyde in rats: effect of vapor on the pulmonary surfactant. Oyo Yakuri 51:33–37.

Kamata, E; Nakadate, M; Uchida, O; et al. (1996b) Effects of formaldehyde vapor on the nasal cavity and lungs of F-344 rats. J Environ Pathol Toxicol Oncol 15:1–8.

Kamata, E; Nakadate, M; Uchida, O; et al. (1997) Results of a 28-month chronic inhalation toxicity study of formaldehyde in male Fisher-344 rats. J Toxicol Sci 22:239–254.

Kane, LE; Alarie, Y. (1977) Sensory irritation to formaldehyde and acrolein during single and repeated exposures in mice. Am Ind Hyg Assoc J 38:509–522.

Kane, LE; Alarie, Y. (1978) Evaluation of sensory irritation from acrolein-formaldehyde mixtures. Am Ind Hyg Assoc J 39:270–274.

Kane, LE; Barrow, CS; Alarie, Y. (1979) A short-term test to predict acceptable levels of exposure to airborne sensory irritants. Am Ind Hyg Assoc J 40:207–229.

Kedderis, GL; Hollenberg, PF. (1983) Steady state kinetics of chloroperoxidase-catalyzed N-demethylation reactions. J Biol Chem 258:12413-12419.

Kelly, JT; Prasad, AK; Wexler, AS. (2000) Detailed flow patterns in the nasal cavity. J Appl Physiol 89:323–337.

Kennedy, G; Slaich, PK; Golding, BT; et al. (1996) Structure and mechanism of formation of a new adduct from formaldehyde and guanosine. Chem Biol Interact 102:93–100.

Kepler, GM; Richardson, RB; Morgan, KT; et al. (1998) Computer simulation of inspiratory nasal airflow and inhaled gas uptake in a rhesus monkey. Toxicol Appl Pharmacol 150:1–11.

Kernan, GJ; Ji, BT; Dosemeci, M; et al. (1999) Occupational risk factors for pancreatic cancer: A case-control study based on death certificates from 24 U.S. states. Am J Ind Med 36:260–270.

Kerns, WD; Pavkov, KL; Donofrio, DJ; et al. (1983) Carcinogenicity of formaldehyde in rats and mice after long-term inhalation exposure. Cancer Res 43:4382–4392.

Khamgaonkar, MB; Fulare, MB. (1991) Pulmonary effects of formaldehyde exposure—an environmental-epidemiological study. Ind J Chest Dis Allied Sci 33:9–13.

Kiernan, JA. (2000) Formaldehyde, formalin, paraformaldehyde and glutaraldehyde: What they are and what they do. Microscopy Today 00-1 pp. 8–12.

Kilburn, KH. (1994) Neurobehavioral impairment and seizures from formaldehyde. Arch Environ Health 49:37–44.

Kilburn, KH; Moro, A. (1985) Reproductive and maternal effects of formaldehyde (HCH0) in rats. Fed Proc 44:535.

Kilburn, KH; Warshaw, R. (1992) Neurobehavioral effects of formaldehyde and solvents on histology technicians: repeated testing across time. Environ Res 58:134–146.

Kilburn, KH; Seidman, BC; Warshaw, R. (1985) Neurobehavioral and respiratory symptoms of formaldehyde and xylene exposure in histology technicians. Arch Environ Health 40:229–233.

Kilburn, KH; Warshaw, R; Thornton, JC. (1987) Formaldehyde impairs memory equilibrium and dexterity in histology technicians effects which persist for days after exposure. Arch Environ Health 42:117–120.

Kim, CW; Song, JS; Ahn, YS; et al. (2001) Occupational asthma due to formaldehyde. Yonsei Med J 42:440–445.

Kim, KC; Hisatsune, A; Kim, DJ; et al. (2003) Pharmacology of airway goblet cell mucin release. J. Pharmacol Sci 92:301–307.

Kimbell, JS; Gross, EA; Joyner, DR; et al. (1993) Application of computational fluid dynamics to regional dosimetry of inhaled chemicals in the upper respiratory tract of the rat. Toxicol Appl Pharmacol 121:253–263.

Kimbell, JS; Godo, MN; Gross, EA; et al. (1997a) Computer simulation of inspiratory airflow in all regions of the F344 rat nasal passages. Toxicol Appl Pharmacol 145:388–398.

Kimbell, JS; Gross, EA; Richardson RB; et al. (1997b) Correlation of regional formaldehyde flux predictions with the distribution of formaldehyde-induced squamous metaplasia in F344 rat nasal passages. Mutat Res 380:143–154.

Kimbell, JS; Subramaniam, RP; Gross, EA; et al. (2001a) Dosimetry modeling of inhaled formaldehyde: comparisons of local flux predictions in the rat, monkey, and human nasal passages. Toxicol Sci 64:100–110.

Kimbell, JS; Overton, JH; Subramaniam, RP; et al. (2001b) Dosimetry modeling of inhaled formaldehyde: binning nasal flux predictions for quantitative risk assessment. Toxicol Sci 64:111–121.

Kimbell, JS. (2006) Nasal dosimetry of inhaled gases and particles: Where do inhaled agents go in the nose? Toxicol Pathol 34:270–273.

Kinzler, KW; Vogelstein, B. (1997) Cancer-susceptibility genes. Gatekeepers and caretakers. Nature 386:761–763.

Kitaev, ÉM; Savchenko, O; Lovchikov, VA; et al. (1984) Embryonic development and various indicators of reproductive function in rats after inhalation of formaldehyde in the preimplantation phase [translation]. Akush Ginekol 10:49–52.

Kitaeva, LV; Kitaev, E; Pimenova, MN. (1990) Cytopathic and cytogenetic effects of chronic inhalation of formaldehyde on the female rat's germ marrow cells [translation]. Tsitologiia 32:1212–1216.

Kitaeva, LV; Mikheeva, EA; Shelomova, LF; et al. (1996) Genotoxic effect of formaldehyde in somatic human cells in vivo [translation]. Genetika 32(9):1287–1290. Translated from Russian by Ted Crump, Translator. NIH Library Translation. National Institutes of Health, Public Health Service, U.S. Department of Health and Human Services; NIH-03-640.

Klaassen, CD. (2001) Casarett and Doull's Toxicology: The Basic Science of Poisons. 6th Edition. McGraw-Hill, New York, NY.

Klein, M; Sinha, B; Subramaniam, RP. (2010) Statistical inferences from formaldehyde DNA-protein cross-link data: Improving methods for characterization of uncertainty. *In Press*, J. Biopharmaceutical Statistics. [To appear in Vol 21(1)]

Kligerman, AD; Phelps, MC; Erexson, GL. (1984) Cytogenetic analysis of lymphocytes from rats following formaldehyde inhalation. Toxicol Lett 21:241–246.

Klonisch, T; Steger, K; Kehlen, A; et al. (2004) INSL-3 ligand-receptor system in the equine testis. Biol Reprod 68:1975–1981.

Koppel, C; Tenczer, J; Peixoto-Menezes, KM. (1991) Formation of formaldehyde adducts from various drugs by use of methanol in a toxicological screening procedure with gas chromatography-mass spectrometry. J Chromatogr 563:73–81.

Korczynski, RE. (1994) Formaldehyde exposure in the funeral industry. Appl Occup Environ Hyg 9:575–579.

Krakowiak, A; Gorski, P; Pazdrak, K; et al. (1998) Airway response to formaldehyde inhalation in asthmatic subjects with suspected respiratory formaldehyde sensitization. Am J Ind Med 33:274–281.

Kramps, JA; Peltenburg, LT; Kerklaan, PR; et al. (1989) Measurement of specific IgE antibodies in individuals exposed to formaldehyde. Clin Exp Allergy 19:509–514.

Krewski, D; Crump, KS; Farmer, J; et al. (1983) A comparison of statistical methods for low dose extrapolation utilizing time-to-tumor data. Fundam Appl Toxicol 3:140–160.

Kriebel, D; Sama, SR; Cocanour, B. (1993) Reversible pulmonary responses to formaldehyde. A study of clinical anatomy students. Am Rev Respir Dis 148:1509–1515.

Kriebel, D; Myers, D; Cheng, M; et al. (2001) Short-term effects of formaldehyde on peak expiratory flow and irritant symptoms. Arch Environ Health 56(1):11–18.

Kreiger, RA; and Garry, VF. (1983) Formaldehyde-induced cytotoxicity and sister-chromatid exchanges in human lymphocyte cultures. Mutat Res 120:51-55.

Krimsky, MM. (1986) Letter to the editor regarding Sallakumar et al., 1985. Toxicol Appl Pharmacol 85:295.

Krzyzanowski, M; Quackenboss, JJ; Lebowitz, MD. (1990) Chronic respiratory effects of indoor formaldehyde exposure. Environ Res 52:117–125.

Kuby, J. (1991) Immunology. 2nd edition. New York, NY: W.H. Freeman and Company.

Kulle, TJ. (1993) Acute odor and irritation response in healthy nonsmokers with formaldehyde exposure. Inhal Toxicol 5:323–332.

Kulle, TJ; Cooper, GP. (1975) Effects of formaldehyde and ozone on the trigeminal nasal sensory system. Arch Environ Health 30:237–243.

Kulle, TJ; Sauder, LR; Hebel, R; et al. (1987) Formaldehyde dose-response in healthy nonsmokers. JAPCA 37:919–924.

Kum, C; Kiral, F; Sekkin, S; et al. (2007) Effects of xylene and formaldehyde inhalations on oxidative stress in adult and developing rats livers. Exp Anim 56:35–42.

Kuper, CF; Hameleers, DMH; Bruijntjes, JP; et al. (1990) Lymphoid and non-lymphoid cells in nasal-associated lymphoid tissue (NALT) in the rat. Cell Tissue Res 259:371–377.

Kuppers, R; Klein, U; Hansmann, M-L; et al. (1999) Cellular origin of human B-cell lymphomas. New Eng J Med 341:1520–1529.

Kuschner, M; Laskin, S; Drew, RT; et al. (1975) Inhalation carcinogenicity of alpha halo ethers. III. Lifetime and limited period inhalation studies with bis(chloromethyl)ether at 0.1 ppm. Arch Environ Health 30:73–77.

Kushch, I; Schwarz, K; Schwentner, L; et al. (2008) Compounds enhanced in a mass spectrometric profile of smokers' exhaled breath versus non-smokers as determined in a pilot study using PTR-MS. J Breath Res 2:1-26.

Laforest, L; Luce, D; Goldberg, P; et al. (2000) Laryngeal and hypopharyngeal cancers and occupational exposure to formaldehyde and various dusts: a case-control study in France. Occup Environ Med 57:767–773.

Lam, CW; Casanova, M; Heck, HD. (1985) Depletion of nasal mucosal glutathione by acrolein and enhancement of formaldehyde-induced DNA-protein cross-linking by simultaneous exposure to acrolein. Arch Toxicol 58:67–71.

Lang, I; Bruckner, T; Triebig, G. (2008) Formaldehyde and chemosensory irritation in humans: A controlled human exposure study. Regul Toxicol Pharmacol 50:23–36.

Lash, LH; Parker, JC. (2001) Hepatic and renal toxicities associated with perchloroethylene. Pharmacol Rev 53:177–208.

Laskin, S; Kuschner, M; Drew, RT; et al. (1971) Tumors of the respiratory tract induced by inhalation of bis(chloromethyl)ether. Arch Environ Health 23:135–136.

Lazutka, JR; Lekevicius, R; Dedonyte, V, et al. (1999) Chromosomal aberrations and sister-chromatid exchanges in Lithuanian populations: effects of occupational and environmental exposures. Mutat Res 445:225-239.

Le Curieux, F; Marzin, D; Erb, F. (1993) Comparison of three short-term assays: results on seven chemicals. Potential contribution to the control of water genotoxicity. Mutat Res 319:223–236.

Leach, C; Sharma, R; Oberg, S. (1983) Immunotoxic and pathologic effects of inhalation of various levels of formaldehyde vapor in F-344 rats. Toxicologist 3:73.

Lee, H; Alarie, Y; Karol, M. (1984) Induction of formaldehyde sensitivity in guinea pigs. Toxicol Appl Pharmacol 75:147–155.

Lee, MH; Kim, YA; Na, TY; et al. (2008) Identification of formaldehyde-responsive genes by suppression subtractive hybridization. Toxicology 243:224–235.

Lemière, C; Desjardins, A; Cloutier, Y; et al. (1995) Occupational asthma due to formaldehyde resin dust with and without reaction to formaldehyde gas. Eur Respir J 8:861–865.

Levine, RJ; Andjelkovich, DA; Shaw, L. (1984) The mortality of Ontario undertakers and a review of formaldehyde-related mortality studies. J Occup Med 26:740–746.

Levy, S; Nocentini, S; and Billardon, C. (1983) Induction of cytogenetic effects in human fibroblast cultures after exposure to formaldehyde or X-rays. Mutat Res 119:309-317.

Li, S.-M.; Anlauf, K. G.; Wiebe, H. A.; Bottenheim, J.W. (1994) Estimating primary and secondary production of HCHO in eastern North America based on gas-phase measurements and principal component analysis. Geophys. Res. Lett. 21: 669-672.

Li, G-Y; Lee, H-Y; Shin, H-S; et al. (2007) Identification of gene markers for formaldehyde exposure in humans. Environ Health Perspect 115:1460–1466.

Li, R; Lu, ZS; Qiao, Y; et al. (2004) Study on the formaldehyde-induced DNA damage with comet assay. Shi Yan Sheng Wu Xue Bao 37:262–268.

Liber, HL; Benforado, K; Crosby, RM; et al. (1989) Formaldehyde-induced and spontaneous alterations in human hprt DNA sequence and mRNA expression. Mutat Res 226:31–37.

Lidén, S; Scheynius, A; Fischer, T; et al. (1993) Absence of specific IgE antibodies in allergic contact sensitivity to formaldehyde. Allergy 48(7):525–529.

Liebling, T; Rosenman, KD; Griffith, RG; et al. (1984) Cancer mortality among workers exposed to formaldehyde. Am J Ind Med 5:423–428.

Liesivuori, J; Savolainen, H. (1987) Urinary formic acid as an indicator of occupational exposure to formic acid and methanol. Am Ind Hyg Assoc J 48:32–34.

Lin, Z; Luo, W; Li, H; et al. (2005) The effect of endogenous formaldehyde on the rat aorta endothelial cells. Toxicol Lett 159:134–143.

Lindbohm, ML; Hemminki, K; Bonhomme, MG; et al. (1991) Effects of paternal occupational exposure on spontaneous abortions. Am J Public Health 81:1029–1033.

Lino dos Santos Franco, A; Damazo, AS; Beraldo de Souza, HR; et al. (2006) Pulmonary neutrophil recruitment and bronchial reactivity in formaldehyde-exposed rats are modulated by mast cells and differentially by neuropeptides and nitric oxide. Toxicol Appl Pharmacol 214:35−42.

Lino dos Santos Franco, A; Domingos, HV; Damazo, AS; et al. (2009) Reduced allergic lung inflammation in rats following formaldehyde exposure: long term effects on multiple effector systems. Toxicology. 256: 157–163.

Liu, KS; Huang, FY; Hayward, SB; et al. (1991) Irritant effects of formaldehyde exposure in mobile homes. Environ Health Perspect 94:91–94.

Liu, L; Hausladen, A; Zeng, M; et al. (2001) A metabolic enzyme for S-nitrosothiol conserved from bacteria to humans. Nature 410:490–494.

Liu, L; Yan, Y; Zeng, M; et al. (2004) Essential roles of S-nitrosothiols in vascular homeostasis and endotoxic shock. Cell 116:617–628.

Liu, Y; Li, CM; Lu, Z; et al. (2006) Studies on formation and repair of formaldehyde-damaged DNA by detection of DNA-protein crosslinks and DNA breaks. Front Biosci 11:991–997.

Logue, J; Barrick, M; Jessup, G. (1986) Mortality of radiologists and pathologists in the radiation registry of physicians. J Occup Med 28:91–99.

Lu, Z; Li, CM; Qiao, Y; et al. (2008) Effect of inhaled formaldehyde on learning and memory in mice. Indoor Air 18:77–83.

Luce, D; Gerin, M; Leclerc, A; et al. (1993) Sinonasal cancer and occupational exposure to formaldehyde and other substances. Int J Cancer 53:224–231.

Luce, D; Leclerc, A; Begin, D; et al. (2002) Sinonasal cancer and occupational exposures: a pooled analysis of 12 case-control studies. Cancer Causes Control 13:147–157.

Luebeck, EG; Grasl-Kraupp, B; Timmermann-Trosiener, I; et al. (1995) Growth kinetics of enzyme-altered liver foci in rats treated with phenobarbital or alpha-hexachlorocyclohexane. Toxicol Appl Pharmacol 130:304–315.

Luebeck, EG; Buchmann, A; Stinchcombe, S; et al. (2000) Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on initiation and promotion of GST-P-positive foci in rat liver: A quantitative analysis of experimental data using a stochastic model. Toxicol Appl Pharmacol 167:63–73.

Luo, W; Li, H; Zhang, Y; et al. (2001) Determination of formaldehyde in blood plasma by high-performance liquid chromatography with fluorescence detection. J Chromatogr B Biomed Sci Appl 753:253-257.

Lyapina, M; Zhelezova, G; Petrova, E; et al. (2004) Flow cytometric determination of neutrophil burst activity in workers exposed to formaldehyde. Int Arch Occup Environ Health 77:335–340.

Lindinger, W; Hansel, A; Jordan, A. (1998) On-line monitoring of volatile organic compounds at pptv levels by means of Proton-Transfer-Reaction Mass-Spectrometry (PTR-MS): Medical applications, food control and environmental research, Review paper. Int J Mass Spectrom. Ion Proc 173:191-241.

Ma, TH; Harris, MM. (1988) Review of the genotoxicity of formaldehyde. Mutat Res 196:37−59.

Mackerer, CR; Angelosanto, FA; Blackburn, GR, et al. (1996) Identification of formaldehyde as the metabolite responsible for the mutagenicity of methyl tertiary-butyl ether in the activiated mouse lymphoma assay. Proc. Soc. exp. Biol. Med 212:338-341.

Magana-Schwencke, N; and Ekert, B. (1978) Biochemical analysis of damage induced in yeast by formaldehyde. II. Induction of cross-links between DNA and protein. Mutat Res 51:11-19.

Main, D; Hogan, TJ. (1983) Health effects of low level exposure to formaldehyde. J Occup Med 25(12):896–900.

Majumder, PK; Kumar, VL. (1995) Inhibitory effects of formaldehyde on the reproductive system of male rats. Indian J Physiol Pharmacol 39:80–82.

Malaka, T; Kodama, AM. (1990) Respiratory health of plywood workers occupationally exposed to formaldehyde. Arch Environ Health 45:288–294.

Malek, FA; Möritz, KU; Fanghänel, J. (2003a) A study on the effect of inhalative formaldehyde exposure on water labyrinth test performance in rats. Ann Anat 185:277–285.

Malek, FA; Möritz, KU; Fanghänel, J. (2003b) Formaldehyde inhalation and open field behaviour in rats. Indian J Med Res 118:90–96.

Malek, FA; Möritz, KU; Fanghänel, J. (2003c) A study on specific behavioral effects of formaldehyde in the rat. J Exp Anim Sci 42:160–170.

Malek, FA; Möritz, KU; Fanghänel, J. (2004) Effects of a single inhalative exposure to formaldehyde on the open field behavior of mice. Int J Hyg Environ Health 207:151–158.

Mannix, RC; Phalen, RF; Walters, RF; et al. (1983) Effects of sulfur dioxide and formaldehyde on particle clearance in the rat. J Toxicol Environ Health 12:429–440.

Marinari, UM; Ferro, M; Sciaba, L, et al. (1984) DNA-damaging activity of biotic and xenobiotic aldehydes in Chinese hamster ovary cells. Cell Biochem Funct 2:243-248.

Marks, TA; Worthy, WC; Staples, RE. (1980) Influence of formaldehyde and sonacide (potentiated acid glutaraldehyde) on embryo and fetal development in mice. Teratology 22:51−58.

Marnett, LJ; Hurd, HK; Hollstein, MC; et al. (1985) Naturally occurring carbonyl compounds are mutagens in Salmonella tester strain TA104. Mutat Res 148:25–34.

Maronpot, RR; Miller, RA; Clarke, WJ; et al. (1986) Toxicity of formaldehyde vapor in B6C3F1 mice exposed for 13 weeks. Toxicology 41:253–266.

Marsh, GM. (1982) Proportional mortality patterns among chemical plant workers exposed to formaldehyde. Br J Ind Med 39:313–322.

Marsh, GM; Youk, AO. (2005) Reevaluation of mortality risks for nasopharyngeal cancer in the formaldehyde cohort study of the National Cancer Institute. Regul Pharmacol Toxicol 42:275−283.

Marsh, GM; Stone, RA; Henderson, VL. (1992a) A reanalysis of the National Cancer Institute study on lung cancer mortality among industrial workers exposed to formaldehyde. J Occup Med 34:42–44.

Marsh, GM; Stone, RA; Henderson, VL. (1992b) Lung cancer mortality among industrial workers exposed to formaldehyde: a Poisson regression analysis of the National Cancer Institute Study. Am Ind Hyg Assoc J 53:681-691.

Marsh, GM; Stone, RA; Esmen, NA; et al. (1994) Mortality patterns among chemical plant workers exposed to formaldehyde and other substances. J Natl Cancer Inst 86:384–386.

Marsh, GM; Stone, RA; Esmen, NA; et al. (1996) Mortality among chemical workers in a factory where formaldehyde was used. Occup Environ Med 53:613–627.

Marsh, GM; Youk, AO; Buchanich, JM; et al. (2002) Pharyngeal cancer mortality among chemical plant workers exposed to formaldehyde. Toxicol Ind Health 18:257–268.

Marsh, GM; Youk, AO; Buchanich, JM; et al. (2007a) Work in the metal industry and nasopharyngeal cancer mortality among formaldehyde-exposed workers. Regul Toxicol Pharmacol 48:308–319.

Marsh, GM; Youk, AO; Morfeld, P. (2007b) Mis-specified and non-robust mortality risk models for nasopharyngeal cancer in the National Cancer Institute formaldehyde worker cohort study. Regul Toxicol Pharmacol 47:59–67.

Martin, CN; McDermid, AC; and Garner, RC. (1978) Testing of known carcinogens and noncarcinogens for their ability to induce unscheduled DNA synthesis in HeLa cells. Cancer Res 38:2621-2627.

Martin, WJ. (1990) A teratology study of inhaled formaldehyde in the rat. Reprod Toxicol 4:237–239.

Mashford, PM; Jones, AR. (1982) Formaldehyde metabolism by the rat: a re-appraisal. Xenobiotica 12:119–124.

Matanoski, GM. (1989) Risks of pathologists exposed to formaldehyde. National Institute for Occupational Safety and Health, Cincinnati, OH. Available from the National Technical Information Service, Springfield, VA; PB91-173682.

Matsunaga, I; Miyake, Y; Yoshida, T; et al. (2008) Ambient formaldehyde levels and allergic disorders among Japanese women: baseline data from the Osaka maternal and child health study. Ann Epidemiol 18:78–84.

Mauderly, JL. (1986) Respiration of F344 rats in nose-only inhalation exposure tubes. J Appl Toxicol 6:25–30.

Maurice, F; Rivory, JP; Larsson, PH; et al. (1986) Anaphylactic shock caused by formaldehyde in a patient undergoing long-term hemodialysis. J Allergy Clin Immunol 77:594–597.

McGhee, JD; von Hippel, PH. (1975a) Formaldehyde as a probe of DNA structure. I. Reaction with exocyclic amino groups of DNA bases. Biochemistry 14:1281–1296.

McGhee, JD; von Hippel, PH. (1975b) Formaldehyde as a probe of DNA structure. II. Reaction with endocyclic imino groups of DNA bases. Biochemistry 14:1297–1303.

McGhee, JD; von Hippel, PH. (1977a) Formaldehyde as a probe of DNA structure. 3. Equilibrium denaturation of DNA and synthetic polynucleotides. Biochemistry 16:3267–3276.

McGhee, JD; von Hippel, PH. (1977b) Formaldehyde as a probe of DNA structure. r. Mechanism of the initial reaction of Formaldehyde with DNA. Biochemistry 16:3276–3293.

McGwin, G; Lienert, J; Kennedy, JI. (2010) Formaldehyde Exposure and Asthma in Children: A Systematic Review. Environ Health Perspect 118:313–317.

McKone, TE; Daniels, JI. (1991) Estimating human exposure through multiple pathways from air, water, and soil. Regul Toxicol Pharmacol 13:36–61.

McLean, JH; Shipley, MT. (1988) Postmitotic, postmigrational expression of tyrosine hydroxylase in olfactory bulb dopaminergic neurons. J Neurosci 8:3658–3669.

McMartin, KE; Martin-Amat, G; Noker, PE; et al. (1979) Lack of a role for formaldehyde in methanol poisoning in the monkey. Biochem Pharmacol 28:645-649.

Meggs, WJ. (1993) Neurogenic inflammation and sensitivity to environmental chemicals. Environ Health Perspect 101:234–238.

Meggs, WJ. (1995) Neurogenic switching: a hypothesis for a mechanism for shifting the site of inflammation in allergy and chemical sensitivity. Environ Health Perspect 103:54–56.

Meng F, Bermudez E, McKinzie PB, Andersen ME, Clewell III HJ, Parsons BL. (2010). Measurement of tumor-associated mutations in the nasal mucosa of rats exposed to varying doses of formaldehyde (2010). Regul Toxicol Pharmacol. Corrected Proof, Available Online, DOI: 10.1016/j.yrtph.2010.03.007.

Merk, O; Speit, G. (1998) Significance of formaldehyde-induced DNA-protein crosslinks for mutagenesis. Environ Mol Mutagen 32:260–268.

Merk, O; and Speit, G. (1999) Detection of crosslinks with the comet assay in relationship to genotoxicity and cytotoxicity. Environ Mol Mutagen 33:167-172.

Merletti, F; Boffetta, P; Ferro, G; et al. (1991) Occupation and cancer of the oral cavity or oropharynx in Turin, Italy. Scand J Work Environ Health 17:248–254.

Mery, S; Gross, EA; Joyner, DR; et al. (1994) Nasal diagrams: a tool for recording the distribution of nasal lesions in rats and mice. Toxicol Pathol 22:353–372.

Metz, B; Kersten, GF; Hoogerhout, P; et al. (2004) Identification of formaldehyde-induced modifications in proteins: reactions with model peptides. J Biol Chem 279:6235–6243.

Metz, B; Kersten, GF; Baart, GJ; et al. (2006) Identification of formaldehyde-induced modifications in proteins: reactions with insulin. Bioconjug Chem 17:815–822.

Migliore, L; Ventura, L; Barale, R; et al. (1989) Micronuclei and nuclear anomalies induced in the gastro-intestinal epithelium of rats treated with formaldehyde. Mutagenesis 4:327–334.

Miretskaia, LV; and Shvartsman, P. (1982) [Chromosome damages in human lymphocytes as affected by formaldehyde. I. Formaldehyde treatment of lymphocytes in culture]. Tsitologiia 24:1056-1060.

Miyachi, T; Tsutsui, T. (2005) Ability of 13 chemical agents used in dental practice to induce sister-chromatid exchanges in Syrian hamster embryo cells. Odontology 93:24–29.

Molotkov, A; Fan, X; Deltour, L; et al. (2002) Stimulation of retinoic acid production and growth by ubiquitously expressed alcohol dehydrogenase Adh3. Proc Natl Acad Sci USA 99:5337–5342.

Monteiro-Riviere, NA; Popp, JA. (1986) Ultrastructural evaluation of acute nasal toxicity in the rat respiratory epithelium in response to formaldehyde gas. Fundam Appl Toxicol 6:251–262.

Monticello, TM; Morgan, KT. (1994) Cell proliferation and formaldehyde-induced respiratory carcinogenesis. Risk Anal 14:313–319.

Monticello, TM; Morgan, KT. (1997) Chemically induced nasal carcinogenesis and epithelial cell proliferation: a brief review. Mutat Res 380:33–41.

Monticello, TM; Morgan, KT; Everitt, JI; et al. (1989) Effects of formaldehyde gas on the respiratory tract of rhesus monkeys. Pathology and cell proliferation. Am J Pathol 134:515−527.

Monticello, TM; Morgan, KT; Hurtt, ME. (1990) Unit length as the denominator for quantitation of cell proliferation in nasal epithelia. Toxicol Pathol 18:24–31.

Monticello, TM; Miller, FJ; Morgan, KT. (1991) Regional increases in rat nasal epithelial cell proliferation following acute and subchronic inhalation of formaldehyde. Toxicol Appl Pharmacol 111:409–421.

Monticello, TM; Swenberg, JA; Gross, EA; et al. (1996) Correlation of regional and nonlinear formaldehyde-induced nasal cancer with proliferating populations of cells. Cancer Res 56:1012–1022.

Moolgavkar, SH. (1994) Biological models of carcinogenesis and quantitative cancer risk assessment. Risk Anal 14:879–882.

Moolgavkar, SH; Venzon, DJ. (1979) Two-event models for carcinogenesis: Incidence curves for childhood and adult tumors. Math Biosci 47:55–77.

Moolgavkar, SH; Knudson, AJ. (1981) Mutation and cancer: A model for human carcinogenesis. J Natl Cancer Inst 66:1037–1052.

Moolgavkar, SH; Luebeck, EG. (1992) Interpretation of labeling indices in the presence of cell death. Carcinogenesis 13:1007–1010.

Moolgavkar, SH; Dewanji, A; Venzon, D. (1988) A stochastic two-stage model for cancer risk assessment. I. The hazard function and the probability of tumor. Risk Anal 8:383–392.

Moolgavkar, SH; Luebeck, EG; Krewski, D; et al. (1993) Radon, cigarette smoke, and lung cancer: A reanalysis of the Colorado plateau uranium miner's data. Epidemiology 4:204–217.

Moore, L; Ogrodnik, EC. (1986) Occupational exposure to formaldehyde in mortuaries. J Environ Health 49:32–35.

Moore, S; Montane-Jaime, K; Shafe, S; et al. (2007) Association of ALDH1 promoter polymorphisms with alcohol-related phenotypes in Trinidad and Tobago. J Stud Alcohol Drugs 68:192–196.

Morgan, KT. (1997) A brief review of formaldehyde carcinogenesis in relation to rat nasal pathology and human health risk assessment. Toxicol Pathol 25:291–307.

Morgan, KT; Patterson, DL; Gross, EA (1983) Localization of areas of inhibition of nasal mucociliary function in rats following in vivo exposure to formaldehyde. Am Rev Respir Dis 127:166–166.

Morgan, KT; Patterson, DL; Gross, EA. (1984a) Frog palate mucociliary apparatus: structure, function, and response to formaldehyde gas. Fundam Appl Toxicol 4:58–68.

Morgan, KT; Jiang, X-Z; Patterson, DL; et al. (1984b) The nasal mucociliary apparatus. Am Rev Respir Dis 130:275–281.

Morgan, KT; Gross, EA; Patterson, DL. (1986a) Distribution, progression, and recovery of acute formaldehyde-induced inhibition of nasal mucociliary function in F-344 rats. Toxicol Appl Pharmacol. 86(3):448–456.

Morgan, KT; Jiang, XZ; Starr, TB; et al. (1986b) More precise localization of nasal tumors associated with chronic exposure of F-344 rats to formaldehyde gas. Toxicol Appl Pharmacol 82:264–271.

Morgan, KT; Patterson, DL; Gross, EA. (1986c) Responses of the nasal mucociliary apparatus of F-344 rats to formaldehyde gas. Toxicol Appl Pharmacol 82:1–13.

Morgan, K; Patterson, DL; Gross, E. (1986d) Responses of the nasal mucociliary apparatus to airborne irritants. In: Barrow, CS; ed. Toxicology of the nasal passages. Washington, DC: Hemisphere Publishing Corporation; pp. 123–133.

Morgan, KT; Kimbell, JS; Monticello, TM; et al. (1991) Studies of inspiratory airflow patterns in the nasal passages of the F344 rat and rhesus monkey using nasal molds: relevance to formaldehyde toxicity. Toxicol Appl Pharmacol 110:223–240.

Moser, B; Bodrogi, F; Eibl, G; et al. (2005) Mass spectrometric profile of exhaled breath-field study by PTR-MS. Respir Physiol Neurobiol 145:295–300.

Muller, W; Engelhart, G; Herbold, B; et al. (1993) Evaluation of mutagenicity testing with Salmonella typhimurium TA102 in three different laboratories. Environ Health Perspect 101 Suppl 3:33–36.

Natarajan, AT; Darroudi, F; Bussman, CJ; et al. (1983) Evaluation of the mutagenicity of formaldehyde in mammalian cytogenetic assays in vivo and vitro. Mutat Res 122:355–360.

NCHS (National Center for Health Statistics). (2002) United States Life Tables, 1999. National Vital Statistics Reports, vol. 50, no. 6, March 21, pp. 7–8.

NCI (National Cancer Institute). (2003) SEER Cancer Statistics Review, 1975–2000. Bethesda, MD. http://seer.cancer.gov/csr/1975_2000/.

NEG (Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals); DECOS (Dutch Expert Committee on Occupational Standards). (2003) 132. Formaldehyde. Stockholm, Sweden: National Institute for Working Life. Available from <http://ebib.arbetslivsinstitutet.se/ah/2003/ah2003_11.pdf>. 2003: 11.

Neri, M; Bonassi, S; Knudsen, LE, et al. (2006) Children's exposure to environmental pollutants and biomarkers of genetic damage. I. Overview and critical issues. Mutat Res 612:1-13.

Nielsen, GD. (1991) Mechanisms of activation of the sensory irritant receptor by airborne chemicals. Crit Rev Toxicol 21:183–208.

Nielsen, GD; Hougaard, KS; Larsen, ST; et al. (1999) Acute airway effects of formaldehyde and ozone in Balb/c mice. Hum Exp Toxicol 18:400–409.

Niinimaa, V; Cole, P; Mintz, S; et al. (1981) Oronasal distribution of respiratory airflow. Respir Physiol 43:69–75.

Nilsson, JA; Hedberg, JJ; Vondracek, M; et al. (2004) Alcohol dehydrogenase 3 transcription associates with proliferation of human oral keratinocytes. Cell Mol Life Sci 61:610–617.

Norbäck, D; Björnsson, E; Janson, C; et al. (1995) Asthmatic symptoms and volatile organic compounds, formaldehyde, and carbon dioxide in dwellings. Occup Environ Med 52(6):388–395.

NRC (National Research Council). (1983) Risk assessment in the federal government: managing the process. Washington, DC: National Academy Press.

NRC (1988) Health risks of radon and other internally deposited alpha-emitters: BEIR IV. Committee on the Biological Effects of Ionizing Radiations. Washington, DC: National Academy Press.

NRC. (1993) Understanding child abuse and neglect. Panel on Research on Child Abuse and Neglect. National Research Council, National academy Press, Washington, DC.

NTP (National Toxicology Program). (2002) Report on Carcinogens. Tenth Edition. Research Triangle Park, NC: National Institute of Environmental Health Services, Public Health Service, U.S. Department of Health and Human Services. Available from: <http://ehp.niehs.nih.gov/roc/tenth/profiles/s089form.pdf>.

NTP. (2005) Control data from NTP Technical Report Series Nos. 210, 267, 272, 306, 311, 314, 329, 346, 363, 371, 375, 377, 379, 380, 386, 421, 437, 440, 447, 448, 450, 451, 453, 454, 461, 462, 466, 467, 468, 471, 472, 475, 482, 486, 487, 490, 492, 499, 400, 507, 513, 515, 519 and control data from the wollastonite calcium silicates study. U.S. Department of Health and Human Services, NTP, Research Triangle Park, NC. Available at http://ntp-apps.niehs.nih.gov/ntp.tox/index.cfm.

Neuss, S; and Speit, G. (2008) Further characterization of the genotoxicity of formaldehyde in vitro by the sister chromatid exchange test and co-cultivation experiments. Mutagenesis 23:355-7.

Nunn, A; Craigen, A; Darbyshire, J; et al. (1990) Six year follow up of lung function in men occupationally exposed to formaldehyde. Br J Ind Med 47:747–752.

Obe, G; and Beek, B. (1979) Mutagenic activity of aldehydes. Drug Alcohol Depend 4:91-94.

O'Connor, PM; and Fox, BW. (1987) Comparative studies of DNA cross-linking reactions following methylene dimethanesulphonate and its hydrolytic product, formaldehyde. Cancer Chemother Pharmacol 19:11-5.

Odeigah, PGC. (1997) sperm head abnormalities and dominant lethal effects of formaldehyde in albino rats. Mutat Res 389:141–148.

O'Donovan, MR; Mee, CD. (1993) Formaldehyde is a bacterial mutagen in a range of Salmonella and Escherichia indicator strains. Mutagenesis 8:577–581.

Oerstavik, D; and Hongslo, JK. (1985) Mutagenicity of endodontic sealers. Biomaterials 6:129-132.

Ohba, Y; Morimitsu, Y; Watarai, A. (1979) Reaction of formaldehyde with calf-thymus nucleohistone. Eur J Biochem 100:285–293.

Ohmichi, K; Komiyama, M; Matsuno, Y; et al. (2006) Relationship between exposure to formaldehyde and immunoglobulin E (IgE) production during the gross anatomy laboratory. J Health Sci 52/5:642–647.

Ohta, T; Watanabe-Akanuma, M; Tokishita, S; et al. (1999) Mutation spectra of chemical mutagens determined by Lac+ reversion assay with Escherichia coli WP3101P-WP3106P tester strains. Mutat Res 440:59–74.

Ohta, T; Watanabe-Akanuma, M; Yamagata, H. (2000) A comparison of mutation spectra detected by the Escherichia coli lac(+) reversion assay and the Salmonella typhimurium his(+) reversion assay. Mutagenesis 15:317–23.

Ohtani, T; Mizuashi, M; Nakagawa, S; et al. (2004a) Diesel exhaust particles and formaldehyde deviate human immune system to Th2-dominant pattern by affecting both T cells and dendritic cells. J Invest Dermatol 122:A121.

Ohtani, T; Kurosawa, W; Mizuashi, M; et al. (2004b) Effects of diesel exhaust particles (DEP) and formaldehyde on human monocyte-derived dendritic cells (MoDCs) and T cells. J Dermatol Sci 34:134.

Ohtsuka, R; Shuto, Y; Fujie, H; et al. (1997) Response of respiratory epithelium of BN and F344 rats to formaldehyde inhalation. Exp Anim 46:279–286.

Ohtsuka, R; Shutoh, Y; Fujie, H; et al. (2003) Rat strain difference in histology and expression of Th1- and Th2-related cytokines in nasal mucosa after short-term formaldehyde inhalation. Exp Toxicol Pathol 54:287–291.

Olin, KL; Cherr, GN; Rifkin, E, et al. (1996) The effects of some redox-active metals and reactive aldehydes on DNA-protein cross-links in vitro. Toxicology 110:1-8.

Olsen, JH; Dossing, M. (1982) Formaldehyde induced symptoms in day care centers. Am Ind Hyg Assoc J 43:366-370.

Olsen, JH; Asnaes, S. (1986) Formaldehyde and the risk of squamous cell carcinoma of the sinonasal cavities. Br J Ind Med 43:769–774.

Olsen, JH; Jensen, SP; Hink, M; et al. (1984) Occupational formaldehyde exposure and increased nasal cancer risk in man. Int J Cancer 34:639–644.

Olsen, GW; Lacy, SE; Bodner, KM; et al. (1997) Mortality from pancreatic and lymphopoietic cancer among workers in ethylene and propylene chlorohydrin production. Occup Environ Med 54:592–598.

Orsière, T; Sari-Minodier, I; Iarmarcovai, G; et al. (2006) Genotoxic risk assessment of pathology and anatomy laboratory workers exposed to formaldehyde by use of personal air sampling and analysis of DNA damage of peripheral lymphocytes. Mutat Res 605:30–41.

Orstavik, D; Hongslo, JK. (1985) Mutagenicity of endodontic sealers. Biomaterials 6:129–132.

Oshino, N; Chance, B; Sies, H; et al. (1973) The role of H2O2 generation in perfused rat liver and the reaction of catalase compound I and hydrogen donors. Arch Biochem Biophys 154:117-131.

Ostojić, L; Bradarić, A; Miše, K; et al. (2006) Pulmonary function in persons who are professionally exposed to formaldehyde fumes. Coll Antropol 30(3):507–511.

Overman, DO. (1985) Absence of embryotoxic effects of formaldehyde after percutaneous exposure in hamsters. Toxicol Lett 24:107–110.

Overton, JH; Kimbell, JS; Miller, FJ. (2001) Dosimetry modeling of inhaled formaldehyde: the human respiratory tract. Toxicol Sci 64:122–134.

Øvrebø, S; Haugen, A; Skaug, V. (2002) Biotransformation of formaldehyde in cultured human bronchus. Environ Res 89:38–42.

Owen, BA; Dudney, CS; Tan, E; et al. (1990a) Comparative Hazard Evaluation, An Approach To Regulation:Formaldehyde in Drinking Water. Health and Safety Research Division, Oak Ridge National Laboratory

Owen, BA; Dudney, CS; Tan, EL; et al. (1990b) Formaldehyde in drinking water: comparative hazard evaluation and an approach to regulation. Regul Toxicol Pharmacol 11:220–236.

Özen, OA; Yaman, M; Sarsilmaz, M; et al. (2002) Testicular zinc, copper and iron concentrations in male rats exposed to subacute and subchronic formaldehyde gas inhalation. J Trace Elem Med Biol 16:119–122.

Özen, OA; Songur, A; Sarsilmaz, M; et al. (2003a) Changes of zinc, copper, and iron levels in the lung of male rats after subacute (4-week) and subchronic (13-week) exposure to formaldehyde. J Trace Elem Exp Med 16:67–74.

Özen, OA; Songur, A; Sarsilmaz, M; et al. (2003b) Zinc, copper and iron concentrations in cerebral cortex of male rats exposed to formaldehyde inhalation. J Trace Elem Med Biol 17:207–209.

Özen, OA; Akpolat, N; Songur, A; et al. (2005) Effect of formaldehyde inhalation on Hsp70 in seminiferous tubules of rat testes: An immunohistochemical study. Toxicol Ind Health 21:249–254.

Palczyński, C; Krakowiak, A; Hanke, W; et al. (1999) Indoor formaldehyde exposure and airway allergic diseases. Int Rev Allergol Clin Immunol 5:65–69.

Park, H-S; Francis, KP; Yu, J; et al. (2003) Membranous cells in nasal-associated lymphoid tissue: a portal of entry for the respiratory mucosal pathogen group A streptococcus. J Immunol 171:2532–2537.

Partanen, T; Kauppinen, T; Hernberg, S; et al. (1990) Formaldehyde exposure and respiratory cancer among woodworkers: an update. Scand J Work Environ Health 16:394–400.

Passegue, E; Jamieson, CH; Ailles, LE; et al. (2003) Normal and leukemic hematopoiesis: are leukemias a stem cell disorder or a reacquisition of stem cell characteristics? Proc Natl Acad Sci U.S.A. 100(Suppl 1):11842–11849.

Pauluhn, J. (1996) Predictive testing for respiratory sensitization Toxicol Lett 86:177–185.

Pauluhn, J. (2003) Issues of dosimetry in inhalation toxicity. Toxicol Lett 140–141:229–238.

Pazdrak, K; Górski, P; Krakowiak, A; et al. (1993) Changes in nasal lavage fluid due to formaldehyde inhalation. Int Arch Occup Environ Health 64:515–519.

Peddada, SD; Dinse, GE; Kissling, GE. (2007) Incorporating historical control data when comparing tumor incidence rates. J Am Stat Assoc 102:1212–1220.

Pellegrino, R; Viegi, G; Brusasco, V; et al. (2005) Interpretative strategies for lung function tests. Eur Respir J 26:948-968.

Pinkerton, LE; Hein, MJ; Stayner, LT. (2004) Mortality among a cohort of garment workers exposed to formaldehyde: an update. Occup Environ Med 61:193–200.

Pitten, FA; Kramer, A; Herrmann, K; et al. (2000) Formaldehyde neurotoxicity in animal experiments. Pathol Res Pract 196:193–198.

Plesner, BH; and Hansen, K. (1983) Formaldehyde and hexamethylenetetramine in Styles' cell transformation assay. Carcinogenesis 4:457-459.

Plopper, CG; Weir, AJ; Nishio, SJ; et al. (1994) Elevated susceptibility to 4-ipomeanol cytotoxicity in immature Clara cells of neonatal rabbits. J Pharmacol Exp Ther 269:867–880.

Poortmans, JR; Kumps, A; Duez, P; et al. (2005) Effect of oral creatine supplementation on urinary methylamine, formaldehyde, and formate. Med Sci Sports Exerc 37:1717-1720.

Portier, CJ; Hedges, JC; Hoel, DG. (1986) Age-specific models of mortality and tumor onset for historical control animals in the National Toxicology Program's carcinogenicity experiments. Cancer Res 46:4372–4378.

Pottern, LM; Heineman, EF; Olsen, JH; et al. (1992) Multiple myeloma among Danish women: employment history and workplace exposures. Cancer Causes Control 3:427–432.

Pourmahabadian, M; Azam, K; Ghasemkhani, M. (2006) Pulmonary function study between formaldehyde exposed and non-exposed staffs at some of the Tehran Educational Hospitals. J Med Sci 6/4:621–625.

Priha, E; Liesivuori, J; Santa, H; et al. (1996) Reactions of hydrated formaldehyde in nasal mucus. Chemosphere 32:1077–1082.

Proctor, BL; Gaulden, ME; Dowd, MA. (1986) Reactivity and fate of benzene and formaldehyde in culture medium with and without fetal calf serum; relevance to in vitro mutagenicity testing. Mutat Res 160:259–266.

Pross, HF; Day, JH; Clark, RH; et al. (1987) Immunologic studies of subjects with asthma exposed to formaldehyde and urea-formaldehyde foam insulation (UFFI) off products. J Allergy Clin Immunol 79:797–810.

Pushkina, N; Gofmekler, V; Klevtsova, G. (1968) Changes in ascorbic-acid and nucleic-acid content during benzol and formaldehyde action rat [translation]. Bull Exp Biol Med 66:51–53.

Pyatt, D; Natelson, E; Golden, R. (2008) Is inhalation exposure to formaldehyde a biologically plausible cause of lymphohematopoietic malignancies? Regul Toxicol Pharmacol 51:119–133.

Que, LG; Liu, L; Yan, Y; et al. (2005) Protection from experimental asthma by an endogenous bronchodilator. Science 308:1618–1621.

Quievryn, G; Zhitkovich, A. (2000) Loss of DNA-protein crosslinks from formaldehyde-exposed cells occurs through spontaneous hydrolysis and an active repair process linked to proteosome function. Carcinogenesis 21:1573–1580.

Ragan, DL; and Boreiko, CJ. (1981) Initiation of C3H/10T1/2 cell transformation by formaldehyde. Cancer Lett 13:325-331.

Rao, GN; Piegorsch, WW; Haseman, JK. (1987) Influence of body weight on the incidence of spontaneous neoplasms in rats and mice of long-term studies. Am J Clin Nutr. 45:252–260.

Rapoport, IA. (1946) Carbonyl compounds and the chemical mechanisms of mutation. Compt Rend Acad Sci (U.S.S.R.) 54:65–67.

Recio, L; Sisk, S; Pluta, L, et al. (1992) p53 mutations in formaldehyde-induced nasal squamous cell carcinomas in rats. Cancer Res 52:6113-6116.

Report of the Federal Panel on Formaldehyde. (1982) Environ Health Perspect 43:139–168.

Reuzel, PGJ; Wilmer, JWGM; Woutersen, RA; et al. (1990) Interactive effects of ozone and formaldehyde on the nasal respiratory lining epithelium in rats. J Toxicol Environ Health 29:279–292.

Riedel, F; Hasenauer, E; Barth, PJ; et al. (1996) Formaldehyde exposure enhances inhalative allergic sensitization in the guinea pig. Allergy 51:94–99.

Ritchie, IM; Lehnen, RG. (1987) Formaldehyde-related health complaints of residents living in mobile and conventional homes. Am J Public Health 77:323–328.

Robins, J; Ambrun, M; Hute, C; et al. (1988) Estimating the effect of formaldehyde exposure on long cancer and non-malignant respiratory disease (NMRD) mortality using a new method to control for the health worker survivor effect. In: Hogstedt, C; Reuterwall, C; eds. Progress in occupational epidemiology. Amsterdam: Elsevier Science; pp. 75–78.

Roemer, E; Anton, HJ; Kindt, R. (1993) Cell proliferation in the respiratory tract of the rat after acute inhalation of formaldehyde or acrolein. J Appl Toxicol 13:103–107.

Rogers, J; Mole, M; Chernoff, N; et al. (2002) Developmental toxicity of inhaled methanol in the CD-1 mouse, with application of quantitative dose-response modeling for estimation of benchmark dosages. U.S. Environmental Protection Agency, Washington, D.C., EPA/600/J-92/350 (NTIS PB93106979).

Rogers, JM; Brannen, KC; Barbee, BD; et al. (2004) Methanol exposure during gastrulation causes holoprosencephaly, facial dysgenesis, and cervical vertebral malformations in C57BL/6J mice Birth Defects B Dev Reprod Toxicol 71:80–88.

Ross, WE; Shipley, N. (1980) Relationship between DNA damage and survival in formaldehyde-treated mouse cells. Mutat Res 79:277–283.

Ross, WE; McMillan, DR; and Ross, CF. (1981) Comparison of DNA damage by methylmelamines and formaldehyde. J Natl Cancer Inst 67:217-221.

Rotstein, J; Sarma, DSR; Farber, E. (1986) Sequential alterations in growth control and cell dynamics of rat hepatocytes in early precancerous steps in hepatocarcinogenesis. Cancer Res 46:2377–2385.

Roush, GC; Walrath, J; Stayner, LT; et al. (1987) Nasopharyngeal cancer, sinonasal cancer, and occupations related to formaldehyde: a case-control study. J Natl Cancer Inst 79:1221–1224.

Rumchev, KB; Spickett, JT; Bulsara, MK; et al. (2002) Domestic exposure to formaldehyde significantly increases the risk of asthma in young children. Eur Respir J 20:403–408.

Rumchev, K; Spickett, J; Bulsara, M; et al. (2004) Association of domestic exposure to volatile organic compounds with asthma in young children. Thorax 59: 746-751.

Rusch, GM; Bolte, HF; Rinehart, WE. (1983a) A 26-week inhalation toxicity study with formaldehyde in the monkey, rat, and hamster. In: Gibson, JE; ed. Formaldehyde toxicity. Washington, DC: Hemisphere Publishing Corporation; pp. 98–110.

Rusch, GM; Clary, JJ; Rinehart, WE; et al. (1983b) A 26-week inhalation toxicity study with formaldehyde in the monkey, rat and hamster. Toxicol Appl Pharmacol 68:329–343.

Rydén, E; Ekström, C; Hellmér, L; et al. (2000) Comparison of the sensitivities of Salmonella typhimurium strains TA102 and TA2638A to 16 mutagens. Mutagenesis 15:495–502.

Sadakane, K; Takano, H; Ichinose, T; et al. (2002) Formaldehyde enhances mite allergen-induced eosinophilic inflammation in the murine airway. J Environ Pathol Toxicol Oncol 21:267–276.

Saillenfait, AM; Bonnet, P; de Ceaurriz, J. (1989) The effects of maternally inhaled formaldehyde on embryonal and foetal development in rats. Food Chem Toxicol 27:545–548.

Saito, Y; Nishio, K; Yoshida, Y, et al. (2005) Cytotoxic effect of formaldehyde with free radicals via increment of cellular reactive oxygen species. Toxicology 210:235-245.

Saladino, AJ; Willey, JC; Lechner, JF, et al. (1985) Effects of formaldehyde, acetaldehyde, benzoyl peroxide, and hydrogen peroxide on cultured normal human bronchial epithelial cells. Cancer Res 45:2522-2526.

Salkie, ML. (1991) The prevalence of atopy and hypersensitivity to formaldehyde in pathologists. Arch Pathol Lab Med 115(6):614–616

Sallmén, M; Lindbohm, ML; Kyyronen, P; et al. (1995) Reduced fertility among women exposed to organic solvents. Am J Ind Med 27(5):699–713.

Salthammer T**,** S Mentese and R Marutzky (2010) Formaldehyde in the Indoor Environment. C*hem. Rev.*, **DOI:** 10.1021/cr800399g

Sandikci, M; Eren, U; Kum, S. (2007) Effects of formaldehyde and xylene on CD4- and CD8-positive T cells in bronchus-associated lymphoid tissue in rats. Toxicol Ind Health 23:471–477.

Sanghani, PC; Stone, CL; Ray, BD; et al. (2000) Kinetic mechanism of human glutathione-dependent formaldehyde dehydrogenase. Biochemistry 39:10720-10729.

Santiago, LY; Hann, MC; Ben-Jebria, A; et al. (2001) Ozone absorption in the human nose during unidirectional airflow. J Appl Physiol 91:725–732.

Sari-Minodier, I; Orsière, T; Auquier, P; et al. (2001) Use of the micronucleus assay in the mutagenic risk assessment: A case of 10 workers occupationally exposed to formaldehyde. Arch Mal Prof 62:75–82.

Sarsilmaz, M; Özen, O; Akpolat, N; et al. (1999) The histopathologic effects of inhaled formaldehyde on Leydig cells of the rats in subacute period [translation]. Firat Univ Med Sci J 13:37–40.

Sarsilmaz, M; Kaplan, S; Songur, A; et al. (2007) Effects of postnatal formaldehyde exposure on pyramidal cell number, volume of cell layer in hippocampus and hemisphere in therat: A stereological study. Brain Res 1145:157–167.

Sarrif, AM; Krahn, DF; Donovan, SM, et al. (1997) Evaluation of hexamethylphosphoramide for gene mutations in Salmonella typhimurium using plate incorporation, preincubation, and suspension assays. Mutat Res 380:167-177.

Sauder, LR; Chatham, MD; Green, DJ; et al. (1986) Acute pulmonary response to formaldehyde exposure in healthy nonsmokers. J Occup Med 28:420–424.

Sauder, LR; Green, DJ; Chatham, MD; et al. (1987) Acute pulmonary response of asthmatics to 3.0 ppm formaldehyde. Toxicol Ind Health 3:569–578.

Schachter, EN; Witek, TJ, Jr; Tosun, T; et al. (1986) A study of respiratory effects from exposure to 2 ppm formaldehyde in healthy subjects. Arch Environ Health 41:229–239.

Schachter, EN; Witek, TJ, Jr; Brody, DJ; et al. (1987) A study of respiratory effects from exposure to 2.0 ppm formaldehyde in occupationally exposed workers. Environ Res 44:188–205.

Schauenstein, E; Esterbauer, H; Zollner, H. (1977) Aldehydes in biological systems. London: Pion Limited.

Schlosser, PM. (1999) Relative roles of convection and chemical reaction for the disposition of formaldehyde and ozone in nasal mucus. Inhal Toxicol 11:967–980.

Schlosser, PM; Lilly, PD; Conolly, RB; et al. (2003) Benchmark dose risk assessment for formaldehyde using airflow modeling and a single-compartment, DNA-protein cross-link dosimetry model to estimate human equivalent doses. Risk Anal 23:473–487.

Schmid, E; Goggelmann, W; Bauchinger, M. (1986) Formaldehyde-induced cytotoxic, genotoxic and mutagenic response in human lymphocytes and Salmonella typhimurium. Mutagenesis 1:427–431.

Schmid, O; Speit, G. (2007) Genotoxic effects induced by formaldehyde in human blood and implications for the interpretation of biomonitoring studies. Mutagenesis 22:69–74.

Schoenberg, JB; Beck, GJ; Bouhuys, A. (1978) Growth and decay of pulmonary function in healthy blacks and whites. Respir Physiol 33:367–393.

Schreiber, H; Bibbo, M; Wied, GL; et al. (1979) Bronchial metaplasia as a benign or premalignant lesion. I. Cytologic and ultrastructural discrimination between acute carcinogen effects and toxin-induced changes. Acta Cytol 23:496–503.

Schuck, EA; Stephens, ER; Middleton, JT. (1966) Eye irritation response at low concentrations of irritants. Arch Environ Health 13(5):570–575.

Schulte-Hermann, R; Bursch, W; Grasl-Kraupp, B; et al. (1997) Concepts of cell death and application to carcinogenesis. Toxicol Pathol 25:89–93.

Schulte-Hermann, R; Bursch, W; Marian, B; et al. (1999) Active cell death (apoptosis) and cellular proliferation as indicators of exposure to carcinogens. IARC Sci.Publ 273–285.

Seidenberg, J; Anderson, DG; Becker, R. (1986) Validation of an in vivo developmental toxicity screen in the mouse. Teratog Carcinog Mutagen 6:361–374.

Seidenberg, J; Becker, R. (1987) A summary of the results of 55 chemicals screened for developmental toxicity in mice. Teratog Carcinog Mutagen 7:17–28.

Seitz, T; Baron, S. (1990) Health hazard evaluation report: Rockcastle Manufacturing, Mount Vernon, Kentucky. National Institute for Occupational Safety and Health, U.S. Department of Health and Human Services, Cincinnati, OH; HETA 87-349-2022.

Selevan, SG; Kimmel, CA; Mendola, P. (2000) Identifying critical windows of exposure for children's health. Environ Health Perspect 108 Suppl 3:451–455.

Sellakumar, AR; Snyder, CA; Solomon, JJ; et al. (1985) Carcinogenicity of formaldehyde and hydrogen chloride in rats. Toxicol Appl Pharmacol 81:401–406.

Senichenkova, IN. (1991) The embryotoxic effect of industrial environmental pollutants formaldehyde and gasoline [translation]. Gig Sanit 9:35–38.

Senichenkova, IN; Chebotar, NA. (1996) Effects of gasoline and formaldehyde on prenatal development of rats with induced iron microelementosis [translation]. Ontogenez 27:108–113.

Shaham, J; Bomstein, Y; Meltzer, A; et al. (1996) DNA-protein crosslinks, a biomarker of exposure to formaldehyde—in vitro and in vivo studies. Carcinogenesis 17:121–125.

Shaham, J; Bomstein, Y; Melzer, A; et al. (1997) DNA-protein crosslinks and sister chromatid exchanges as biomarkers of exposure to formaldehyde. Int J Occup Environ Health 3:95–104.

Shaham, J; Gurvich, R; Kaufman, Z. (2002) Sister chromatid exchange in pathology staff occupationally exposed to formaldehyde. Mutat Res 514:115–123.

Shaham, J; Bomstein, Y; Gurvich, R; et al. (2003) DNA-protein crosslinks and p53 protein expression in relation to occupational exposure to formaldehyde. Occup Environ Med 60:403–409.

Shangina, O; Brennan, P; Szeszenia-Dabrowska, N; et al. (2006) Occupational exposure and laryngeal and hypopharyngeal cancer risk in central and eastern Europe. Am J Epidemiol 164(4):367–375.

Sharpe, JA; Hostovsky, M; Bilbao, JM; et al. (1982) Methanol optic neuropathy. A histopathological study. Neurology 32:1093–1100.

Shelby, M; Portier, C; Goldman, L; et al. (2004) NTP-CERHR expert panel report on the reproductive and developmental toxicity of methanol. Reprod Toxicol 18:303-390.

Sheppard, D; Eschenbacher, W; Epstein, J. (1984) Lack of bronchomotor response to up to 3 ppm formaldehyde in subjects with asthma. Environ Res 35:133–139.

Sheveleva (1971) In Toxicology of New Industrial Chemicals. 12:78–86.

Shibamoto, T. (2006) Analytical methods for trace levels of reactive carbonyl compounds formed in lipid peroxidation systems. J Pharm Biomed Anal 41:12-25.

Shin, HS; Ahn, HS; Lee, BH. (2007) Determination of thiazolidine-4 carboxylates in urine by chloroformate derivatization and gas chromatography-electron impact mass spectrometry. J Mass Spectrom 42:1225–1232.

Shipkovitz, HD. (1966) Formaldehyde vapor in the permanent-press fabrics industry. Occupational Health Program, Public Health Service, U.S. Department of Health, Education, and Welfare, Cincinnati, OH; Report-TR-52.

Singh, GD; McNamara, JA Jr; Lozanoff, S. (1998) Morphometry of the midfacial complex in subjects with class III malocclusions: Procrustes, Euclidean, and cephalometric analyses. Clin Anat 11:162–170.

Skrzydlewska, E. (1996) Decreased antioxidant status and increased lipid peroxidation in rats after methanol intoxication. Rocz Akad Med Bialymst 41:397–404.

Slater, TF. (1984) Free-radical mechanisms in tissue injury. Biochem J 222:1-15.

Slikker, WJ; Andersen, M; Bogdanffy, M; et al. (2004) Dose-dependent transitions in mechanisms of toxicity: Case studies. Toxicol Appl Pharmacol 201:226–294.

Smedje, G; Norbäck, D. (2001) Incidence of asthma diagnosis and self-reported allergy in relation to the school environment—a four-year follow-up study in schoolchildren. Int J Tuberc Lung Dis 5:1059–1066.

Smith, AE; Coit, DW. (1995) Penalty Functions. In Environmental Computation 2. Eds: Back, T; Fogel, DB; Michalewicz., Z. 41–48. Institute of Physics Publishing and Oxford University Press, New York, NY.

Smith, D; Španěl, P. (2005) Selected ion flow tube mass spectrometry (SIFT-MS) for on-line trace gas analysis. Mass Spectrom Rev 24:661-700.

Smith, D; Španěl, P. (2007) The challenge of breath analysis for clinical diagnosis and therapeutic monitoring. Analyst 132:390-396.

Snedecor, GW; Cochran, WG. (1980) Statistical Methods, Seventh Edition. Iowa State University Press, Ames, IA.

Snyder, RD; van Houten, B. (1986) Genotoxicity of formaldehyde and an evaluation of its effects on the DNA repair process in human diploid fibroblasts. Mutat Res 165:21–30.

Songur, A; Kus, I; Sahin, S; Sogut, S; Ozen, OA; Yaman, M; Sarsilmaz, M. (2005) The changes of zinc, copper, and iron levels in lung tissue after formaldehyde inhalation during the early postnatal period of rats. Eur J Gen Med 2(2):62-68.

Songur, A; Sarsilmaz, M; Özen, OA. (2008) The effects of inhaled formaldehyde on oxidant and antioxidant systems of rat cerebellum during the postnatal development process. Toxicol Mechan Methods 18:569–574.

Sorg, BA; Hochstatter, T. (1999) Behavioral sensitization after repeated formaldehyde exposure in rats. Toxicol Ind Health 15:346–355.

Sorg, BA; Willis, JR; See, RE; et al. (1996) Repeated low-level formaldehyde exposure produces long-term cross-sensitization to cocaine: relevance to an animal model for multiple chemical sensitivity. Soc Neurosci Abstr 22:2064

Sorg, BA; Willis, JR; See, RE; et al. (1998) Repeated low-level formaldehyde exposure produces cross-sensitization to cocaine: Possible relevance to chemical sensitivity in humans. Neuropsychopharm 18:385–394.

Sorg, BA; Bailie, TM; Tschirgi, ML; et al. (2001) Exposure to repeated low-level formaldehyde in rats increases basal corticosterone levels and enhances the corticosterone response to subsequent formaldehyde. Brain Res 898:314–320.

Sorg, BA; Swindell, S; Tschirgi, ML. (2004) Repeated low level formaldehyde exposure produces enhanced fear conditioning to odor in male, but not female, rats. Brain Res 1008:11–19.

Španěl, P and Smith, D. (2008) Quantification of trace levels of the potential cancer biomarkets formaldehyde, acetaldehyde and propanol in breath by SIFT-MS. J. Breath Res. 2: 1-10.

Speit, G; Merk, O. (2002) Evaluation of mutagenic effects of formaldehyde in vitro: detection of crosslinks and mutations in mouse lymphoma cells. Mutagenesis 17:183–187.

Speit, G; Schmid, O. (2006) Local genotoxic effects of formaldehyde in humans measured by the micronucleus test with exfoliated epithelial cells. Mutat Res 613:1–9.

Speit, G; Schutz, P; Merk, O. (2000) Induction and repair of formaldehyde-induced DNA-protein crosslinks in repair-deficient human cell lines. Mutagenesis 15:85–90.

Speit, G; Schmid, O; Fröhler-Keller, M; et al. (2007a) Assessment of local genotoxic effects of formaldehyde in humans measured by the micronucleus test with exfoliated buccal mucosa cells. Mutat Res 627:129–135.

Speit, G; Schutz, P; Hogel, J, et al. (2007b) Characterization of the genotoxic potential of formaldehyde in V79 cells. Mutagenesis 22:387-394.

Speit, G; Schmid, O; Neuss, S, et al. (2008) Genotoxic effects of formaldehyde in the human lung cell line A549 and in primary human nasal epithelial cells. Environ Mol Mutagen 49:300-307.

Speit, G; Zeller, J; Schmid, O, et al. (2009) Inhalation of formaldehyde does not induce systemic genotoxic effects in rats. Mutat Res. (In Press)

Squire, RA; Cameron, LL. (1984) An analysis of potential carcinogenic risk from formaldehyde. Regul Toxicol Pharmacol 4:107–129.

Staab, CA; Hellgren, M; Höög, J-O. (2008a) Dual functions of alcohol dehydrogenase 3: Implications with focus on formaldehyde dehydrogenase and S-nitrosoglutathione reductase activities. Cell Mol Life Sci 65:3950–3960.

Staab, CA; Ålander, J; Brandt, M; et al. (2008b) Reduction of S-nitrosoglutathione by alcohol dehydrogenase 3 is facilated by substrate alcohols via direct cofactor recycling and leads to GSH-controlled formation of glutathione transferase inhibitors. Biochem J 41:493–504.

Staab, CA; Hellgren, M; Grafström, RC; et al. (2009) Medium-chain fatty acids and glutathione derivatives as inhibitors of S-nitroglutathione reduction mediated by alcohol dehydrogenase 3. Chem Biol Interact 180:113–118.

Staffelbach T, Neftel A, Stauffer B, et al. 1991. A record of the atmospheric methane sink from formaldehyde in polar ice cores. Nature 349:603-605.

Stayner, LT; Elliot, L; Blade, L; et al. (1988) A retrospective cohort mortality study of workers exposed to formaldehyde in the garment industry. Am J In Med 13:667–681.

Stedman's Medical Dictionary. (2005) 28th edition. Lippincott Williams, and Wilkins, New York, NY.

Steinhagen, WH; Barrow, CS. (1984) Sensory irritation structure-activity study of inhaled aldehydes in B6C3F1 and Swiss-Webster mice. Toxicol Appl Pharmacol 72:495–503.

Stellman, SD; Demers, PA; Colin, D; et al. (1998) Cancer mortality and wood dust exposure among participants in the American cancer Society cancer prevention study-II (CPS-II). Am J Ind Med 34:229–237.

Sterling, TD; Weinkam, JJ. (1988) Reanalysis of lung cancer mortality in a National Cancer Institute study on mortality among industrial workers exposed to formaldehyde. J Occup Med 30:895–901.

Sterling, TD; Weinkam, JJ. (1989a) Reanalysis of lung cancer mortality in a National Cancer Institute study on "Mortality among industrial workers exposed to formaldehyde." Exp Pathol 37:128–132.

Sterling, TD; Weinkam, JJ. (1989b) Reanalysis of lung cancer mortality in a National Cancer Institute study of "Mortality among industrial workers exposed to formaldehyde:" additional discussion. J Occup Med 31:881–884.

Sterling, TD; Weinkam, JJ. (1994) Mortality from respiratory cancers (including lung cancer) among workers employed in formaldehyde industries. Am J Ind Med 25:593–602.

Stewart, PA; Blair, A; Cubit, DA; et al. (1986) Estimating historical exposures to formaldehyde in a retrospective mortality study. Appl Ind Hyg 1:34–41.

Stewart, PA; Cubit, D; Blair, A. (1987) Formaldehyde levels in seven industries. Appl Ind Hyg 2:231–236.

Stewart, PA; Herrick, RF; Feigley, CE; et al. (1992) Study design for assessing exposures of embalmers for a case-control study. Part I. Monitoring results. Appl Occup Environ Hyg 7:532–540.

Stone, DM; Grillo, M; Margolis, FL; et al. (1991) Differential effect of functional olfactory bulb deafferentation on tyrosine hydroxylase and glutamic acid decarboxylase messenger RNA levels in rodent juxtaglomerular neurons. J Comp Neurol 311:223–233.

Stroup, NE; Blair, A; Erikson, GE. (1986) Brain cancer and other causes of death in anatomists. J Natl Cancer Inst 77:1217–1224.

Subramaniam, RP; Richardson, RB; Morgan, KT; et al. (1998) Computational fluid dynamics simulations of inspiratory airflow in the human nose and nasopharynx. Inhal Toxicol 10:92–120.

Subramaniam, RP; Crump, KS; Van Landingham, C; et al. (2007) Uncertainties in the CIIT model for formaldehyde-induced carcinogenicity in the rat: A limited sensitivity analysis-I. Risk Anal 27:1237–1254.

Subramaniam, RP, Chen, C; Crump, KS; et al. (2008) Uncertainties in biologically-based modeling of formaldehyde-induced respiratory cancer risk: Identification of key issues. Risk Anal 28:907–923.

Sul, D; Kim, H; Oh, E; et al. (2007) Gene expression profiling in lung tissues from rats exposed to formaldehyde. Arch Toxicol 81:589–597.

Suruda, A; Schulte, P; Boeniger, M; et al. (1993) Cytogenetic effects of formaldehyde exposure in students of mortuary science. Cancer Epidemiol Biomarkers Prev 2:453–460.

Suskov, II; and Sazonova, LA. (1982) Cytogenetic effects of epoxy, phenolformaldehyde and polyvinylchloride resins in man. Mutat Res 104:137-140.

Sweeney, MH; Beaumont, JJ; Waxweiler, RJ; et al. (1986) An investigation of mortality from cancer and other causes of death among workers employed at an East Texas chemical plant. Arch Environ Health 41:23–28.

Swenberg, JA; Kerns, WD; Mitchell, RI; et al. (1980) Induction of squamous cell carcinomas of the rat nasal cavity by inhalation exposure to formaldehyde vapor. Cancer Res 40:3398–3402.

Swenberg, JA; Gross, EA, Martin, J; et al. (1983) Mechanisms of formaldehyde toxicity. In: Formaldehyde toxicity, Ed: Gibson, JE. Hemisphere Publishing Corp. Washington, D.C.

Swenberg, JA; Gross, EA; Randall, HW. (1986) Localization and quantitation of cell proliferation following exposure to nasal irritants. In Toxicology of Nasal Passages, ed: Barrow, CS. Hemisphere Publishing Corp. Washington, D.C.

Swiecichowski, AL; Long, KJ; Miller, ML; et al. (1993) Formaldehyde-induced airway hyperreactivity in vivo and ex vivo in guinea pigs. Environ Res 61:185–199.

Szarvas, T; Szatloczky, E; Volford, J; et al. (1986) Determination of endogenous formaldehyde level in human blood and urine by dimedone-14C radiometric method. J Radioanaly Nucl Chem Letters 106:357-367.

Takahashi, K; Morita, T; Kawazoe, Y. (1985) Mutagenic characteristics of formaldehyde on bacterial systems. Mutat Res 156:153–161.

Takahashi, M; Hasegawa, R; Furukawa, F; et al. (1986) Effects of ethanol, potassium metabisulfite, formaldehyde and hydrogen peroxide on gastric carcinogenesis in rats after initiation with N-methyl-N'-nitro-N-nitrosoguanidine. Jpn J Cancer Res 77:118–124.

Takahashi, S; Tsuji, K; Fujii, K; et al. (2007) Prospective study of clinical symptoms and skin test reactions in medical students exposed to formaldehyde gas. J Dermatol 34(5):283–289.

Takigawa, T; Usami, M; Yamasaki, Y; et al. (2005) Reduction of indoor formaldehyde concentrations and subjective symptoms in a gross anatomy laboratory. Bull Environ Contam Toxicol 74:1027–1033.

Tang, X; Bai, Y; Duong, A, et al. (2009) Formaldehyde in China: Production, consumption, exposure levels, and health effects. Envir. International. 35:1210-1224.

Tarkowski, M; Gorski, P. (1995) Increased IgE antiovalbumin level in mice exposed to formaldehyde. Int Arch Allergy Immunol 106:422–424.

Tarone, RE. (1975) Tests for trend in life table analysis. Biometrika 62:679–682. (as cited in Battelle Columbus Laboratories [1981])

Tarone, R; McLaughlin, JK. (2005) Re: “mortality from solid cancers among workers in formaldehyde industries.” Am J Epidemiol 161:1089–1091.

Taskinen, H; Kyyronen, P; Hemminki, K. (1994) Laboratory work and pregnancy outcome. J Occup Med 36:311-319.

Taskinen, HK; Kyyronen, P; Sallmen, M; et al. (1999) Reduced fertility among female wood workers exposed to formaldehyde. Am J Ind Med 36:206–212.

Tavernier, G; Fletcher, G; Gee, I; et al. (2006) IPEADAM study: Indoor endotoxin exposure, family status, and some housing characteristics in English children. J Allergy Clin Immunol 117:656–662.

Temcharoen, P; Thilly, WG. (1983) Toxic and mutagenic effects of formaldehyde in Salmonella typhimurium. Mutat Res 119:89–93.

Teng, S; Beard, K; Pourahmad, J; et al. (2001) The formaldehyde metabolic detoxification enzyme systems and molecular cytotoxic mechanism in isolated rat hepatocytes. Chem Biol Interact 130–132:285–296.

Thomas, RS; Allen, BC; Nong, A; et al. (2007) A method to integrate benchmark dose estimates with genomic data to assess the functional effects of chemical exposure. Toxicol Sci 98(1):240–248.

Thompson, CM; Grafström, RC. (2008) Mechanistic considerations for formaldehyde-induced bronchoconstriction involving S-nitrosoglutathione reductase. J Toxicol Environ Health A 71:244–248.

Thompson, CM; Subramaniam, RP; Grafström, RC. (2008) Mechanistic and dose considerations for supporting adverse pulmonary physiology in response to formaldehyde. Toxicol Appl Pharmacol 233:355–359.

Thompson, CM; Sonawane, B; Grafström, RC. (2009) The ontogeny, distribution and regulation of alcohol dehydrogenase 3: implications for pulmonary physiology. Drug Metab Dispos. [Epub ahead of print]

Thomson, EJ; Shackleton, S; Harrington, JM. (1984) Chromosome aberrations and sister-chromatid exchange frequencies in pathology staff occupationally exposed to formaldehyde. Mutat Res 141:89–93.

Thorndike, J; Beck, WS. (1977) Production of formaldehyde from N5-methyltetrahydrofolate by normal and leukemic leukocytes. Cancer Res 37:1125-1132.

Thrasher, JD; Wojdani, A; Cheung, G; et al. (1987) Evidence for formaldehyde antibodies and altered cellular immunity in subjects exposed to formaldehyde in mobile homes. Arch Environ Health 42:347–350.

Thrasher, JD; Broughton, A; Gard, Z. (1988) Indoor formaldehyde and the elderly. Clin Gerontol 7(3-4):63–66.

Thrasher, JD; Broughton, A; Madison, R. (1990) Immune activation and autoantibodies in humans with long-term inhalation exposure to formaldehyde. Arch Environ Health 45:217–223.

Thun, MJ; Lakat, M; Altman, R. (1982) Symptom survey of residents of homes insulated with urea formaldehyde foam. Environ Res 29(2):320–334.

Til, HP; Woutersen, RA; Feron, VJ; et al. (1988) Evaluation of the oral toxicity of acetaldehyde and formaldehyde in a 4-week drinking-water study in rats. Food Chem Toxicol 26:447–452.

Til, HP; Woutersen, RA; Feron, VJ; et al. (1989) Two-year drinking-water study of formaldehyde in rats. Food Chem Toxicol 27:77–87.

Titenko-Holland, N; Levine, AJ; Smith, MT; et al. (1996) Quantification of epithelial cell micronuclei by fluorescence in situ hybridization (FISH) in mortuary science students exposed to formaldehyde. Mutat Res 371:237–248.

Tobe, M; Kaneko, Y; Uchida, Y; et al. (1985) Studies of the inhalation toxicity of formaldehyde. National Sanitary and Medical Laboratory Service, Toxicity, Tokyo, Japan; T-85-0236. (cited in IPCS, 2002)

Tobe, M; Naito, K; Kurokawa, Y. (1989) Chronic toxicity study on formaldehyde administered orally to rats. Toxicology 56:79–86.

Togias, A. (1999) Mechanisms of nose-lung interaction. Allergy 54:94–105.

Trezl, L; Hullan, L; Jaszay, ZM; et al. (2003) Antagonistic reactions of arginine and lysine against formaldehyde and their relation to cell proliferation, apoptosis, folate cycle and photosynthesis. Mol Cell Biochem 244:167–176.

Tsubone, H; Kawata, M. (1991) Stimulation to the trigeminal afferent nerve of the nose by formaldehyde, acrolein, and acetaldehyde gases. Inhal Toxicol 3:211–222.

Tsukahara, S; Yamamoto, S; Tin-Tin-Tin-Shwe; et al. (2006) Inhalation of low-level formaldehyde increases the Bcl-2/Bax expression ratio in the hippocampus of immunologically sensitized mice. Neuroimmunomodulation 13:63–68.

Turner, C; Parekh, B; Walton, C; et al. (2008) An exploratory comparative study of volatile compounds in exhaled breath and emitted by skin using selected ion flow tube mass spectrometry. Rapid Commun Mass Spectrom. 22(4):526–532.

Tuthill, RW. (1984) Woodstoves, formaldehyde, and respiratory disease. Am J Epidemiol 120:952–955.

Tyihák, E; Albert, L; Nemeth, ZI; et al. (1998) Formaldehyde cycle and the natural formaldehyde generators and capturers. Acta Biol Hung 49:225-238.

Tyihák, E; Bocsi, J; Timár, F; et al. (2001) Formaldehyde promotes and inhibits the proliferation of cultured tumour and endothelial cells. Cell Prolif 34:135–141.

U.S. EPA (U.S. Environmental Protection Agency). (1985) Health and environmental effects profile for formaldehyde. Prepared by the U.S. Environmental Protection Agency, Office of Research and Development, Cincinnati, OH for the Office of Solid Waste and Emergency Response, Washington, D.C.; EPA 600/X-85/362.

U.S. EPA (Environmental Protection Agency). (1986a) Guidelines for the health risk assessment of chemical mixtures. Federal Register 51(185):34014-34025. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (1986b) Guidelines for mutagenicity risk assessment. Federal Register 51(185):34006-34012. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (1987) Assessment of health risks to garment workers and certain home residents from exposure to formaldehyde. Office of Pesticides and Toxic Substances, Washington, DC.

U.S. EPA. (1988) Recommendations for and documentation of biological values for use in risk assessment. Prepared by the Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Cincinnati, OH for the Office of Solid Waste and Emergency Response, Washington, DC; EPA 600/6-87/008. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (1989) Review of the national ambient air quality standard for ozone, assessment for scientific and technical information. U.S. Environmental Protection Agency, RTP, NC, EPA-450/2-92.

U.S. EPA. (1991a) Guidelines for developmental toxicity risk assessment. Federal Register 56(234):63798-63826. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (1991b) Formaldehyde Risk Assessment Update. Prepared by the Office of Toxic Substances, Washington, DC.

U.S. EPA. (1991c) Formaldehyde. Integrated Risk Information System (IRIS). National Center for Environmental Assessment, Washington, DC. Available online at http://www.epa.gov/iris.

U.S. EPA. (1994a) Interim policy for particle size and limit concentration issues in inhalation toxicity studies. Federal Register 59(206):53799. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (1994b) Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. Office of Research and Development, Washington, DC; EPA/600/8-90/066F. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (1995) Use of the benchmark dose approach in health risk assessment. Risk Assessment Forum, Washington, DC; EPA/630/R-94/007. Available from: <http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=42601>.

U.S. EPA. (1996) Guidelines for reproductive toxicity risk assessment. Federal Register 61(212):56274-56322. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (1998) Guidelines for neurotoxicity risk assessment. Federal Register 63(93):26926-26954. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (2000a) Science policy council handbook: risk characterization. Office of Science Policy, Office of Research and Development, Washington, DC; EPA 100-B-00-002. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (2000b) Benchmark dose technical guidance document [external review draft]. Risk Assessment Forum, Washington, DC; EPA/630/R-00/001. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (2000c) Supplementary guidance for conducting for health risk assessment of chemical mixtures. Risk Assessment Forum, Washington, DC; EPA/630/R-00/002. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (2002a) A review of the reference dose and reference concentration processes. Risk Assessment Forum, Washington, DC; EPA/630/P-02/0002F. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (2002b) Toxicological Review and IRIS Summary of 1,3-Butadiene. Integrated Risk Information System, National Center for Environmental Assessment, Washington, DC. Accessed at http://www.epa.gov/EPA/iris/subst/0139.htm.

U.S. EPA. (2002c) Memorandum: Interim genomics policy. Office of the Administrator Science Policy Council.

U.S. EPA. (2004) Potential implications of genomics for regulatory and risk assessment applications at EPA. Prepared by the Genomics Task Force Workgroup. EPA 100/B-04/002.

U.S. EPA. (2005a) Guidelines for carcinogen risk assessment. Risk Assessment Forum, Washington, DC; EPA/630/P-03/001B. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (2005b) Supplemental guidance for assessing susceptibility from early-life exposure to carcinogens. Risk Assessment Forum, Washington, DC; EPA/630/R-03/003F. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (2006a) Science policy council handbook: peer review. Third edition. Office of Science Policy, Office of Research and Development, Washington, DC; EPA/100/B-06/002. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (2006b) A framework for assessing health risk of environmental exposures to children. National Center for Environmental Assessment - Washington, DC, EPA/600/R-05/093F. Available from: <http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=158363>.

U.S. EPA. (2006c) Technology transfer network 1999 national-scale air toxics assessment. Available from http://www.gov/ttn/atw/nata/.

U.S. EPA. (2006d) Air quality criteria for ozone and related photochemical oxidants (Final). U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-05/004aF-cF.

U.S. EPA (2008) Reregistration eligibility decision for formaldehyde and paraformaldehyde. Office of Pesticide Programs. Available from: http://www.epa.gov/pesticides/reregistration/formaldehyde/.

U.S. EPA (2009a) Formaldehyde: Chemical Report. TRI Explorer. Available from http://www.epa.gov/triexplorer/chemical.htm.

U.S. EPA (2009b) AirData. Available from http://www.epa.gov/air/data/index.html.

Uba, G; Pachorek, D; Bernstein, J; et al. (1989) Prospective study of respiratory effects of formaldehyde among healthy and asthmatic medical students. Am J Ind Med 15(1):91–101.

Uotila, L; Koivusalo, M. (1974a) Formaldehyde dehydrogenase from human liver. Purification, properties, and evidence for the formation of glutathione thiol esters by the enzyme. J Biol Chem 249:7653–7663.

Uotila, L, Koivusalo, M. (1974b) Purification and properties of S-formylglutathione hydrolase from human liver. J Biol Chem 249:7664–7672.

Ura, H; Nowak, P; Litwin, S; et al. (1989) Effects of formaldehyde on normal xenotransplanted human tracheobronchial epithelium. Am J Pathol 134:99–106.

Usanmaz, SE; Akarsu, ES; Vural, N. (2002) Neurotoxic effects of acute and subacute formaldehyde exposures in mice. Environ Toxicol Pharmacol 11:93–100.

USDA (U.S. Department of Agriculture). (1979) Food intakes: Individuals in 48 States, Year 1977–78, Nationwide Food Consumption Survey 1977-78, Rep No I-1. Technical report no. PB91-103523INZ.

van Birgelen, APJM; Chou, BJ; Renne, RA; et al. (2000) Effects of glutaraldehyde in a 2-year inhalation study in rats and mice. Toxicol Sci 55:195–205.

Van Schoor, J; Joos, GF; Pauwels, RA. (2000) Indirect bronchial hyperresponsiveness in asthma: mechanisms, pharmacology and implications for clinical research. Eur Respir J 16:514–533.

Vandenplas, O; Fievez, P; Delwiche, JP; et al. (2004) Persistent asthma following accidental exposure to formaldehyde. Allergy 59:115–116.

Vargová, M; Janota, S; Karelová, J; et al. (1992) Analysis of the health risk of occupational exposure to formaldehyde using biological markers. Analysis 20:451–454.

Vasudeva, N; Anand, C. (1996) Cytogenetic evaluation of medical students exposed to formaldehyde vapor in the gross anatomy dissection laboratory. J Am Coll Health 44:177–179.

Vaughan, TL; Strader, C; Davis, S; et al. (1986a) Formaldehyde and cancers of the pharynx, sinus and nasal cavity: I. Occupational exposures. Int J Cancer 38:677–683.

Vaughan, TL; Strader, C; Davis, S; et al. (1986b) Formaldehyde and cancers of the pharynx, sinus and nasal cavity: II. Residential exposures. Int J Cancer 38:685–688.

Vaughan, TL; Stewart, PA; Teschke, K; et al. (2000) Occupational exposure to formaldehyde and wood dust and nasopharyngeal carcinoma. Occup Environ Med 57:376–384.

Venkatakrishna-Bhatt, H; Panchal, GM. (1992) Behavioural change in rats due to chronic oral and systemic formaldehyde. Ind J Physiol Pharmacol 36:270–272.

Venkatakrishna-Bhatt, H and Panchal, GM (1997). Chronic exposure to formaldehyde in drinking water and behavioral changes in rats. Ind. J. Physioil. & Allied Sci. 51(4):181-184.

Vogelstein, B; Fearon, ER; Hamilton, SR; et al. (1988) Genetic alterations during colorectal-tumor development. N Engl J Med 319:525–532.

Von Hippel, PH; and Wong, KY. (1971) Dynamic aspects of native DNA structure: kinetics of the formaldehyde reaction with calf thymus DNA. J Mol Biol 61:587-613.

Wahlberg, JE. (1993) Measurement of skin-fold thickness in the guinea pig. Assessment of edema-inducing capacity of cutting fluids, acids, alkalis, formalin and dimethyl sulfoxide. Contact Dermatitis 28:141–145.

Walker, JF. (1975) Formaldehyde. 3rd edition. Huntington, NY: R.E. Krieger Publishing Company.

Walrath, J; Fraumeni, JF, Jr. (1983) Mortality patterns among embalmers. Int J Cancer 31:407–411.

Walrath, J; Fraumeni, JF, Jr. (1984) Cancer and other causes of death among embalmers. Cancer Res 44:4638-4641.

Wang, B; and Liu, DD. (2006) [Detection of formaldehyde induced developmental toxicity assessed with single cell gel electrophoresis]. Fen Zi Xi Bao Sheng Wu Xue Bao 39:462-466.

Wang, M; Cheng, G; Villalta, PW; et al. (2007) Development of liquid chromatography electrospray ionization tandem mass spectrometry methods for analysis of DNA adducts of formaldehyde and their application to rats treated with N-nitrosodimethylamine or 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. Chem Res Toxicol 20:1141–1148.

Wang, R; Zhang, Y; Lan, Q; et al. (2009) Occupational exposure to solvents and risk of non-Hodgkin lymphoma in Connecticut women. Amer. J. Epidem. 169(2):176-185.

Wang TS, Pysanenko A, Dryahina K, Španěl P, Smith D. (2008) Analysis of breath, exhaled via the mouth and nose, and the air in the oral cavity. J. Breath Res. 2:1-13.

Wangenheim, J; and Bolcsfoldi, G. (1988) Mouse lymphoma L5178Y thymidine kinase locus assay of 50 compounds. Mutagenesis 3:193-205.

Wantke, F; Demmer, CM; Tappler, P; et al. (1996a) Exposure to gaseous formaldehyde induces IgE-mediated sensitization to formaldehyde in school-children. Clin Exp Allergy 26:276–280.

Wantke, F; Focke, M; Hemmer, W; et al. (1996b) Formaldehyde and phenol exposure during an anatomy dissection course: a possible source of IgE-mediated sensitization. Allergy 51:837–841.

Ward Jr, BJ; Legator, MS; Pereira, MA; et al. (1983) Evaluation in man and animals of tests for the detection of population exposures to genotoxic chemicals. In: Waters, MD; Sandhu, SS; Lewtas, J; et al.; eds. Short-Term Bioassays in the Analysis of Complex Environmental Mixtures, New York: Plenum Press, 461-484.

Ward, Jr., JB; Hokanson, JA; Smith, ER; et al. (1984) Sperm count, morphology and fluorescent body frequency in autopsy service workers exposed to formaldehyde. Mutat Res 30:417–424.

Watanabe, K; Sakamoto, K; Sasaki, T. (1996) Comparisons on chemically-induced mutagenicity among four bacterial strains, Salmonella typhimurium TA102 and TA2638, and Escherichia coli WP2/pKM101 and WP2 uvrA/pKM101: collaborative study I. Mutat Res 361:143–155.

Waydhas, C; Weigl, K; Sies, H. (1978) The disposition of formaldehyde and formate arising from drug N-demethylations dependent on cytochrome P-450 in hepatocytes and in perfused rat liver. Eur J Biochem 89:143-150.

Weber-Tschopp, A; Fischer, T; Grandjean, E. (1977) Irritating effects of formaldehyde on man. Int Arch Occup Environ Health 39(4):207–218.

Wehinger, A; Schmid, A; Mechtcheriakov, S; et al. (2007) Lung cancer detection by proton transfer reaction mass-spectrometric analysis of human breath gas. Int J Mass Spectrometry 265:49-59.

Weibel, ER. (1963) Morphometry of the human lung. Springer-Verlag, Berlin.

Weiler, E; Apfelbach, R. (1992) Age-related differences in olfactory sensitivity in Wistar rats after low-level formaldehyde gas exposure. Chem Senses 17:717–718.

Weiss, B; Stern, S; Soderholm, SC; et al. (1996) Developmental neurotoxicity of methanol exposure by inhalation in rats. Research Report (Health Effects Institute) 73:1–64.

Weisskopf, M; Morozova, N; O'Reilly, EJ; et al. (2009) Prospective study of chemical exposures and amyotrophic lateral sclerosis mortality. J Neurol Neurosurg Psychiatry 80:558–561.

West, GB, and James HB. (2005) The origin of allometric scaling laws in biology from genomes to ecosystems: towards a quantitative unifying theory of biological structure and organization. The Journal of Experimental Biology 208(9): 1575-1592. Doi:10.1242/jeb.01589.

West, S; Hildesheim, A; Dosemeci, M. (1993) Non-viral risk factors for nasopharyngeal carcinoma in the Philippines: results from a case-control study. Int J Cancer 55:722–727.

Westerlund, M; Galter, D; Carmine, A; et al. (2005) Tissue- and species-specific expression patterns of class I, III, and IV ADH and ALDH1 mRNAs in rodent embryos. Cell Tissue Res 322:227–236.

WHO (World Health Organization). (1987) Laboratory manual for the examination of human semen and sperm-cervical mucus interaction. Cambridge, UK: Cambridge University Press.

Wilcox, AJ; Horney, LF. (1984) Accuracy of spontaneous abortion recall. Am J Epidemiol 120:727–733.

Wilcox, P; Naidoo, A; Wedd, DJ; et al. (1990) Comparison of Salmonella typhimurium TA102 with Escherichia coli WP2 tester strains. Mutagenesis 5:285–291.

Wilhelmsson, B; Holmström, M. (1992) Possible mechanisms of formaldehyde-induced discomfort in the upper airways. Scand J Work Environ Health 18:403–407.

Wilkins, RJ; and Macleod, HD. (1976) Formaldehyde induced DNA-protein crosslinks in Escherichia Coli. Mutat Res 36:11-6.

Williams, GM; Mori, H; and McQueen, CA. (1989) Structure-activity relationships in the rat hepatocyte DNA-repair test for 300 chemicals. Mutat Res 221:263-286.

Wilmer, JW; Woutersen, RA; Appelman, LM; et al. (1987) Subacute (4-week) inhalation toxicity study of formaldehyde in male rats: 8-hour intermittent versus 8-hour continuous exposures. J Appl Toxicol 7:15–16.

Wilmer, JW; Woutersen, RA; Appelman, LM; et al. (1989) Subchronic (13-week) inhalation toxicity study of formaldehyde in male rats: 8-hour intermittent versus 8-hour continuous exposures. Toxicol Lett 47:287–293.

Witek, TJ, Jr; Schachter, EN; Tosun, T. (1986) Controlled human studies on the pulmonary effects of indoor air pollution: experiences with sulfur dioxide and formaldehyde. Environ Int 12:129–135.

Witek, TJ, Jr; Schachter, EN; Tosun, T; et al. (1987) An evaluation of respiratory effects following exposure to 2.0 ppm formaldehyde in asthmatics: lung function, symptoms, and airway reactivity. Arch Environ Health 42:230-237.

Wolf, DC; Gross, EA; Lyght, O, et al. (1995) Immunohistochemical localization of p53, PCNA, and TGF-alpha proteins in formaldehyde-induced rat nasal squamous cell carcinomas. Toxicol Appl Pharmacol 132:27-35.

Wolfe, RK. (1986) Effects of airborne pollutants on mucociliary clearance. Environ Health Perspect 66: 223–237.

Wong, O. (1983) An epidemiologic mortality study of a cohort of chemical workers potentially exposed to formaldehyde, with a discussion on SMR and PMR. In: Gibson, J; ed. Formaldehyde toxicity. Washington, DC: Hemisphere Publishing Corporation; pp. 256–272.

Wong, EY; Ray, R; Gao, DL; et al. (2006) Reproductive history, occupational exposures, and thyroid cancer risk among women textile workers in Shanghai, China. Int Arch Occup Environ Health 79:251–158.

Wood, RW; Coleman, JB. (1995) Behavioral evaluation of the irritant properties of formaldehyde. Toxicol Appl Pharmacol 130:67–72.

Wortley, P; Vaughan, TL; Davis, S; et al. (1992) A case-control study of occupational risk factors for laryngeal cancer. Br J Ind Med 49:837–844.

Woutersen, RA; Appelman, LM; Wilmer, JW; et al. (1987) Subchronic (13-week) inhalation toxicity study of formaldehyde in rats. J Appl Toxicol 7:43–49.

Woutersen, RA; Garderen-Hoetmer, A; Bruijntjes, JP; et al. (1989) Nasal tumours in rats after severe injury to the nasal mucosa and prolonged exposure to 10 ppm formaldehyde. J Appl Toxicol 9:39–46.

Wu, H-Y; Nguyen, HH; Russell, MW. (1997) Nasal lymphoid tissue (NALT) as a mucosal immune inductive site. Scand J Immunol 46:506–513.

Wu, H; Romieu, I; Sienra-Monge, J-J; et al. (2007) Genetic variation in S-nitrosoglutathione reductase (GSNOR) and childhood asthma. J Allergy Clin Immunol 120:322–328.

Xing S-Y; Ye. L; Wang, N-N. (2007) Toxic effect of formaldehyde on reproduction and heredity in male mice. J Jilin Univ 33:716–718.

Xu, B; Aoyama, K; Takeuchi, M; et al. (2002) Expression of cytokine mRNAs in mice cutaneously exposed to formaldehyde. Immunol Lett 84:49–55.

Yager, JW; Cohn, KL; Spear, RC; et al. (1986) Sister-chromatid exchanges in lymphocytes of anatomy students exposed to formaldehyde-embalming solution. Mutat Res 174:135–139.

Yang, X; Zhang, YP; Chen, D; et al. (2001) Eye irritation caused by formaldehyde as an indoor air pollution—a controlled human exposure experiment. Biomed Environ Sci 14:229–236.

Yang, YH; Xi, ZG; Chao, FH; et al. (2005) Effects of formaldehyde inhalation on lung of rats. Biomed Environ Sci 18:164–168.

Ye, X; Yan, W; Xie, H; et al. (2005) Cytogenetic analysis of nasal mucosa cells and lymphocytes from high-level long-term formaldehyde exposed workers and low-level short-term exposed waiters. Mutat Res 588:22–27.

Yi C, Ke K, Xiaohua L and Xu Y (2007) Up-regulation of GSNO reductase in mice lungs by formaldehyde inhalation. Bioinform Biomed Engineering 6-8: 294-297.

Ying, CJ; Yan, WS; Zhao, MY; et al. (1997) Micronuclei in nasal mucosa, oral mucosa and lymphocytes in students exposed to formaldehyde vapor in anatomy class. Biomed Environ Sci 10:451–455.

Ying, CJ; Ye, XL; Xie, H; et al. (1999) Lymphocyte subsets and sister-chromatid exchanges in the students exposed to formaldehyde vapor. Biomed Environ Sci 12:88–94.

Yokley, KA; Tran, H; Schlosser, PM. (2008) Sensory irritation response in rats: modeling, analysis and validation. Bull Math Biol 70:555–588.

Young, JT. (1981) Histopathologic examination of the rat nasal cavity. Fundam Appl Toxicol 1:309–312.

Yu, L-Q; Jiang, S-F; Leng, S-G; et al. (2005) Early genetic effects on workers occupationally exposed to formaldehyde. Chin J Prevent Med 39:392–395.

Yu, PH; Zuo, DM. (1996) Formaldehyde produced endogenously via deamination of methylamine. A potential risk factor for initiation of endothelial injury. Atherosclerosis 120:189-197.

Yu, PH; Lai, CT; Zuo, DM. (1997) Formation of formaldehyde from adrenaline in vivo; a potential risk factor for stress-related angiopathy. Neurochem Res 22:615-620.

Yu, PH. (1998) Increase of formation of methylamine and formaldehyde in vivo after administration of nicotine and the potential cytotoxicity. Neurochem Res 23:1205-1210.

Zhang, L; Steinmaus, C; Eastmond, DA; et al. (2009) Formaldehyde exposure and leukemia: A new meta-analysis and potential mechanisms. Mutat Res 681:150–168.

Zhang, L; Tang, X; Rothman, N; et al. (2010a) Occupational exposure to formaldehyde, hematotoxicity, and leukemia-specific chromosome changes in cultured myeloid progenitor cells. Cancer Epidemiol Biomarkers Prev 19:80-88.

Zhang, L; Freeman, LE; Nakamura, J; et al. (2010b) Formaldehyde and leukemia: epidemiology, potential mechanisms, and implications for risk assessment. Environ Mol Mutagen 51:181-191.

Zhao, Z; Zhang, Z; Wang, Z; et al. (2008) Asthmatic symptoms among pupils in relation to winter indoor and outdoor air pollution in schools in Taiyaun, China. Environ Health Perspect 116:90–97.

Zhitkovich, A; and Costa, M. (1992) A simple, sensitive assay to detect DNA-protein crosslinks in intact cells and in vivo. Carcinogenesis 13:1485-1489.

Zhong, W; Que Hee, S. (2004a) Quantitation of normal and formaldehyde-modified deoxynucleosides by high-performance liquid chromatography/UV detection. Biomed Chromatogr 18:462–469.

Zhong, W; Que Hee, SS. (2004b) Formaldehyde-induced DNA adducts as biomarkers of in vitro human nasal epithelial cell exposure to formaldehyde. Mutat Res 563:13–24.

Zhou, DX; Qiu, SD; Zhang, J; et al. (2006) Reproductive toxicity of formaldehyde to adult male rats and the functional mechanism concerned. Sichuan Da Xue Xue Bao Yi Xue Ban 37:566–569.

Zielenska, M; Guttenplan, JB. (1988) Mutagenic activity and specificity of N-nitrosomethylaniline and N-nitrosodiphenylamine in Salmonella. Mutat Res 202:269–76.

Zuercher, AW; Cebra, JJ. (2002) Structural and functional differences between putative mucosal inductive sites of the rat. Eur J Immunol 32:3191–3196.

Zuercher, AW; Coffin, SE; Thurnheer, MC; et al. (2002) Nasal-associated lymphoid tissue is a mucosal inductive site for virus-specific humoral and cellular immune responses. J Immunol 168:1796–1803.

Zwart, A; Woutersen, RA; Wilmer, JW; et al. (1988) Cytotoxic and adaptive effects in rat nasal epithelium after 3-day and 13-week exposure to low concentrations of formaldehyde vapour. Toxicology 51:87–99.

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TOXICOLOGICAL REVIEW OF FORMALDEHYDE - INHALATION ASSESSMENT

(CAS No. 50-00-0)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

VOLUME IV of IV

Appendices

June 2, 2010

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Appendix A

1

APPENDIX A

SUMMARY OF EXTERNAL PEER REVIEW
AND PUBLIC COMMENTS AND DISPOSITIONS

[NOTE: This is a placeholder for Appendix A which will be drafted following External Peer review and receipt of public comments.]

Appendix B

1

APPENDIX B

SIMULATIONS OF INTERINDIVIDUAL AND ADULT-TO-CHILD VARIABILITY IN REACTIVE GAS UPTAKE IN A SMALL SAMPLE OF PEOPLE (GARCIA ET AL., 2009)

Garcia et al. (2009) used computational fluid dynamics to study human variability in the nasal dosimetry of model reactive, water-soluble gases in 5 adults and 2 children, aged 7 and 8 years old. They considered two model categories of gases, corresponding to maximal and moderate absorption at the nasal lining. This Appendix was developed in response to EPA reviewers' suggestions that results from the Garcia et al. (2009) work should be used to inform the uncertainty factor considered for interhuman variability in this document. Furthermore the tumor incidence in F344 rats have been used to extrapolate the risk of cancer in the human respiratory tract. This extrapolation was based on internal dose metrics derived using a CFD model constructed from the nasal passages of a single individual (Subramaniam et al. 1998). The adults considered in the Garcia et al. study included that individual.

Garcia et al. (2009) mapped out the nasal airway (including the nasopharynx) geometries of these individuals using magnetic resonance imaging or computed tomography scans. The scans chosen for the analysis were from individuals who had normal nasal anatomies with no pathology (as per a review carried out by a ear-nose-throat surgeon). The minute volumes of these individuals were ranged from 6.8 to 9.0 L/min (adults) and 5.5 to 5.8 L/min (children). The sample size in this study is too small to consider the results representative of the population as a whole (as also recognized by the authors). Nonetheless, various comparisons with the characteristics of other study populations add to the strength of this study; we therefore evaluated this study further in this document partly with the goal of impacting on research directions and future interpretations for specific gases. The range of adult minute volumes in this study is reported by the authors to be in good agreement with that obtained in many other studies in the literature. Minute volumes for the children in the study were found to be similar to the average minute volume of 6.1 ± 1.7 L/min obtained by Bennett and Zeman (2004) in a study of 36 children aged 6 to 13 years; the range of nasal surface area values for the adults agreed well with that obtained by Guilmette et al. (1997) for 45 adults; and the range of values for the surface area to volume ratio is in good agreement with that obtained for 40 adult Caucasians studied by Yokley (2006). The surface area to volume ratio is useful for comparing the rate of diffusional transport of a gas out of different cavities; however in the case of the highly nonhomogeneously shaped nasal lumen, this should only be considered a gross indicator.

We focus here only on the "maximum uptake" simulations in Garcia et al. (2009). In this case, the model gas was considered so highly reactive and soluble that it was reasonable to assume an infinitely fast reaction of the absorbed gas with compounds in the airway lining. Although such a gas could be reasonably considered a proxy for formaldehyde, these results cannot be utilized to inform quantitative estimates of formaldehyde dosimetry (and that does not appear to have been the intent of the authors either). This is because the same boundary condition corresponding to maximal uptake was applied on the vestibular section as well as on the respiratory and transitional epithelial lining of the nasal cavity. This is not appropriate for formaldehyde as the lining on the nasal vestibule is made of keratinized epithelium which is considerably less absorbing than the transitional or respiratory epithelium (Kimbell et al., 2001a).

Table B-1 provides results obtained by Garcia et al. (2009) for gas nasal uptake in five adults and two children for the maximal uptake scenario. Although the nasal cavities of the children were smaller in surface area, volume and length, the surface-area-to-volume ratios were similar in the two age groups. Overall uptake efficiency, average flux (rate of gas absorbed per unit surface area of the nasal lining) and maximum flux levels over the entire nasal lining did not vary substantially between adults (1.6-fold difference in average flux and much less in maximum flux), and the mean values of these quantities were comparable between adults and children. The comparisons between adults and children are in agreement with conclusions reached by Ginsberg et al. (2005) that overall extrathoracic absorption of highly and moderately reactive and soluble gases (corresponding to category 1 and 2 reactive gases as per the scheme in EPA [1994]) is similar in adults and children. However, the interindividual variations in each of these three quantities alone are limited in their ability to characterize variability in the interaction of the gas with the nasal lining. For a very reactive and soluble gas, regional absorption of the gas is highly nonhomogeneously distributed over the nasal lining; interindividual variations due to differing spatial patterns of this distribution between individuals could potentially be diluted when flux is averaged over the whole nose. Estimates of maximum gas flux, on the other hand, correspond to extremely small localized regions of hot spots (see Chapter 3), and thus interindividual differences in this quantity provide limited perspective on interindividual variability in flux distribution patterns over the whole nose. Furthermore, numerical error in the calculation (such as mass balance and irregularly shaped elements of the finite-element mesh) is likely to be most pronounced when estimates are considered over extremely small regions. We do not know to what extent these errors impact upon the accuracy in calculations of maximum flux.

Table B-1. Variations in overall nasal uptake, whole nose flux, and key parameters

	% nasal uptake	MV (L)	SA/V (1/mm)	Avg flux 10^{-8} kg/(s.m^2)		Maximum flux 10^{-8} kg/(s.m^2)	
				left cavity	right cavity	left cavity	right cavity
adult1	93.5	9	1.12	1.8	1.5	10.8	10.0
adult1	92.4	6.8	1.09	1.5	1.5	10.8	10.4
adult1	93.1	9	0.88	1.6	1.3	11	10.6
adult1	89.2	7.1	0.87	1.2	1.2	10.6	10.2
adult1	91.5	6.9	0.95	1.4	1.5	10.8	10.0
child1	92	5.5	1.13	1.9	1.5	11.8	11.0
child2	88.2	5.8	0.95	1.6	1.5	12.3	11.6

MV = minute volume, SA = nasal surface area, V = nasal volume.
Source: Garcia et al. (2009).

On the other hand, Figure 6A of Garcia et al. (2009), reproduced here as Figure B-1, provides a different perspective on interhuman variability in flux values at specific points on the nasal walls. In this figure gas flux across the nasal lining is plotted as function of distance from the nostril along the septal axis of the nose, normalized by the total nasal length along the septal axis of each subject. The local flux of formaldehyde varies among individuals by a factor of 3 to 5 at various normalized distances along the septal axis of the nose. However, interpretation of the values in this plot is problematic for reasons explained in their paper:[1]

> The greater variability among individuals seen for wall fluxes at specific sites of the nasal passages (Figure 6) in comparison to the minimal variability in total uptake (Table 2) and whole-nose dose (Tables 3 and Tables 4) indicates that fluxes of equal magnitude do not exactly overlay the same anatomical regions of the nasal cavity in each individual. This implies that specific anatomical regions subtended by maximum flux could be offset from one individual to another.

Notwithstanding this difficulty in interpretation, we believe the extents of vertical bars on each point plotted in Figure B-1 provide a better perspective of the interindividual (adult) variability in local flux than the variation in whole nose average or in maximum flux presented in Table B-1.

[1] The figures and tables in the cited text refer those in Garcia et al. (2009).

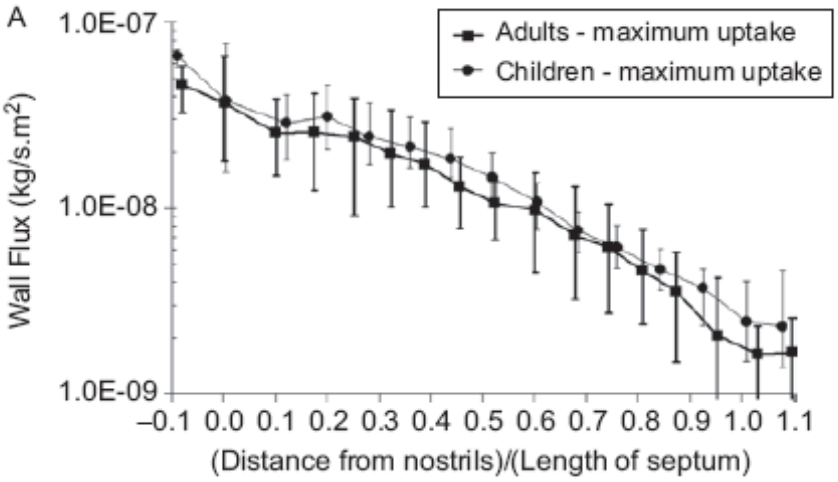

Figure B-1. Gas flux across the nasal lining for the case of a "maximum uptake" gas in Garcia et al. (2009) as a function of axial distance from the nostril. The vertical bars show range of variation. See the paper for further details. Figure is reproduced from Garcia et al. (2009).

Clearly, multiple measures of variability in dose can be developed depending on the adverse response. The advantage of models such as that developed by Garcia et al. is that they make it possible to explicitly carry out these calculations. For example, if deficit in pulmonary function is the adverse response, and the mechanism of action was a function of total dose to the lung, then interindividual variation in mean whole nose flux or overall nasal uptake efficiency would be most useful. It is possible to conceive of allergic or irritation responses being triggered by some threshold value of local flux. In such a case it may be preferable to calculate the variability associated with the net surface area receiving flux values greater than that threshold. On the other hand, the probability of developing a tumor at a nasal site may be nonlinearly related to the flux at that site and linearly related to the number of cells at that site. In this case, the appropriate metric may be the nasal surface area associated with some intermediate levels of local flux (see appendix in Subramaniam et al., 2008).

Various caveats presented by the authors as limitations of their study should be noted: Possible nonuniform distribution of epithelial types, enzymes, glands and other cellular metabolic or clearance machinery were not considered in the model; only effects pertaining to resting breathing were considered; the study sample size was small; children younger than 7 years old were not studied; and, the model assumed a rigid nasal geometry.

Garcia et al. (2009) conclude their paper as follows:

> "…, our simulations predicted no differences in the nasal dosimetry of reactive, water-soluble gases between children and adults, suggesting that the risk factor of 10 typically used to accommodate interhuman variability is adequate."

In addition to the caveats already recognized by the authors, the above conclusion needs further qualification:

1. While the uncertainty factor of 10 that is typically applied for interhuman variability is generally considered to be protective of children, it is not based on variations between children and adults. (If there is reasonable evidence that children are more sensitive than adults, the 10-fold factor may be considered inadequate.)
2. Assuming that the adverse response under consideration is one for which the localized nature of reactive gas flux across the nasal lining is important, the calculations such as those shown in Figure B-1 for the model gas are very relevant to the discussion of interindividual variability. The 3 to 5-fold variation in the local gas flux between adults (and also between the children) in the small sample size in this simulation may be compared with the value of 3.3 used for the pharmacokinetic component of the uncertainty factor for interhuman variability in susceptibility. (EPA practice is often to split this 10-fold uncertainty factor into pharmacokinetic and pharmacodynamic components of 3.3 each.)

Appendix C

1

APPENDIX C

LIFETABLE ANALYSIS

A spreadsheet illustrating the extra risk calculation for the derivation of the lower 95% bound on the effective concentration associated with a 0.05% extra risk (LEC_{0005}) for nasopharyngeal carcinoma (NPC) incidence is presented in Table C-1.

Table C-1. Extra risk calculation[a] for environmental exposure to 0.0461 ppm formaldehyde (the LEC0005 for NPC incidence)[b] using a log-linear exposure-response model based on the cumulative exposure trend results of Hauptmann et al. (2004), as described in Section 5.2.2

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Interval number (i)	**Age interval**	**All cause mortality ($\times 10^5$/yr)**	**NPC incidence ($\times 10^5$/yr)**	**All cause hazard rate (h*)**	**Prob of surviving interval (q)**	**Prob of surviving up to interval (S)**	**NPC cancer hazard rate (h)**	**Cond prob of NPC incidence in interval (Ro)**	**Exp duration mid interval (xtime)**	**Cum exp mid interval (xdose)**	**Exposed NPC hazard rate (hx)**	**Exposed all cause hazard rate (h*x)**	**Exposed prob of surviving interval (qx)**	**Exposed prob of surviving up to interval (Sx)**	**Exposed cond prob of NPC in interval (Rx)**
1	<1	728.7	0	0.0073	0.9927	1.0000	0.00000	0.000000	0	0.0000	0.0000	0.0073	0.9927	1.0000	0.00000
2	1−4	32.9	0.05	0.0013	0.9987	0.9927	0.00000	0.000002	0	0.0000	0.0000	0.0013	0.9987	0.9927	0.00000
3	5−9	16.4	0.03	0.0008	0.9992	0.9914	0.00000	0.000001	0	0.0000	0.0000	0.0008	0.9992	0.9914	0.00000
4	10−14	20.9	0.09	0.0010	0.9990	0.9906	0.00000	0.000004	0	0.0000	0.0000	0.0010	0.9990	0.9906	0.00000
5	15−19	68.2	0.12	0.0034	0.9966	0.9896	0.00001	0.000006	2.5	0.3506	0.0000	0.0034	0.9966	0.9896	0.00001
6	20−24	96	0.16	0.0048	0.9952	0.9862	0.00001	0.000008	7.5	1.0517	0.0000	0.0048	0.9952	0.9862	0.00001
7	25−29	99	0.23	0.0050	0.9951	0.9815	0.00001	0.000011	12.5	1.7528	0.0000	0.0050	0.9951	0.9815	0.00001
8	30−34	116.3	0.48	0.0058	0.9942	0.9766	0.00002	0.000023	17.5	2.4539	0.0000	0.0058	0.9942	0.9766	0.00003
9	35−39	162.2	0.55	0.0081	0.9919	0.9710	0.00003	0.000027	22.5	3.1550	0.0000	0.0081	0.9919	0.9710	0.00003
10	40−44	237.3	1.14	0.0119	0.9882	0.9631	0.00006	0.000055	27.5	3.8561	0.0001	0.0119	0.9882	0.9631	0.00008
11	45−49	356	1.3	0.0178	0.9824	0.9518	0.00007	0.000061	32.5	4.5572	0.0001	0.0178	0.9823	0.9517	0.00009
12	50−54	518.6	1.72	0.0259	0.9744	0.9350	0.00009	0.000079	37.5	5.2583	0.0001	0.0260	0.9744	0.9349	0.00012
13	55−59	801.8	1.69	0.0401	0.9607	0.9111	0.00008	0.000075	42.5	5.9594	0.0001	0.0401	0.9607	0.9110	0.00012
14	60−64	1257.9	1.9	0.0629	0.9390	0.8753	0.00010	0.000081	47.5	6.6605	0.0002	0.0630	0.9390	0.8751	0.00014
15	65−69	1928.2	2.87	0.0964	0.9081	0.8219	0.00014	0.000112	52.5	7.3616	0.0003	0.0965	0.9080	0.8217	0.00021
16	70−74	2968.1	2.1	0.1484	0.8621	0.7464	0.00011	0.000073	57.5	8.0627	0.0002	0.1485	0.8620	0.7461	0.00014
17	75−59	4556.6	2.19	0.2278	0.7963	0.6434	0.00011	0.000063	62.5	8.7638	0.0002	0.2279	0.7962	0.6431	0.00013
18	80−84	7399.6	1.98	0.3700	0.6907	0.5123	0.00010	0.000042	67.5	9.4649	0.0002	0.3701	0.6907	0.5120	0.00009
							Ro =	0.000725						**Rx =**	0.001225
Extra Risk = (Rx−Ro)/(1−Ro) = 0.0005															

Table C-1. Extra risk calculation[a] for environmental exposure to 0.0461 ppm formaldehyde (the LEC0005 for NPC incidence)[b] using a log-linear exposure-response model based on the cumulative exposure trend results of Hauptmann et al. (2004), as described in Section 5.2.2 (continued)

Column B: 5-year age interval (except <1 and 1−4) up to age 85.
Column C: all-cause mortality rate for interval i (× 10^5/year) (2000 data from NCHS).
Column D: NPC incidence rate for interval i (× 10^5/year) (1996−2000 SEER data).
Column E: all-cause hazard rate for interval i (h^*_i) (= all-cause mortality rate × number of years in age interval).[c]
Column F: probability of surviving interval i without being diagnosed with NPC (q_i) (= $\exp(-h^*_i)$).
Column G: probability of surviving up to interval i without having been diagnosed with NPC (S_i) ($S_1 = 1$; $S_i = S_{i-1} \times q_{i-1}$, for $i > 1$).
Column H: NPC incidence hazard rate for interval i (h_i) (= NPC incidence rate × number of years in interval).
Column I: conditional probability of being diagnosed with NPC in interval i (= $(h_i/h^*_i) \times S_i \times (1-q_i)$), i.e., conditional upon surviving up to interval i without having been diagnosed with NPC [Ro, the background lifetime probability of being diagnosed with NPC = the sum of the conditional probabilities across the intervals].
Column J: exposure duration (in years) at mid-interval (xtime).
Column K: cumulative exposure mid-interval (xdose) (= exposure level (i.e., 0.0461 ppm) × 365/240 × 20/10 × xtime) [365/240 × 20/10 converts continuous environmental exposures to corresponding occupational exposures].
Column L: NPC incidence hazard rate in exposed people for interval i (hx_i) (= $h_i \times (1 + \beta \times \text{xdose})$, where $\beta = 0.05183 + (1.645 \times 0.01915) = 0.08333$) [0.05183 per ppm × year is the regression coefficient obtained, along with its SE of 0.01915, from Dr. Hauptmann (see Section 5.2.2.1). To estimate the LEC_{0005}, i.e., the 95% lower bound on the continuous exposure giving an extra risk of 0.05%, the 95% upper bound on the regression coefficient is used, i.e., MLE + 1.645 × SE].
Column M: all-cause hazard rate in exposed people for interval i (h^*x_i) (= $h^*_i + (hx_i - h_i)$).
Column N: probability of surviving interval i without being diagnosed with NPC for exposed people (qx_i) (= $\exp(-h^*x_i)$).
Column O: probability of surviving up to interval i without having been diagnosed with NPC for exposed people (Sx_i) ($Sx_1 = 1$; $Sx_i = Sx_{i-1} \times qx_{i-1}$, for $i > 1$).
Column P: conditional probability of being diagnosed with NPC in interval i for exposed people (= $(hx_i/h^*x_i) \times Sx_i \times (1-qx_i)$) [Rx, the lifetime probability of being diagnosed with NPC for exposed people = the sum of the conditional probabilities across the intervals].

[a] Using the methodology of BEIR IV (1988).
[b] The estimated 95% lower bound on the continuous exposure level of TCE that gives a 0.05% extra lifetime risk of NPC.
[c] For the cancer incidence calculation, the all-cause hazard rate for interval i should technically be the rate of either dying of any cause or being diagnosed with the specific cancer during the interval, i.e., (the all-cause mortality rate for the interval + the cancer-specific incidence rate for the interval—the cancer-specific mortality rate for the interval [so that a cancer case isn't counted twice, i.e., upon diagnosis and upon death]) × number of years in interval. This adjustment was ignored here because the NPC incidence rates are small compared with the all-cause mortality rates.

MLE = maximum likelihood estimate, SE = standard error

Appendix D

1

APPENDIX D

MODEL STRUCTURE & CALIBRATION IN CONOLLY ET AL. (2003, 2004)

The various studies indicated in Section 5.4.1 were followed by the development of a biologically motivated dose-response model for formaldehyde-induced cancer in the respiratory tract. These efforts are represented in a series of papers and in a health assessment report (CIIT model) (Conolly et al., 2004, 2003, 2000; Conolly, 2002; Kimbell et al., 2001a, b; Overton et al., 2001; CIIT, 1999). The CIIT modeling and available data, and alternatives based on their original model were evaluated extensively for the purpose of this assessment and utilized in calculating the cancer potency. EPA's cancer guidelines (U.S. EPA, 2005a) suggest using a BBDR model for extrapolation when data permits since it facilitates the incorporation of MOA in risk assessment

In Conolly et al. (2003), tumor incidence data in the above long-term bioassays were modeled by using an approximation of the two-stage clonal growth model (Moolgavkar et al., 1988) and allowing formaldehyde to have directly mutagenic action. Conolly et al. (2003) combined these data with historical control data on 7,684 animals obtained from National Toxicology Program (NTP) bioassays. These models are based on the Moolgavkar, Venzon, and Knudson (MVK) stochastic two-stage model of cancer (Moolgavkar et al., 1988; Moolgavkar and Knudson, 1981; Moolgavkar and Venzon, 1979), which accounts for growth of a pool of normal cells, mutation of normal cells to initiated cells, clonal expansion and death of initiated cells, and mutation of initiated cells to fully malignant cells.

The MVK model for formaldehyde accounted for two MOAs that may be relevant to formaldehyde carcinogenicity:

- An indirect MOA in which the regenerative cell proliferation in response to formaldehyde cytotoxicity increased the probability of errors in DNA replication. This MOA was modeled by using labeling data on normal cells in nasal mucosa of rats exposed to formaldehyde.
- A possible direct mutagenic MOA, based on information indicating that formaldehyde is mutagenic (Speit and Merk, 2002; Heck et al., 1990; Grafstrom et al., 1985), was modeled by using rat and rhesus monkey data on formaldehyde production of DPXs.

The human model for formaldehyde carcinogenicity (Conolly et al., 2004) is conceptually very similar to the rat model. The model uses, as input, results from a dosimetry model for an anatomically realistic representation of the human upper airways and an idealized

representation of the lower airways. However, the model does not incorporate any data on human responses to formaldehyde exposure. The rat and human formaldehyde models are detailed further below.

The following notations are used in the rest of this appendix:

N cell, normal cell

I cell, initiated cell

LI, labeling index (number of labeled cells/(number labeled + unlabeled cells)

ULLI, unit length labeling index (number labeled cells/length of basement membrane)

N, number of normal cells that are eligible for progression to malignancy

α_N, division rate of normal cells ($hours^{-1}$)

μ_N, rate at which an initiated cell is formed by mutation of a normal cell (per cell division of normal cells)

$\alpha_{I,}$ division rate of an initiated cell ($hours^{-1}$)

$\beta_{I,}$ death rate of an initiated cell ($hours^{-1}$)

$\mu_{I,}$ rate at which a malignant cell is formed by mutation of an initiated cell (per cell division of initiated cells)

A novel contribution of the CIIT model is that cell replication rates and DPX concentrations are driven by local dose, which is formaldehyde flux to each region of nasal tissue expressed as $pmol/mm^2$-hour. This dosimetry is predicted by computational fluid dynamics (CFD) modeling using anatomically accurate representations of the nasal passages (see Chapter 3). Such a feature is important to incorporating site-specific toxicity in the case of a highly reactive gas like formaldehyde, for which uptake patterns are spatially localized and significantly different across species (see Chapter 3). In the CIIT model, each of these parameters is characterized by local flux. The inputs to the two-stage cancer modeling consisted of results from other model predictions as well as empirical data as follows:

- Regional uptake of formaldehyde in the respiratory tract predicted by using CFD modeling in the F344 rat and human (Kimbell et al., 2001a, b; Overton et al., 2001; Subramaniam et al., 1998)
- Concentrations of DPXs predicted by a PBPK model (Conolly et al., 2000) calibrated to fit the DPX data in F344 rat and rhesus monkey (Casanova et al., 1994, 1991) and subsequently scaled up to humans

- α_N inferred from LI data on rats exposed to formaldehyde (Monticello et al., 1996, 1991, 1990)

D.1. DPX AND MUTATIONAL ACTION

Formaldehyde interacts with DNA to form DPXs. These cross-links are considered to induce mutagenic as well as clastogenic effects. Casanova et al. (1994, 1989) carried out two studies of DPX measurements in F344 rats. In the first study, rats were exposed to concentrations of 0.3, 0.7, 2, 6, and 10 ppm for 6 hours and DPX measurements were made over the whole respiratory mucosa of the rat, while, in the second study, the exposure was to 0.7, 2, 6, or 15 ppm formaldehyde for 3 hours and measurements were made at "high" and "low" tumor sites. Overall, these studies showed statistically significantly elevated levels of DPXs at concentrations ≥2 ppm, with the trend also indicating elevated DPXs at 0.7 ppm. In Conolly et al. (2003), DPX formation is considered proportional to the intracellular dose that induces mutations. Conolly et al. (2000) used data from the second study to develop a PBPK model that predicted the time course of DPX concentrations as a function of regional formaldehyde flux (estimated in the CFD modeling and expressed as pmol/mm^2-hour). In Conolly et al. (2003), this PBPK model was then used to predict regional DPX concentrations (that is, as a function of regional formaldehyde flux) (see Figure 5-11, Chapter 5). These data were incorporated into the two-stage clonal expansion model by defining the mutation rate of normal and initiated cells as the same linear function of DPX concentration as follows:

$$\mu_N = \mu_I = \mu_{Nbasal} + KMU \times DPX \qquad \text{(D-1)}$$

The unknown constants μ_{Nbasal} and KMU were estimated by fitting model predictions to the tumor bioassay data.

D.2. CALIBRATION OF MODEL

The rat model in Conolly et al. (2003) involved six unknown statistical parameters that were estimated by fitting the model to the rat formaldehyde bioassay data shown in Table 5-24 in Chapter 5 (Monticello et al., 1996; Kerns et al., 1983) plus historical data from several thousand control animals from **all** the rat bioassays conducted by the NTP. These NTP bioassays were conducted from 1976 through 1999 and included 7,684 animals with an incidence of 13 SCCs (i.e., 0.17% incidence). The resulting model predicts the probability of a nasal SCC in the F344 rat as a function of age and exposure to formaldehyde. The fit of the Conolly et al. (2003) model to the tumor incidence data is shown in Figure 5-12, Chapter 5.

D.3. FLUX BINS

The spatial distribution of formaldehyde over the nasal lining was characterized by partitioning the nasal surface by formaldehyde flux to the tissue (rate of gas absorbed per unit surface area of the nasal lining), resulting in 20 "flux bins" (see Figure 5-13, Chapter 5). Each bin is comprised of elements (not necessarily contiguous) of the nasal surface that receive a particular interval of formaldehyde flux per ppm of exposure concentration (Kimbell et al., 2001a). The spatial coordinates of elements comprising a particular flux bin are fixed for all exposure concentrations, with formaldehyde flux in a bin scaling linearly with exposure concentration (ppm). The number of cells at risk varies across the bins, as shown in Figure 5-14, Chapter 5.

D.4. USE OF LABELING DATA

Cell replication rates in Conolly et al. (2003) were obtained by pooling labeling data from two phases of a labeling study in which male F344 rats were exposed to formaldehyde gas at similar concentrations (0, 0.7, 2.0, 6.0, 10.0, or 15.0 ppm). The first phase employed injection labeling with a 2-hour pulse labeling time, and animals were exposed to formaldehyde for early exposure periods of 1, 4, and 9 days and 6 weeks (Monticello et al., 1991). The second phase used osmotic minipumps for labeling with a 120-hour labeling time to quantify labeling in animals exposed for 13, 26, 52, and 78 weeks (Monticello et al., 1996). The combined pulse and continuous labeling data were expressed as one exposure TWA over all sites for each exposure concentration. α_N was calculated from these labeling data by using an approximation from Moolgavkar and Luebeck (1992). A dose-response curve for normal cell replication rates (i.e., α_N as a function of formaldehyde flux) was then calculated as shown in Figure D-1. These steps are carefully detailed and evaluated in Subramaniam et al. (2008), and discussion of the data will continue in Appendix E in the section on uncertainties in characterizing cell replication rates.

D.5. UPWARD EXTRAPOLATION OF NORMAL CELL DIVISION RATE

The extensive labeling data collected by Monticello et al. (1996, 1991) present an opportunity to use precursor data in assessing cancer risk. However, these empirical data could be used to determine α_N(flux) only for the lower flux range, 0–9,340 pmol/mm^2-hour (see Subramaniam et al. [2008] for the reasons), as shown by the solid line in Figure D-1, whereas the highest computed flux at 15.0 ppm exposure was 39,300 pmol/mm^2-hour. Therefore Conolly et al. (2003) introduced an adjustable parameter, α_{max}, that represented the value of α_N(flux) at the maximum flux of 39,300 pmol/mm^2-hour. α_{max} was estimated by maximizing the likelihood of

the two-stage model fit to the tumor incidence data. For 9,340 < flux ≤ 39,300 pmol/mm^2-hour, α_N(flux) was determined by linear interpolation from α_N(9,340) to α_{max}, as shown by the dashed line in Figure D-1.

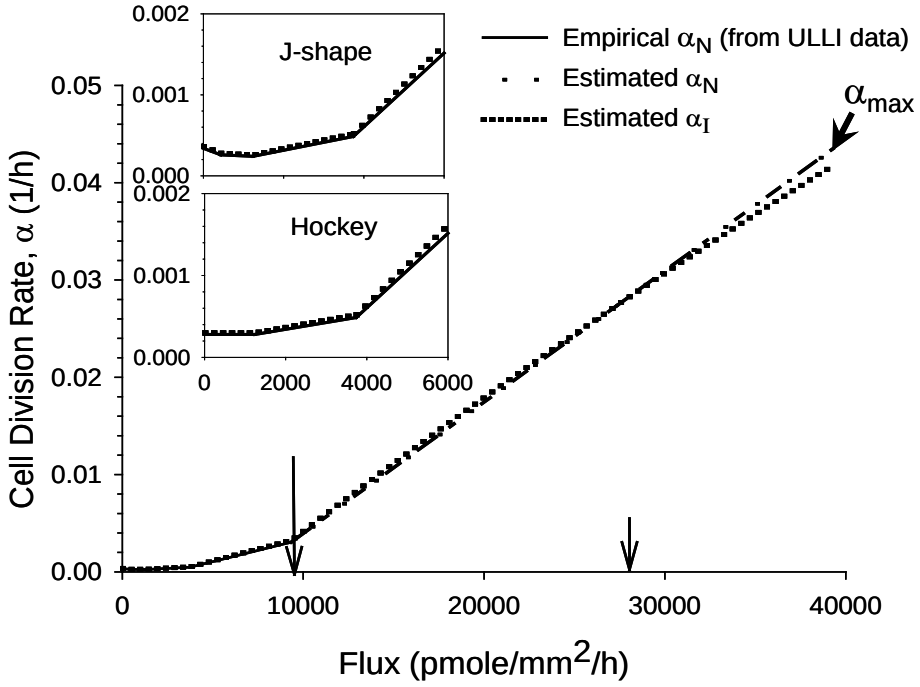

Figure D-1. Dose response of normal (α_N) and initiated (α_I) cell division rate in Conolly et al. (2003).

Note: Empirically derived values of α_N (TWA over six sites) from Table 1 in Conolly et al. (2003) and optimized parameter values from their Table 4 were used. The main panel is for the J-shape dose response. Insets show J-shape and hockey-stick shape representations at the low end of the flux range. The long arrow denotes the upper end of the flux range for which the empirical unit-length labeling data are available for use in the clonal growth model. α_{max} is the value of α_N at the maximum formaldehyde flux delivered at 15 ppm exposure and estimated by optimizing against the tumor incidence data. $\alpha_I < \alpha_N$ for flux greater than the value indicated by the small vertical arrow. Conolly et al. (2004, 2003) assumed $\beta_I = \alpha_N$ at all flux values.

Source: Subramaniam et al. (2008).

D.6. INITIATED CELL DIVISION AND DEATH RATES

The pool of cells used for obtaining the LI data in Monticello et al. (1996, 1991) consists of largely normal cells with perhaps increasing numbers of initiated cells at higher exposure concentrations. Since the division rates of initiated cells in the nasal epithelium, either background or formaldehyde exposed, could not be inferred from the available empirical data, Conolly et al. (2003) made what they perceived to be a biologically reasonable assumption for α_I, assuming α_I to be linked to α_N via a two-parameter function:

$$\alpha_I = \alpha_N \times \{\text{multb} - \text{multc} \times \max[\alpha_N - \alpha_{N(\text{basal})}, 0]\} \quad \text{(D-2)}$$

where $\alpha_N \equiv \alpha_N(\text{flux})$, $\alpha_{N(\text{basal})}$ is the estimated average cell division rate in unexposed normal cells, and multb and multc are unknown parameters estimated by likelihood optimization against the tumor data.[2] The value of $\alpha_{N(\text{basal})}$ was equal to 3.39×10^{-4} hours^{-1} as determined by Conolly et al. (2003) from the raw averaged unit length labeling index data. The ratio α_I/α_N is plotted against flux in Figure D-2, and $\alpha_I(\text{flux})$ is shown by the dotted line in Figure D-1.

Death rates of Initiated cells (β_I) are assumed to equal the division rates of Normal cells (α_N) for all formaldehyde flux values, that is

$$\beta_I(\text{flux}) = \alpha_N(\text{flux}) \quad \text{(D-3)}$$

Conolly et al. (2003) stated that this formulation for α_I and β_I provided the best fit of the model to the tumor data.

D.7. STRUCTURE OF THE CIIT HUMAN MODEL

Subsequent to the BBDR model for modeling rat cancer, Conolly et al. (2004) developed a corresponding model for humans for the purpose of extrapolating the risk estimated by the rat model to humans. Also, rather than considering only nasal tumors (as in the rat model), the human model was used to predict the risk of all human respiratory tumors. The human model for formaldehyde carcinogenicity (Conolly et al., 2004) is conceptually very similar to the rat model and follows the schematic in Figure 5-11, Chapter 5. The following points need to be noted:

[2] multb and multc were equal to 1.072 and 2.583, respectively (J-shaped α_N), and 1.070 and 2.515, respectively (hockey-stick shaped α_N).

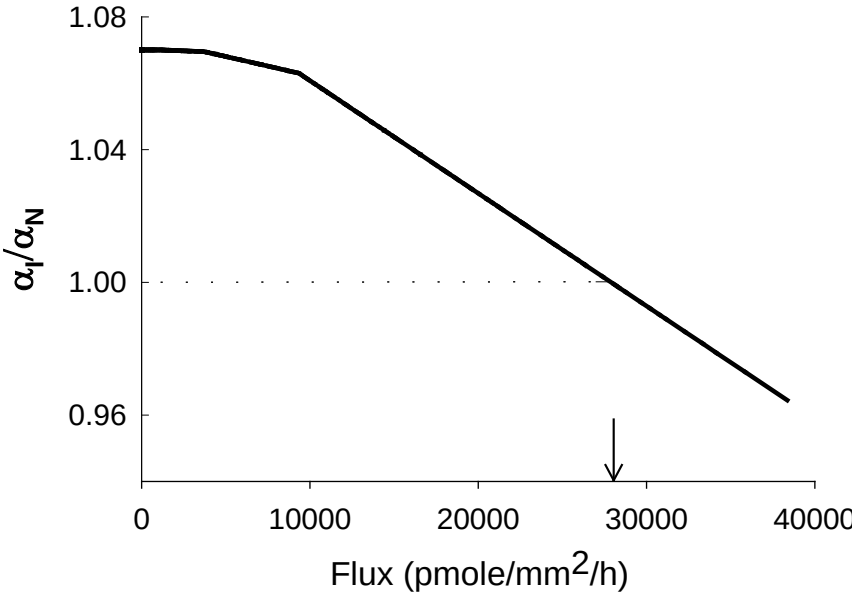

Figure D-2. Flux dependence of ratio of initiated and normal cell replication rates (α_I/α_N) in CIIT model.
Note: Cell replication rate of initiated cells is less than normal cell replication rate at flux exceeding the value denoted by the arrow. By assumption, the Y-axis also represents (α_I/β_I).

Source: Subramaniam et al. (2008).

- The model does not incorporate any data on human responses to formaldehyde exposure.
- The model is based on an anatomically realistic representation of the human nasal passages in a single individual and an idealized representation of the LRT. Local formaldehyde flux to the tissue is estimated by a CFD model for humans (Subramaniam et al., 1998; Kimbell et al., 2001a; Overton et al., 2001).
- Rates of cell division and cell death are, with a minor modification, assumed to be the same in humans as in rats.
- The concentration of formaldehyde-induced DPXs in humans is estimated by scaling up from values obtained from experiments in the F344 rat and rhesus monkey. This scaling up was discussed in chapter 3.
- The statistical parameters for the human model are either estimated by fitting the model to the human background data, assumed to have the same value as obtained in the rat model, or, in one case, fixed at a value suggested by the epidemiologic literature. The delay, D, is fixed at 3.5 years, based on a fit to the incidence of lung cancer in a cohort of British doctors (Doll and Peto, 1978). The two other parameters in the rat model that affect the background rate of cancer (multb and μ_{basal}) are estimated by fitting to U.S. cancer incidence or mortality data. These parameters affect the baseline values for the

human $\alpha_{I,}$ μ_N, and $\mu_{I.}$ Since α_{max}, multfc, and KMU do not affect the background cancer rate, they cannot be estimated from the (baseline) U.S. cancer incidence rates. Therefore, in Conolly et al. (2004, 2003), α_{max} and multfc are assumed to have the same values in humans as in rats, and the human value for KMU is obtained by assuming that the ratio KMU/μ_{basal} is invariant across species. Thus,

$$KMU_{(human)} = KMU_{(rat)} \times \frac{\mu_{Nbasal(human)}}{\mu_{Nbasal(rat)}} \quad \text{(D-4)}$$

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Appendix E

1

APPENDIX E

EVALUATION OF BBDR MODELING OF NASAL CANCER IN THE F344 RAT: CONOLLY ET AL. (2003) AND ALTERNATIVE IMPLEMENTATIONS

A biologically based dose-response model for formaldehyde-induced cancer was developed in a series of papers and in a health assessment report (CIIT model) (Conolly et al., 2004, 2003, 2000; Conolly, 2002; Kimbell et al., 2001a, b; Overton et al., 2001; CIIT, 1999). The model structure, notations, and calibration have been described in Appendix D. In Chapter 5, an evaluation of the uncertainties of this model and alternative approaches based on its conceptual framework was presented in a summary form. This Appendix provides the relevant details of that evaluation and presents a range of dose-response curves for tumor risk in the rat. It is divided into the following major sections. First, an overview of all the issues that were evaluated is provided in tabular form. The rest of the Appendix then presents only those issues which have a significant impact on model predictions: the use of history controls, the uncertainty and variability in the dose-response for normal cell-replication rates, and sensitivity of model results to uncertainty in the kinetics of initiated cells. These issues have significant impact on inferences regarding mode of action, and this is discussed in some detail in this Appendix. Assumptions and uncertainties related to the human formaldehyde model are discussed in Appendix F.

E.1. TABULATION OF ALL ISSUES EVALUATED IN THE RAT MODELS

Table E-1 summarizes model uncertainties and their impact as evaluated by EPA. The key uncertainties are discussed in considerably more detail in additional sections in this Appendix and in published manuscripts (Klein et al., 2010; Crump et al., 2008; Subramaniam et al., 2008, 2007). The results in Subramaniam et al. (2007) and Crump et al. (2008) have been debated further in the literature (Conolly et al., 2009; Crump et al., 2009). Other alternatives to the CIIT biological modeling (but based on that original model) are also further explored and evaluated in this appendix.

Table E-1. Evaluation of assumptions and uncertainties in the CIIT model for nasal tumors in the F344 rat

Assumptions, approach, and characterization of input data in model	Rationale for assumption/ approach	EPA evaluation	Further elaboration of evaluation[a]
Hoogenveen et al. (1999) solution method, which is valid only for time-independent parameters, is accurate enough.	Errors due to this assumption thought to be significant only at high concentration and not at human exposures.	EPA implemented a solution method valid for time-dependent parameters. Results did not differ significantly from those obtained assuming Hoogenveen et al.(1999) solutions. However, impact was not evaluated for the case where cell replication rates vary in time.	Crump et al. (2005); Subramaniam et al. (2007)
All observed SCC tumors are rapidly fatal; none are incidental tumors.	Death is expected to occur typically within 1–2 weeks of observed tumor (personal communication with R. Conolly).	1) Overall, assumption does not impact model calibration or prediction. 2) However, since 57 animals were observed to have tumors at interim sacrifice times, EPA implementation distinguished between incidental and fatal tumors. Time lag between observable tumor and time of death was significant compared to time lag between first malignant cell and observable tumor.	Subramaniam et al. (2007)
Historical controls from entire NTP database were lumped with concurrent controls in studies.	Large number of control animals (7,684). Intercurrent mortality was not expected to be substantial.	1) Tumor incidence in "all NTP" 10-fold higher than in "all inhalation NTP" controls. Including all NTP controls is considered inappropriate. 2) Low-dose response curve is very sensitive to use of historical controls. 3) Model inference regarding relevance of formaldehyde's mutagenic potential to its carcinogenicity varies from "insignificant" to "highly significant," depending on controls used. (See Appendix F for impact on human risk.)	Table E-2; Subramaniam et al. (2007); Sec E.3.1
LI was derived from experimentally measured ULLI.	Derived from correlating ULLI to LI measured in same experiment.	Significant variation in number of cells per unit length of basement membrane. Spread in ULLI/LI ~25%. Impact on risk not evaluated.	Subramaniam et al. (2008);

Table E-1. Evaluation of assumptions and uncertainties in the CIIT model for nasal tumors in the F344 rat (continued)

Assumptions, approach, and characterization of input data in model	Rationale for assumption/approach	EPA evaluation	Further elaboration of evaluation[a]
Pulse and continuous labeling data were combined in deriving α_N from LI.	All continuous LI values were normalized by mean ratio of pulse to continuous LI for controls.	Formula used for deriving α_N from LI is not applicable for pulse labeling data. Pulse labeling is measure of number of cells in S-phase, not of their recruitment rate into S-phase; not enough information to derive α_N from pulse data. Impact on risk predictions could not be evaluated.	Subramaniam et al. (2008); Section E.3.2.2
To construct dose response for α_N, labeling data were weighted by exposure time (t) and averaged over all nasal sites (TWA). At an exposure concentration, flux was averaged over all nasal sites.	Site-to-site variation in LI was large and did not vary consistently with flux. No reasonable approach was available for extrapolating observed time variation in labeling in rats to humans.	1) TWA assigns low weight to early time LI values, but α_N for early time (t) is very important to the cancer process. Since pulse ULLI was used for $t < 13$ weeks, impact of these ULLIs on risk could not be evaluated. 2) Time dependence in α_N derived from continuous ULLI does not significantly impact model predictions. 3) Site-to-site variation of α_N is at least 10-fold and has major impact on model calibration. Variation in tumor incidence data across sites is 10-fold. 4) Large differences in number of cells across nasal sites (see Table E-3), so averaging over sites is problematic. 5) TWA is also problematic because histologic changes, thickening of epithelium and metaplasia occur at later times for the higher dose and would affect replication rate.	Figures E-1, E-2, E-3**;** Subramaniam et al. (2008); Section E.3.2.3
Steady-state flux estimates are not affected by airway and tissue reconfiguration due to long-term dosing.	Histopathologic changes not likely to be rate-limiting factors in dosimetry.	1) Thickening of epithelium and squamous metaplasia occurring at later times for the higher dose (Kimbell et al. 1997b) will reduce tissue flux. Not incorporated in model. 2) These effects will push regions of higher flux to more posterior regions of respiratory tract. Likely to affect calibration of rat model. Uncertainty not evaluated quantitatively. 3) Calibration of PBPK model for DPXs was seen to be highly sensitive to tissue thickness.	Subramaniam et al. (2008); Cohen-Hubal et al. (1997); Klein et al. (2010).

Table E-1. Evaluation of assumptions and uncertainties in the CIIT model for nasal tumors in the F344 rat (continued)

Assumptions, approach, and characterization of input data in model	Rationale for assumption/approach	EPA evaluation	Further elaboration of evaluation[a]
TWA α_N(flux) rises above baseline levels only at cytolethal dose. Above such dose, α_N(flux) rises sharply due to regenerative proliferation.	Variability in α_N(flux) is partly represented by also considering hockey-stick (threshold in dose) when TWA indicates J-shape (inhibition of cell division) description of α_N(flux).	1) Uncertainty and variability in α_N were quantitatively evaluated to be large. In addition, there are several qualitative uncertainties in characterization of α_N(flux) from LI. 2) Several dose-response shapes, including a monotonic increasing curve without a threshold, were considered in order to adequately describe highly dispersed cell replication data. This has substantial impact on low dose risk.	Figures E-1, E-2, E-3, E-4, E-5; Subramaniam et al. (2008); Section E.3.2
Dose response for α_I was obtained from α_N, assuming ratio (α_I /α_N) to be a two-parameter function of flux (see Figures 5-7, 5-9). Parameters were estimated by optimizing model predictions against tumor incidence data.	(α_I /α_N) was >1.0 in line with the notion of I cells possessing a growth advantage over N cells. Satisfies Occam's razor principle (Conolly et al., 2009).	1) α_I /α_N in CIIT modeling is <1.0 (growth disadvantage) for higher flux values and is >1.0 only at lower end of flux range in model (see Figure 5-9). 2) Since there are no data to inform α_I, sensitivity of risk estimates to various functional forms was evaluated. Risk estimates for the rat were extremely sensitive to alternate biologically plausible assumptions for α_I(flux) and varied by many orders of magnitude at ≤1 ppm, including values lower than baseline risk. All these models described tumor incidence data and cell replication and DPX data equally well.	Figures D-2, E-5, E-6; Subramaniam et al. (2008); Crump et al. (2009, 2008); Section E.3.3
Death rate of I cells is assumed equal to division rate of N cells i.e. β_I(flux) = α_N(flux).	Based on homeostasis (α_N = β_N) and assumption that formaldehyde is equally cytotoxic to N cells and I cells. Satisfies Occam's Razor principle (Conolly et al., 2009).	1) In general, data indicate I cells are more resistant to cytolethality and that ADH3 clearance capacity is greater in transformed cells. Therefore, plausibility of model assumption, that β_I = α_N, is tenuous. 2) Alternate assumption, β_I proportional to α_I, was examined. Risk estimates were extremely sensitive to assumptions on β_I (see Figure 5-12).	Subramaniam et al. (2008); Crump et al. (2009, 2008); Section E.3.3

Table E-1. Evaluation of assumptions and uncertainties in the CIIT model for nasal tumors in the F344 rat (continued)

Assumptions, approach, and characterization of input data in model	Rationale for assumption/approach	EPA evaluation	Further elaboration of evaluation[a]
DPX is dose surrogate for formaldehyde's mutagenic potential. DPX clearance is rapid and complete in 18 hours.	Casanova et al. (1994).	Half-life for DPX clearance in in vitro experiments on transformed cell lines was 7-times longer than estimated by Conolly et al. (2004, 2003) and perhaps 14-times longer with normal (nontransformed) human cells. Some DPX accumulation is therefore likely. However, model calibration and dose response in rat was insensitive to this uncertainty. See section E.3 for effect on scale-up of model to humans.	Quievryn and Zhitkovich, (2000); Subramaniam et al. (2007); Section 3.6.6.3
Formaldehyde's mutagenic action takes place only while DPX's are in place.		DNA lesions may remain after DPX repair and incomplete repair of DPX can lead to mutations (Barker et al. 2005). There is some potential for formaldehyde-induced mutation after DPX clearance. Thus, it is possible that formaldehyde mutagenicity may be underrepresented in model. Could not quantitatively evaluate uncertainty (no data on clearance of secondary lesions).	Subramaniam et al. (2008); Section 4.3.3.3

[a]References stated here are in addition to Conolly et al. (2004, 2003).

Note: Risk estimates discussed in this table are for the F344 rat.

E.2. STATISTICAL METHODS USED IN EVALUATION

Parameters of the alternate models shown here were estimated by maximizing the likelihood function defined by the data (Cox and Hinkley, 1974). Such estimates are referred to as maximum likelihood estimates (MLEs). Statistical confidence bounds were computed by using the profile likelihood method (Crump, 2002; Cox and Oakes, 1984; Cox and Hinkley, 1974). In this approach, an asymptotic $100(1 - \alpha)\%$ upper (lower) statistical confidence bound for a parameter, β, in the animal cancer model is calculated as the largest (smallest) value of β that satisfies

$$2[L_{max} - L^*(\beta)] = x_{1-2\alpha} \quad \text{(E-1)}$$

where L indicates the likelihood of the rat bioassay data, L_{max} is its maximum value, $L^*(\beta)$ is, for a fixed value of β, the maximum value of the log-likelihood with respect to all of the remaining parameters, and $x_{1-2\alpha}$ is the $100(1-2\alpha)$ percentage point of the chi-square distribution with one degree of freedom. The required bound for a parameter, β, was determined via a numerical search for a value of β that satisfies this equation.

The additional risk is defined as the probability of an animal dying from an SCC by the age of 790 days, in the absence of other competing risks of death, while exposed throughout life to a prescribed constant air concentration of formaldehyde, minus the corresponding probability in an animal not exposed to formaldehyde. The MLE of additional risk is the additional risk computed using MLEs of the model parameters.

The method described above for computing profile likelihood confidence bounds cannot be used with additional risk because additional risk is not a parameter in the cancer model. Instead, an asymptotic $100(1 - \alpha)\%$ upper (lower) statistical confidence bound for additional risk was computed by finding the parameter values that presented the largest (smallest) value of additional risk, subject to the inequality

$$2[L_{max} - L] \leq x_{1-2\alpha} \quad \text{(E-2)}$$

being satisfied, with the resulting value of additional risk being the required bound. This procedure was implemented through use of penalty functions (Smith and Coit, 1995). For example, the profile upper bound on additional risk was computed by maximizing the "penalized added risk," defined as (*additional risk – penalty*), where

$$penalty = W \times \{[(L_{max} - L) - x_{1-2\alpha}/2]^+\}^2 \quad \text{(E-3)}$$

and $[]^+$ equals the quantity in the brackets whenever it is positive and zero otherwise. The multiplicative weight, *W*, was selected by trial and error so that the final solution satisfied the following equation sufficiently well.

$$2(L_{max} - L) = x_{1-2\alpha} \quad \text{(E-4)}$$

The computer code was written in Microsoft Excel® 2002 SP3 Visual Basic. Either the regular Excel Solver or the Frontline Systems Premium Solver® was used to make the required function optimizations. Computation of confidence bounds was highly computationally intensive, and, consequently, confidence bounds were computed only for selected parameters in selected runs. For select cases, the bootstrap method was also used to calculate confidence bounds in order to confirm their accuracy. Values so calculated were found to be in agreement with those calculated by using the likelihood method.

E.3. PRIMARY UNCERTAINTIES IN BBDR MODELING OF THE F344 RAT DATA

In their evaluation, Subramaniam et al. (2007) first attempted to reproduce the Conolly et al. (2003) results under similar conditions and assumptions as employed in their paper, which included the assumption that tumors were rapidly fatal. Figure 5-12 in Chapter 5 shows the results for this case. The predicted probabilities shown in this figure were obtained by Subramaniam et al. (2007) by using the source code made available by Dr. Conolly. These are compared with the best-fitting model and plotted against the Kaplan-Meier (KM) probabilities. Although the results are largely similar, there are some residual differences, and these are detailed in Subramaniam et al. (2007).

Given the scope of issues to examine for the uncertainty analyses, the evaluation proceeded in stages. First, the Hoogenveen et al. (1999) solution was replaced by one that is valid for a model with time varying parameters (Crump et al., 2005; first entry in Table E-1), and tumors found at scheduled sacrifices were assumed to be incidental rather than fatal (second entry in Table E-1). Second, weekly averaged solutions for DPX concentration levels were used instead of hourly varying solutions (predicted by a PBPK model). The log-likelihood values and tumor probabilities remained essentially unchanged. Upon quantitative evaluation, these factors, although important from a methodological point of view, were not found to be major determinants of either calibration or prediction of the model for the F344 rat data (Subramaniam et al., 2007).

Following Georgieva et al. (2003), Subramaniam et al. (2007) used the DPX clearance rate constant obtained from in vitro data instead of the assumption in Conolly et al. (2003) that

all DPXs cleared within 18 hours (Subramaniam et al., 2007). With this revision, weekly average DPX concentrations were larger than those in Conolly et al. (2003) by essentially a constant ratio equal to 4.21 (range of 4.12–4.36) when averaged over flux bin and exposure concentrations. Accordingly, cancer model fits to the rat tumor incidence data using the two sets of DPX concentrations (everything else remaining the same) provided very similar parameter estimates, except that the parameter KMU_{rat} in eq D-1 (and eq D-4) (Appendix D) was 4.23 times larger with the Conolly et al. (2003) DPX concentrations. In other words, the product KMU × DPX remained substantially unchanged. However, it is important to note that the different clearance rate does significantly impact the scale-up of the two-stage clonal growth model to the human since the parameter KMU_{human} is not estimated separately but related to KMU_{rat} (see eq D-4).

After making the above modifications, the impact of the other uncertainties in Table E-1 were examined. Of the issues in Table E-1, the following uncertainties had large impacts on the modeling of the F344 rat data, and will be discussed in considerably more detail:

1. use of historical controls,
2. uncertainty and variability in characterizing cell replication rates from the labeling data, and
3. uncertainty in model specification of initiated cell kinetics.

E.3.1. Sensitivity to Use of Historical Controls

E.3.1.1. *Use of Historical Controls*

Conolly et al. (2003) combined the historical controls arising from the entire NTP database of bioassays. Tumor and survival rates in control groups from different NTP studies are known to vary due to genetic drift in animals over time and differences in laboratory procedures, such as diet, housing, and pathological procedures (Haseman, 1995; Rao et al., 1987). In order to minimize extra variability when historical control data are used, the current NTP practice is to limit the historical control data, as far as possible, to studies involving the same route of exposure and to use historical control data from the most recent studies (Peddada et al., 2007).

Bickis and Krewski (1989) analyzed 49 NTP long-term rodent cancer bioassays and found a large difference in determinations of carcinogenicity, depending on the use of historical controls with concurrent control animals. The historical controls used in the CIIT modeling controls came from different rat colonies and from experiments conducted in different laboratories over a wide span of years, so it is clearly problematic to assume that background

rates in these historical control animals are the same as those in the concurrent control group. There are considerable differences among the background tumor rates of SCCs in all NTP controls (13/7,684 = 0.0017), NTP inhalation controls (1/4,551 = 0.0002), and concurrent controls (0/341 = 0.0). The rate in all NTP controls is significantly higher than that in NTP inhalation controls (p = 0.01, Fisher's exact test). Given these differences, the inclusion of any type of historical controls is problematic and is thought to have limited value if these factors are not controlled for (Haseman, 1995).

E.3.1.2. *Influence of Historical Controls on Model Calibration and on Human Model*

To investigate the effect of including historical controls in the CIIT model, the analyses in Subramaniam et al. (2007) were conducted by using the following sets of data for controls (the fraction of animals with SCCs is denoted in parentheses):

a) only concurrent controls (0/341),

b) concurrent controls plus all the NTP historical control data used by Conolly et al. (2003) (13/8,031),

c) concurrent controls plus data from historical controls obtained from NTP inhalation studies (1/4,949) (NTP, 2005).[3]

The results of the evaluation are shown in Table E-2. For these analyses, the same normal cell replication rates and the same relationship (see eq D-2 in Appendix D) between initiated cell and normal cell replication rates as used in Conolly et al. (2003) were used. In all cases, weekly averaged values of DPX concentrations were used. Model fits to the tumor incidence data were similar in all cases to that shown in Figure 5-12 (see Subramaniam et al. [2007] for a more complete discussion). The biggest influence of the control data was seen to be on the estimated basal mutation rate in rats, $\mu_{Nbasal(rat)}$, which, in turn, influences the estimated mutation effect in humans through eq D-4 (Appendix D). α_{max} was also seen to be a sensitive parameter and is discussed later. See Subramaniam et al. (2007) for other parameters in the calibration.

[3] Three animals in the inhalation historical controls were diagnosed with nasal SCC. Of these, two of the tumors were determined to have originated in tissues other than the nasal cavity upon further review (Dr. Kevin Morgan and Ms. Betsy Gross Bermudez, personal communication). These two tumors were therefore not included on the advice of Dr. Morgan. See Subramaniam et al. (2007) for more details.

Table E-2. Influence of control data in modeling formaldehyde-induced cancer in the F344 rat

Case	A	D	B	E	C	F
Control animals (combined with concurrent controls)	**All NTP historical[a]**	**All NTP historical[a]**	**NTP inhalation historical[a]**	**NTP inhalation historical[a]**	**Concurrent only[a]**	**Concurrent only[a]**
Cell replication dose response	**J-shaped**	**Hockey stick**	**J-shaped**	**Hockey stick**	**J-shaped**	**Hockey stick**
Log-likelihood	−1692.65	−1693.68	−1,493.21	−1,493.35	−1,474.29	−1,474.29
μ_{Nbasal}	1.87×10^{-6}	2.12×10^{-6}	7.32×10^{-7}	9.32×10^{-7}	0.0	0.0
KMU	1.12×10^{-7}	0.0	6.84×10^{-7}	6.18×10^{-7}	1.20×10^{-6}	1.20×10^{-6}
KMU/μ_{Nbasal}	0.06 (0.0, 0.40)	0.0 (0.0, 0.25)	0.94 (0.26, 6.20)	0.66 (0.2, 5.20)	∞ (0.42, ∞)	∞ (0.41, ∞)
α_{max}	0.045 (0.029, 0.045)	0.045 (0.029, 0.045)	0.045 (0.026, 0.045)	0.045 (0.027, 0.045)	0.045 (0.027, 0.045)	0.045 (0.027, 0.045)

[a]Values in parentheses denote lower and upper 90% confidence bounds.

Source: Adapted from Subramaniam et al. (2007).

The ratio KMU/μ_{Nbasal} is of particular interest because extrapolation to human in Conolly et al. (2004) assumed its invariance as given by eq D-4 (Appendix D). Now, μ_{Nbasal} in the human is estimated independently by fitting a scaled-up version of the two-stage model to human baseline rates of tumor incidence. Thus, a decrease in the value of μ_{Nbasal} estimated in the rat modeling increases the formaldehyde-induced mutational effect in the human.

The MLE of $KMU_{rat}/\mu_{Nbasal(rat)}$ is zero in Conolly et al. (2003). However, in the various cases examined in Subramaniam et al. (2007) it takes a range of values from 0 to 0.9 mm^3/pmol and undefined (or infinite, when $\mu_{Nbasal} = 0$). The 95% upper confidence bound on this ratio ranges from 0.25–6.2 (these values would be four times larger had the Conolly et al. [2003] DPX concentrations been used) to infinite. Thus, the extrapolation to human risk by using the approach in Conolly et al. (2004) becomes particularly problematic when only concurrent controls are used, because then the mutational contribution to formaldehyde-induced risk in humans becomes unbounded. This issue will be discussed again toward the end of the discussion on historical controls.

It may be noted, however, that absence of tumors in the limited number of concurrent animals does not imply that the calculation will necessarily predict a zero background probability of tumor (i.e., a parameter estimate of $\mu_{Nbasal} = 0$). Subramaniam et al. (2007) observed such a counterexample estimate for μ_{Nbasal} in simulations involving the alternate dose-response curves for α_N and α_I that are discussed in Section E.3.4. Nonetheless, when $\mu_{Nbasal} = 0$, an upper bound for μ_{Nbasal} using the concurrent controls could be inferred. Accordingly, the 90% statistical lower confidence bound on the ratio KMU/μ_{Nbasal} is also reported in Table E-2. Such a value would of course provide a lower bound on risk by using this model and would therefore not be conservative.

Conolly et al. (2003) estimated KMU to be zero for both their hockey-stick and J-shape dose response models for cell replication. However, the estimate for the coefficient KMU (obtained using the solution of Crump et al. [2005]) is zero only for the case of the model with the hockey-stick curve for cell replication and with control data as used by Conolly et al. (2003). It is positive in all other cases and statistically significantly so in all cases in which either NTP inhalation control data or concurrent controls were used. With concurrent controls only and the J-shape cell replication model, the MLE estimate for KMU (1.2×10^{-6}) is larger than the statistical upper bound obtained by Conolly et al. (2003) (8.2×10^{-7}). It should also be kept in mind that the estimate would be about 4.2 times larger still had the Conolly et al. (2003) DPX model been used.

E.3.1.3. *Influence of Historical Controls on Dose-Response Curve*

Subramaniam et al. (2007) showed that inclusion of historical controls had a strong impact on the tumor probability curve below the range of exposures over which tumors were observed in the formaldehyde bioassays. As shown there, the MLE probabilities for occurrence of a fatal tumor at exposure concentrations below 6 ppm were roughly an order of magnitude higher when all the NTP historical controls were used, compared with MLE probabilities predicted when historical controls were drawn only from inhalation bioassays, and many orders of magnitude higher than MLE probabilities predicted when only concurrent controls were used in the analysis. (Note that this comparison should not be inferred to apply to upper bound risk estimates since there were many fewer concurrent than historical controls, so error bounds could be much larger in the case where concurrent controls were used.)

However, as shown by these authors, model fits to the tumor data in the 6–15 ppm exposure concentration range were qualitatively indifferent to which of these control data sets was used. This observation emphasizes the statistical aspect of the CIIT modeling—that significant interplay among the various adjustable parameters allows the model to achieve a good fit to the tumor incidence data independent of the control data used. On the other hand, the results in Subramaniam et al. (2007) show that changes in the control data affect parameter KMU, resulting in significantly different tumor predictions at lower exposure concentrations. Therefore, the strong influence of using all the NTP historical controls on the low-dose region of the time-to-tumor curves presented in Subramaniam et al. (2007) suggests that large uncertainties may arise in extrapolating to both human and rat (in the low-dose region) from such considerations alone.

E.3.1.4. *Problem Including 1976 Study for Inhalation Historical Control*

A crucial point needs to be noted with regard to the use of inhalation NTP historical controls (i.e., cases B and E) in the two-stage clonal growth modeling. The single relevant tumor in the NTP inhalation studies came from the very first NTP inhalation study, dated 1976, and the animals in this study were from Hazelton Laboratories, whereas the concurrent animals were all from Charles River Laboratories. Similar problems arise with inclusion of several other NTP inhalation studies. As mentioned before, genetic and other time-related variation can lead to different tumor and survival rates, and in general it is recommended that use of historical controls be restricted to the same kind of bioassays and to studies within a 5–7 year span of the concurrent animals (Peddada et al., 2007). Thus, it is problematic to assume that the tumor in the 1976 NTP study is representative of the risk of SCCs in the formaldehyde bioassays. Even if it were appropriate to consider the 1976 study, this leads to the unstable situation in which,

despite all of the "upstream" mechanistic information used to construct the BBDR model, the only piece of data that might keep the model predictions of human risk bounded is a single tumor found among several thousand rats from NTP bioassays (Crump et al., 2008). In summary, although it can be argued that the rate of SCCs among the controls in the rat bioassay is probably not zero, it is also problematic to assume that this rate can be adequately represented by the background rate in NTP historical controls or even in NTP inhalation historical controls.

E.3.1.5. *Effect of Control Data on MOA Inferences*

Subramaniam et al. (2007) also examined the contribution of the DPX component (which represents the directly mutagenic potential of formaldehyde in the model) to the calculated tumor probability, choosing for their case study the optimized models that use the NTP inhalation control data. In the range of exposures where tumors were observed (6.0–15.0 ppm), the DPX term was found to be responsible for 58–74% of the added tumor probability. Below 6.0 ppm the estimated DPX contribution was extremely sensitive to whether the hockey-stick shape or J-shape was used to characterize the dose response for cell replication, and varied between 2% and 80%.

The CIIT BBDR cancer modeling has contributed to the weight-of-evidence process in various formaldehyde risk assessment efforts and papers by lending weight to the argument that the direct mutations induced by formaldehyde are relatively irrelevant compared to the importance of cytotoxicity-induced cell proliferation in explaining the observed tumorigenicity in rodent bioassays and in projecting those observations to human exposures (Conolly et al., 2004, 2003; Slikker et al., 2004; Bogdanffy et al., 2001, 1999; Conolly, 1995). The reanalyses in Subramaniam et al. (2007) (in particular, the results in the above paragraph) indicate that, if the CIIT mathematical modeling were utilized to inform this debate, it would in fact indicate the contrary—that a large contribution from formaldehyde's mutagenic potential may be needed to explain formaldehyde carcinogenicity. This discussion is resumed in the context of uncertainties in model specification for initiated cells.

E.3.2. Characterization of Uncertainty-Variability in Cell Replication Rates

E.3.2.1. *Dose-Response for α_N as Used in the CIIT Clonal Growth Modeling*

Monticello et al. (1996, 1991) used unit length labeling index (ULLI) to quantify cell replication within the respiratory epithelium. ULLI is a ratio between a count of labeled cells and the corresponding length (in millimeters) of basal membrane examined, whereas the per-cell labeling index (LI) is the ratio of labeled cells to all epithelial cells, in this case, along some length of basal membrane and its associated layer of epithelial cells. Monticello et al. (1996,

1991) published ULLI values averaged over replicate animals for each combination of exposure concentration, exposure time, and nasal site. These values are plotted in Figure E-1.

In order to utilize the ULLI data in clonal growth modeling, ULLI needed to be related to LI, and thereby to cell replication rate (α_N) of normal cells. Conolly et al. (2003) adopted the following procedure in using these values (Subramaniam et al., 2008):

1. The injection labeled ULLI data were first normalized by the ratio of the average minipump ULLI for controls to the average injection labeled ULLI for controls.
2. Next, these ULLI average values were weighted by the exposure times in Monticello et al. (1996, 1991) and averaged over the nasal sites. Thus, the data were combined into one TWA for each exposure concentration.
3. LI was linearly related to the measured ULLI by using data from a different experiment (Monticello et al., 1990) where both quantities had been measured for two sites in the nose.
4. Cell replication rates of normal cells (α_N) were then calculated as $\alpha_N = (-0.5/t)\log(1 - LI)$ (Moolgavkar and Luebeck, 1992), where LI is the labeling index and t is the period of labeling.
5. This was repeated for each exposure concentration of formaldehyde, resulting in one value of α_N for each exposure concentration.
6. Correspondingly, for a given exposure concentration, the steady-state formaldehyde flux into tissue, computed by CFD modeling, was averaged over all nasal sites. Thus, the α_N(flux) constructed by Conolly et al. (2003) consisted of a single α_N and a single average flux for each of six exposures.

This yielded a J-shaped dose-response curve for cell replication (when viewed on a nontransformed scale for α_N), as shown in Figure D-1 (Appendix D) for the full range of flux values used in their modeling. The authors also considered a hockey-stick threshold representation of their J-shaped curve for α_N in order to make a health-protective choice, and the differences between the two can be seen from the insets in Figure D-1. In these curves, the cell replication rate is less than or the same as the baseline cell replication rate at low formaldehyde flux values. The shape of the dose-response curve for cell replication as characterized in Conolly et al. (2003) is seen as representing regenerative cell proliferation secondary to the cytotoxicity of formaldehyde (Conolly, 2002). Considerable uncertainty and variability, both quantitative and qualitative, exist in the use and interpretation of these labeling data for characterizing a dose response for cell replication rates. The primary issues are discussed here. Unlike the preceding sections, these have largely not been published elsewhere, so more details are provided.

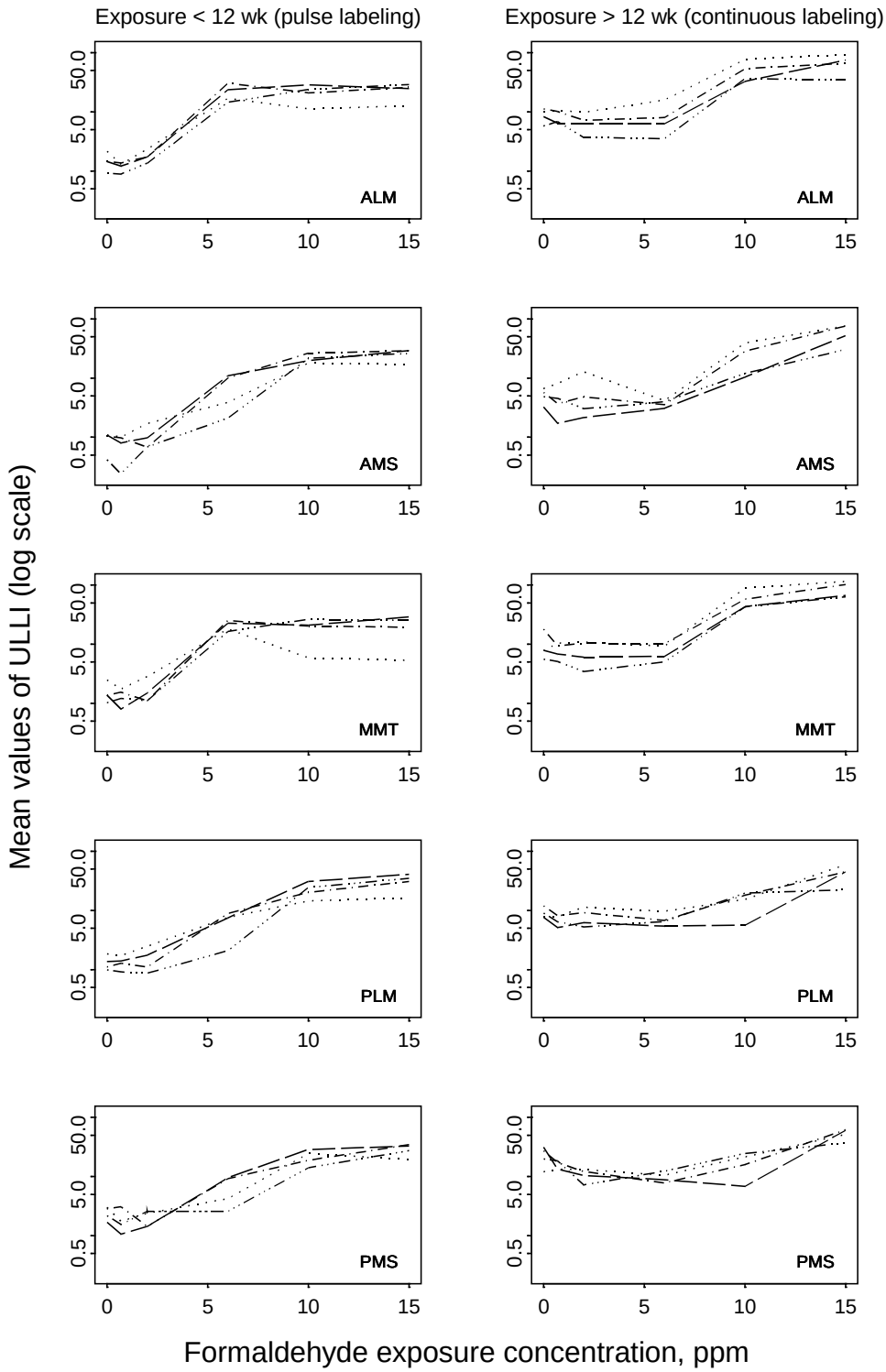

Figure E-1. ULLI data for pulse and continuous labeling studies.

Note: Data are from pulse labeling study, left-hand side, at 1–42 days of exposure and from the continuous-labeling study, right-hand side, at 13–78 weeks of exposure for five nasal sites ALM, AMS, MMT, PLM, and posterior mid septum [PMS]). Within each graph, lines with more breaks correspond to shorter exposure times. Data source: Monticello et al. (1996, 1991).

E.3.2.2. *Time Variability in Labeling Data*

E.3.2.2.1. ***Short-time exposure effects on cell replication.***

Figure E-1 shows the site and time variation in the raw unit-length labeling index (ULLI) data for 1 day to 78 weeks of exposure duration. The temporal variation in ULLI is quite different between the "early time" (left panel) and "later time" (right panel) and these early-time effects may be quite important to the cancer modeling. At the earliest times in the left panel, the data show an increased trend in labeling at 2 ppm for the sites anterior lateral meatus (ALM), anterior medial septum (AMS), posterior lateral meatus (PLM), and medial maxilloturbinate (MMT) relative to control. Such an increase is generally indicated for low flux values also for the 13-week exposure time. This can be seen in the dose-response plotted as a function of flux in Figure E-4.

The early times would be important if, say, repeated episodic exposures were considered, where adequate time has not elapsed for adaptive effects to take place. Such an exposure scenario may be the norm in the human context. In the CIIT cancer modeling, the LI was weighted by exposure time. As a consequence, the contribution of the early-time labeling data is minimized in their modeling.

E.3.2.2.2. ***Uncertainty due to combining pulse and continuous labeled data.***

The formula used for obtaining α_N from LI in Conolly et al. (2003) was due to Moolgavkar and Luebeck (1992) who derived this formula for continuous LI, cautioning that it is not applicable for pulse labeled data. However, Conolly et al. (2003) applied this formula to the injection (pulse) labeled data also. Such an application is problematic because 2-hour pulse labeled data represent the pool of cells in S-phase rather than the rate at which cells are recruited to the pool, and because the baseline values of α_N obtained in this manner from both data sets differ considerably. As such, we are not aware of any reasonable manner to derive cell replication rates from these pulse data without acquisition of data at additional time points. Because of these problems in incorporating the pulse-labeled data, further quantitative analysis of cell replication rates is restricted in this document to the continuous labeled data (Monticello et al., 1996), which do not include measurements made before 13 weeks of exposure. It is unfortunate that the continuous labeled data do not include any early measurements.

E.3.2.3. *Site and Time Variability in Derived Cell Replication Rate*

In the remainder of this section, the factors that are considered in order to represent the uncertainty and variability in the cell replication data when developing alternate dose-response curves for α_N(flux) will be elaborated.

The ULLI data for individual animals were provided by CIIT, which were transformed to LI values using the linear relationship from step 3 in Section E.3.2.1. For these replicate data, cell replication rates of normal cells (α_N) were then calculated as $\alpha_N = (-0.5/t)\log(1 - LI)$ as in Step 4. Figure E-2 (adapted from Subramaniam et al., 2008) shows the variability in α_N due to replicated animals, exposure times, and nasal sites in the continuous labeled data obtained by Monticello et al. (1996). In this figure, log α_N versus site-specific flux are plotted for six sites and four exposure times for four to six replicate animals in each case. (The mean ULLI over these replicates were shown in Figure E-1 for each site and time as a function of exposure concentration.) It needs to be noted that these nasal sites differ considerably in the number of cells estimated at these locations as shown in Table E-3. Each point in Figure E-2 represents data from a single site for a single animal at a given time. For comparison, the α_N(flux) in Conolly et al. (2003) is also plotted in this figure at their averaged flux values (filled circles). For flux >9,340 pmol/mm^2-hour, Conolly et al. (2003) extrapolated this empirically derived α_N(flux) by using a scheme discussed in Appendix D (see Section D.5) on the upward extrapolation of cell replication rate. The curves shown connecting the filled circles in the figure represent their linear interpolation (long dashes) between the six points. Their linear extrapolation for flux value >9,340 pmol/mm^2-hour is also shown (short dashes). Note that the linear interpolation and extrapolation are shown transformed to a logarithmic scale in this plot.

As discussed, the raw labeling data plotted in Figure E-1 indicates considerable temporal variability. In Figures E-3, fitted dose-response curves showing $\log_{10}(\alpha_N)$ versus flux with simultaneous confidence limits separately for each time point for two of the largest sites in Table E-3 (ALM and PLM) are plotted for the continuous labeled data. Note that flux levels are different at each site. Simple polynomial models in flux (as a continuous predictor), with time included as a factor (i.e., a class or indicator variable, τ_i representing the effect of the ith time) were used as follows:

$$\log(\alpha_N) = a + b \times flux + c \times flux^2 + d \times flux^3 + \tau_i \qquad \text{(E-5)}$$

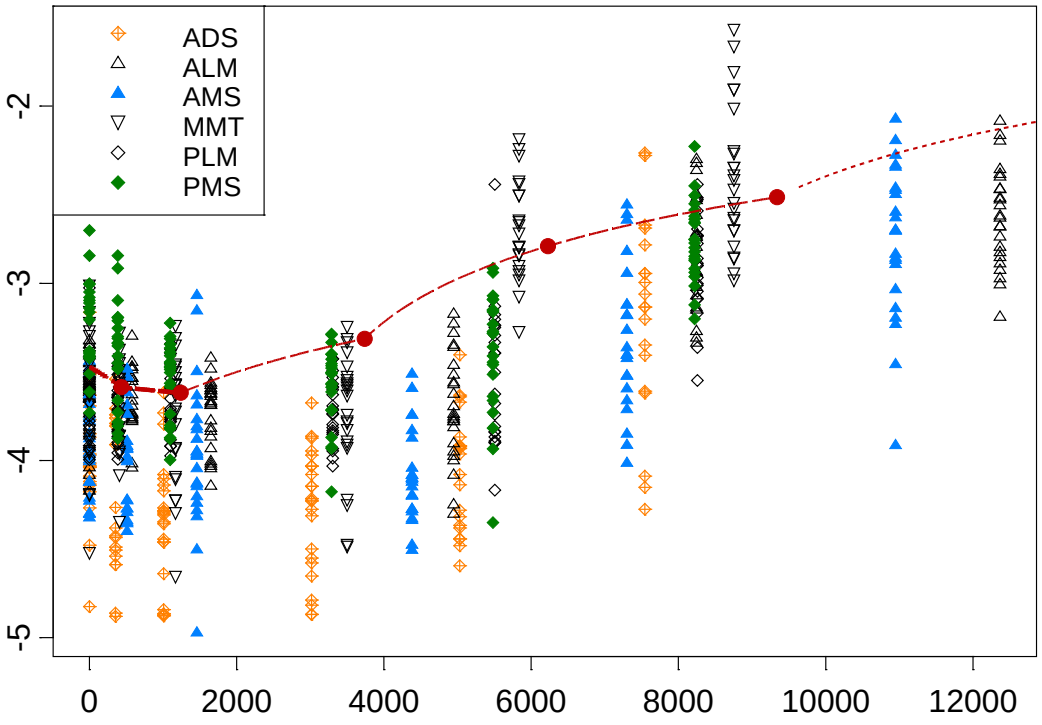

Figure E-2. Logarithm of normal cell replication rate α_N versus formaldehyde flux (in units of pmol/mm^2-hour) for the F344 rat nasal epithelium.

Note: Values were derived from continuous unit length labeled data obtained by Monticello et al. (1996) for four to six individual animals at all six nasal sites (legend, sites as denoted in original paper) and four exposure durations (13, 26, 52, 78 weeks). Each point represents a measurement for one rat, at one nasal site, and at a given exposure time. Filled red circles: α_N(flux) used in Conolly et al. (2003) plotted at their averaged flux values (see text for details). Long dashed lines: their linear interpolation between points. Short dashed line: their linear extrapolation for flux value >9,340 pmol/mm^2-hour (see Figure D-1 for full range of extrapolation). Linear interpolation/extrapolation is shown with Y-axis transformed to logarithmic scale.

Source: Subramaniam et al. (2008).

Table E-3. Variation in number of cells across nasal sites in the F344 rat

Nasal site	No. of cells
Anterior lateral meatus	976,000
Posterior lateral meatus	508,000
Anterior mid septum	184,000
Posterior mid septum	190,000
Anterior dorsal septum	128,000
Anterior medial maxilloturbinate	104,000

Note: Mean number of cells in each side of the nose of control animals.

Source: Monticello et al. (1996).

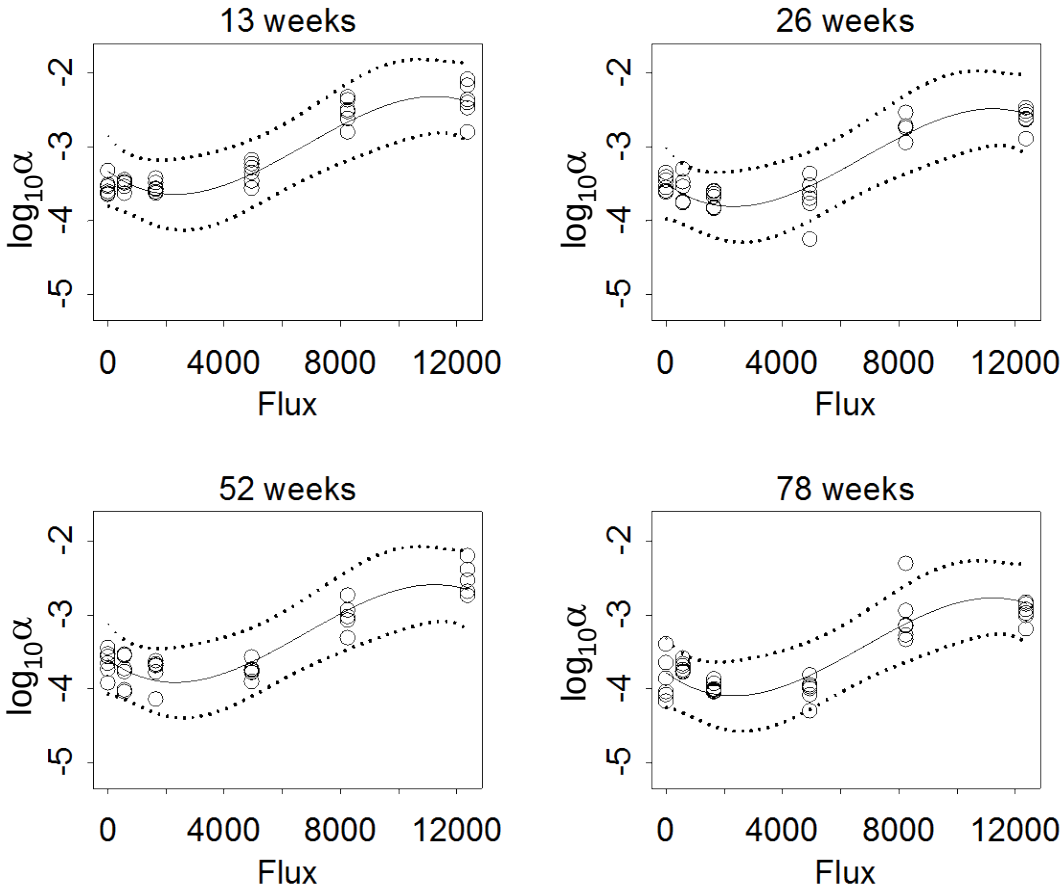

Figure E-3A. Logarithm of normal cell replication rate versus formaldehyde flux with simultaneous confidence limits for the ALM.

Source: Subramaniam et al. (2008).

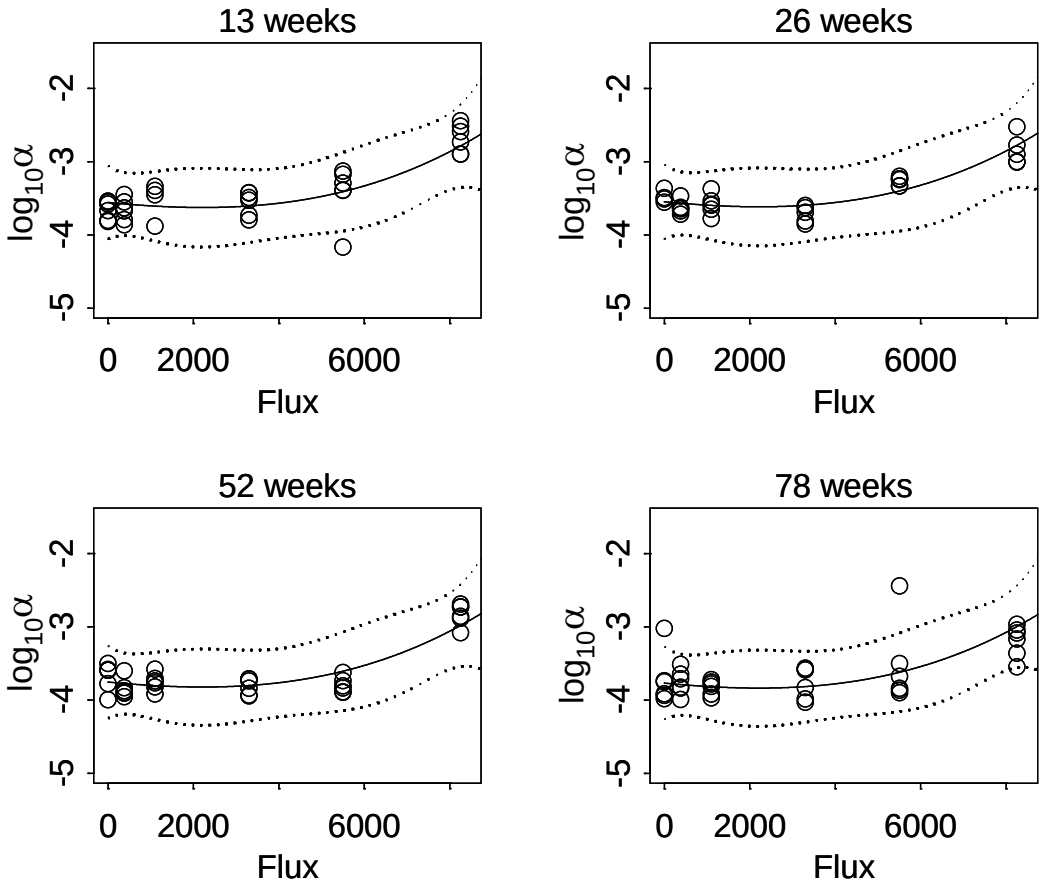

Figure E-3B. Logarithm of normal cell replication rate versus formaldehyde flux with simultaneous confidence limits for the PLM.

Source: Subramaniam et al. (2008).

The variability considered is that among animals and any measurement error as well as any other design-related components of error. Simultaneous 95% confidence limits for $\log(\alpha_N)$ were produced using Scheffe's method (Snedecor and Cochran, 1980). These 95% confidence limits span a range of 0.96 in $\log10(\alpha_N)$, or nearly a 10-fold range in median α_N. There is additional dispersion in these data that does not appear in Figures E-2 and E-3 for α_N, derived using the mean value of ULLI/LI; due to variation in the number of cells per mm basement membrane, the ratio of ULLI/LI had a spread of approximately ±25% (0.45 to 0.71, mean 0.60) among the eight observations considered in Monticello et al. (1990). Thus:

1. As suggested by Table E-3, and Figures E-2 and E-3, the shape of α_N(flux) in Conolly et al. (2003) is therefore likely to be very sensitive to how α_N is weighted and averaged over site and time.
2. Averaging of sites could significantly affect model calibration because of substantial nonlinearity in model dependence on α_N at the 10 and 15 ppm doses associated with high cancer incidence.
3. Monticello et al. (1996) found a high correlation between tumor rate and the ULLI weighted by the number of cells at a site. Therefore, considering these factors while regressing α_N against tissue dose would be important in the context of site differences in tumor response.
4. A further complexity arises because of histologic changes and thickening that occurs in the nasal epithelium over time in the higher dose groups (Morgan, 1997), factors that are likely to affect estimates of local formaldehyde flux, uptake, and replication rates (Subramaniam et al., 2008).

It is clear from Figures E-1 and E-3 that the time dependence in cell replication is significant. It would also be useful to examine if this time dependence affects the results of the time-to-tumor modeling and if early temporal changes in replication rate are important to consider because of the generally cumulative nature of cancer risk. The time window over which formaldehyde-induced cancer risk is most influenced is not known, but the time weighting used by Conolly et al. (2003) assigns a relatively low weight to labeling observed at early times compared with those observed at later time points. Finally, initiated cells are likely to be replicating at higher rates than normal cells as evidenced in several studies on premalignant lesions (Coste et al., 1996; Dragan et al., 1995; Rotstein et al., 1986). Therefore, LI data as an estimator of normal cell replication rate would be most reliable at early times when the mix of cells sampled include fewer preneoplastic or neoplastic cells.

The more relevant question, therefore, is whether the α_N(flux) derived in Conolly et al. (2003) by a TWA over all sites has an effect on low-dose risk estimates. Given the above uncertainties and variability not characterized in CIIT (1999) or in Conolly et al. (2003), it is important to examine whether additional dose-response curves that fit the cell replication data reasonably well have an impact on estimated risk. Such sensitivity analyses are carried out in the sections that follow.

E.3.2.4. *Alternate Dose-Response Curves for Cell Replication*

Clearly, a large number of alternative α_N(flux) can be developed. In conjunction with the other uncertainties, mainly the use of control data and alternative model structures for initiated cell kinetics, the number of plausible clonal growth models to be exercised soon require a

prohibitively large investment of time. Therefore, detailed analyses were restricted to a select set of biologically plausible choices of curves for α_N(flux), which would allow the identification of a range of plausible risk estimates (MLEs and statistical bounds). This discussion is further informed by recently published dose response data for cell replication (Meng et al., 2010), detailed in section F.2.3.

Six alternative equations for α_N were developed by regression analysis of the Monticello et al. (1996) ULLI data. The replicate data corresponding to the summary data presented in this paper were kindly provided to EPA by CIIT for further analyses. In each of these equations, α_N is expressed as a function of formaldehyde flux to nasal tissue (pmol/mm^2-hour) and, in one equation (see eq E-11) that explored time-dependence, the duration of exposure to formaldehyde in weeks. All the graphs use flux/10,000 for the X-axis, and the Y-axis expresses $\log_{10}\alpha_N$.

One source of uncertainty in the cell replication dose response in Conolly et al. (2003) is the large value of α_{max} (the cell replication rate corresponding to the upper end of the flux range at 15 ppm exposure) in the upward extrapolation from the empirically-determined α_N(flux) (see Figure D-1 and surrounding text in Section D.5). The optimal value of α_{max} was found by Conolly et al. (2003) to be 0.0435 hour^{-1}. As noted by the authors, an argument in support of this value is that it corresponds to the inverse of the fastest cell cycle times found in the literature. Since the model treats the induced replication rates as being time invariant, this means that cells in the high-flux region(s) divide at the highest cell turnover rate ever observed throughout most of an animal's life. This does not seem to be biologically plausible (Subramaniam et al., 2008).

Our analysis found that a 20% increase or decrease in the estimated value for α_{max} degraded the fit to the tumor incidence data considerably. Because of the interplay between the parameters estimated by optimization, this sensitivity of the model to α_{max} indicates that it is necessary to examine if other plausible values of α_{max} are also indicated by the data and to what extent low dose estimates of risk are influenced by the uncertainty in its value. The need for such an analysis is also indicated by Figure E-2. The value of α_{max} ($\log_{10}\alpha_{max} = -1.37$) in Conolly et al. (2003) is roughly an order of magnitude greater than the values of α_N(flux) at the highest flux levels in this figure. If the data pooled over all sites and times are to be used for α_N(flux), then, based solely on the trend in α_N(flux) in Figure E-2, it appears unlikely that α_N(flux) could increase up to this value of α_{max}. Visually, these empirically derived data collectively suggest that α_N versus flux could be leveling off rather than increasing 10-fold. Therefore, as an alternative to the approach taken in Conolly et al. (2003) of estimating α_{max} via likelihood optimization against the tumor data, regressions of the empirical cell replication data

in Figure E-2 were used to extrapolate α_N(flux) outside the range of observation (recognizing the uncertainty and model dependence that still results from extrapolating well outside the range of observed data).

In fitting dose-response curves to the cell replication data, a functional form was used that was flexible to allow a variety of monotonic and nonmonotonic shapes, with a parameter that determined the asymptotic behavior of the dose-response function. This allowed the extrapolation of α_N(flux) to higher flux levels by only relying on the empirical cell replication data. Then, there is no need for an adjustable parameter to be estimated by fitting to the tumor data. However, the plausible asymptotes obtained in this manner spanned a large range. In one case below, the asymptote suggested by the fit to the empirical cell replication data was judged to be abnormally high. In this case, the α_N versus flux curve was followed until the biological maximum of α_{max} (as given in Conolly et al. [2003]) was reached.

In three of the six regression models below, the data were restricted to the earliest exposure time (13 weeks) in Monticello et al. (1996) for which the cell proliferation rate (α_N) could be calculated. The interest in using only the 13-week exposure time arises from observations (Monticello et al., 1996, 1991) that at later times there were more frequent and severe histologic changes, which may have altered formaldehyde uptake and cell proliferation response. Consequently, given that the data in Monticello et al. (1991) for times earlier than 13 weeks could not be utilized as explained in Section E.3.2.3, the 13-week responses might better represent proliferation rates for use in a two-stage model of the cancer process than the rest of the Monticello et al.(1996) data.

Second, the LI data showed considerable variation among nasal sites, which may be related to the variation in tumor response among sites. Since the cell replication dose-response curves used in the cancer model represent all of the sites, it was attempted to include this variation by weighting the regression by the relative cell populations at risk at each of the sites. This was carried out for some of the models as stated below.

Finally, in one of the regression models, derived from fitting to all of the Monticello et al. (1996) ULLI data, time-dependence of α_N was considered by using weeks of exposure as a covariate. In this model, time was a regression (continuous) predictor, not a class variable, and its coefficient represents the change in $\log_{10} \alpha_N$ per week of exposure

The following regression models for α_N versus flux, denoted in the equations below as N1–N6 and shown in Figure E-4, as well as the hockey-stick and J-shaped curves used by Conolly et al. (2003), shown in Figure D-1, Appendix D, were next used as inputs to the clonal growth model for cancer:

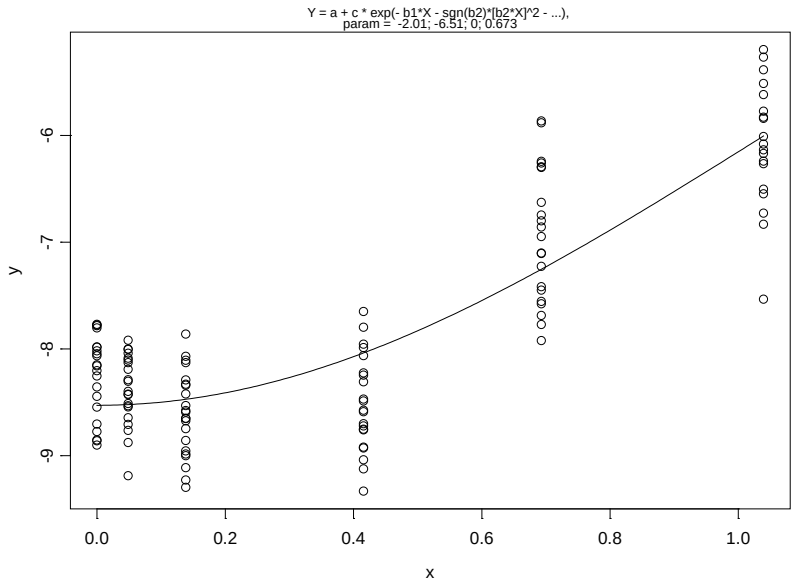

Figure E-4, N1. Various dose-response modeling of normal cell replication rate.

Note: See text for definitions of N1–N6. N1: Quadratic; monotone increasing in flux, derived from fit to all of the Monticello et al. (1996) ULLI data.

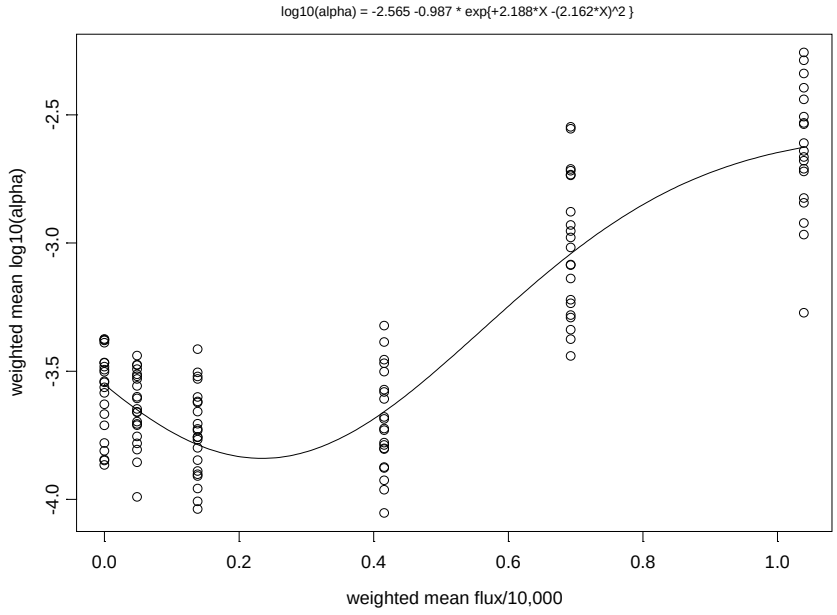

Figure E-4, N2. Various dose-response modeling of normal cell replication rate.

Note: See text for definitions of N1–N6. N2: Linear-quadratic; decreasing in flux for small values of flux, derived from fit to all of the Monticello et al. (1996) ULLI data.

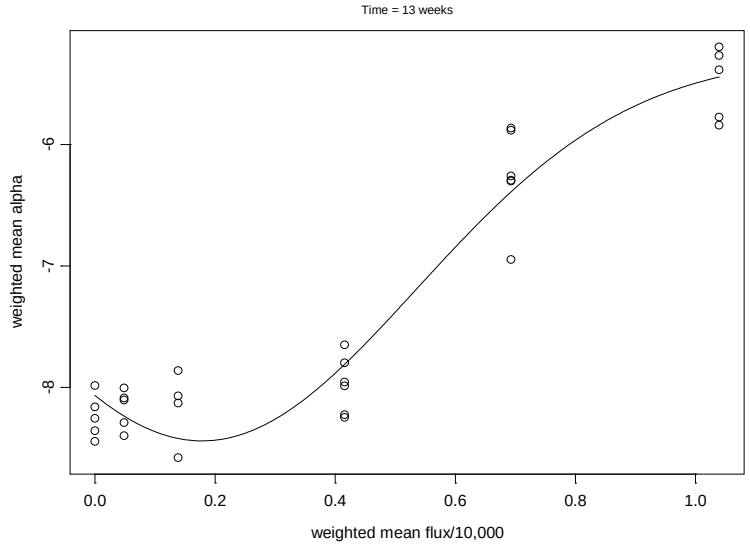

Figure E-4, N3. Various dose-response modeling of normal cell replication rate.
Note: See text for definitions of N1–N6. N3: Linear-quadratic; decreasing in flux for small values of flux, derived from fit to the 13-week Monticello et al. (1996) ULLI data, using average flux over all sites for a given ppm exposure and weighting regression by estimates of the numbers of cells at each of five sites.

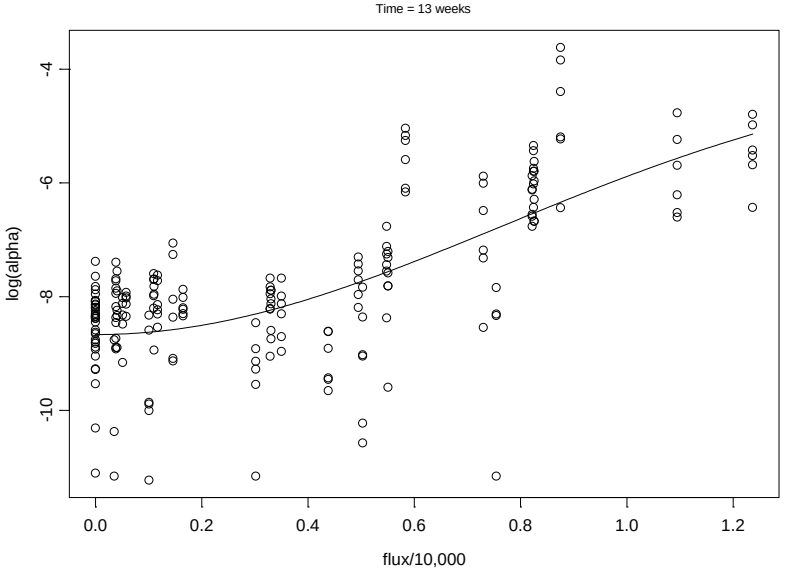

Figure E-4, N4. Various dose-response modeling of normal cell replication rate.
Note: See text for definitions of N1–N6. N4: Quadratic; monotone increasing in flux, derived from unweighted fit to 13-week Monticello et al. (1996) ULLI data.

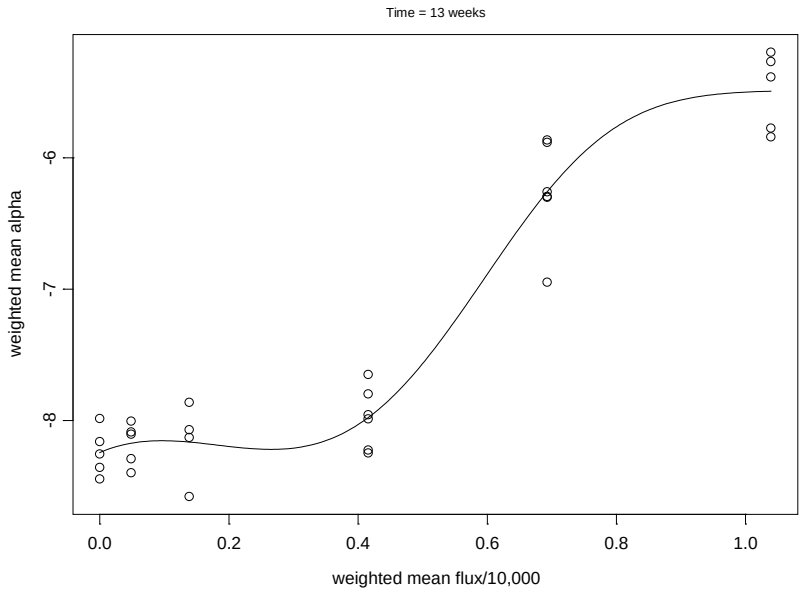

Figure E-4, N5. Various dose-response modeling of normal cell replication rate.

Note: See text for definitions of N1–N6. N5: Linear-quadratic-cubic; initially increasing slightly with increasing flux, then decreasing slightly, and finally increasing, derived from fit to 13-week Monticello et al. (1996) ULLI data, using average flux over all sites for a given ppm exposure and weighting regression by estimates of the numbers of cells at each of five sites.

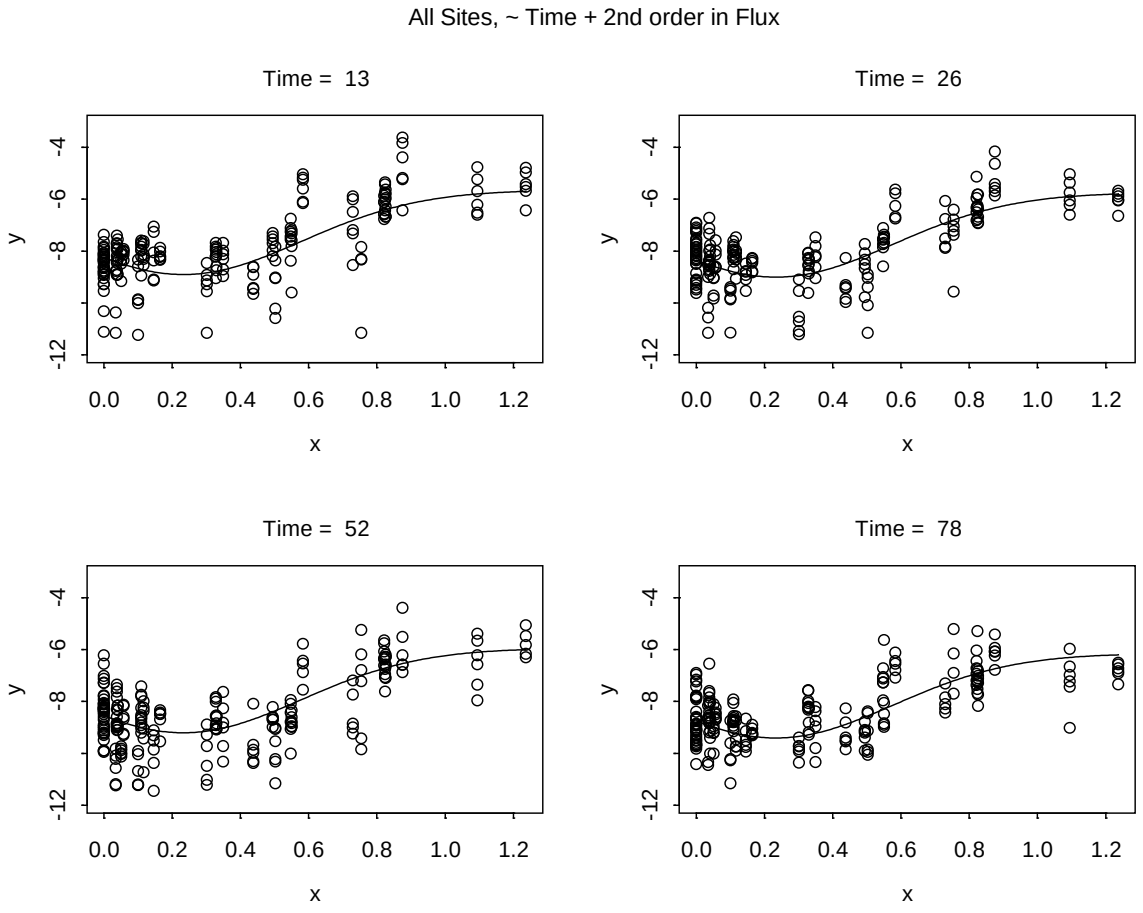

Figure E-4, N6. Various dose-response modeling of normal cell replication rate.

Note: See text for definitions of N1–N6. N6: Linear-quadratic-cubic; initially increasing slightly with increasing flux, then decreasing slightly, and finally increasing, derived from fit to all Monticello et al. (1996) ULLI data, using weeks of exposure as a covariate. In this model, time was a regression (continuous) predictor, not a class variable, and its coefficient represents the decrease in $\log_{10}$ α_N per week of exposure time.

N1: Quadratic; monotone increasing in flux, derived from fit to all of the Monticello et al. (1996) ULLI data.

$$\alpha_N = Exp\{-2.015 - 6.513 \times Exp[-(6.735\times10^{-4} \times flux)^2]\} \quad \text{(E-6)}$$

N2: Linear-quadratic; decreasing in flux for small values of flux, derived from fit to all of the Monticello et al. (1996) ULLI data.

$$\alpha_N = Exp\{-5.906 - 2.272 \times Exp[2.188\times10^{-4} \times flux - (2.162\times10^{-4} \times flux)^2]\} \quad \text{(E-7)}$$

N3: Linear-quadratic; decreasing in flux for small values of flux, derived from fit to the 13-week Monticello et al. (1996) ULLI data, using average flux over all sites for a given ppm exposure and weighting regression by estimates of the numbers of cells at each of five sites.

. $$\alpha_N = Exp\{-5.274 - 2.792 \times Exp[1.407\times10^{-4} \times flux - (1.986\times10^{-4} \times flux)^2]\} \quad \text{(E-8)}$$

N4: Quadratic; monotone increasing in flux, derived from unweighted fit to 13-week Monticello et al. (1996) ULLI data.

$$\alpha_N = Exp\{-3.858 - 4.809 \times Exp[-(9.293\times10^{-5} \times flux)^2]\} \quad \text{(E-9)}$$

N5: Linear-quadratic-cubic; initially increasing slightly with increasing flux, then decreasing slightly, and finally increasing, derived from fit to 13-week Monticello et al. (1996) ULLI data, using average flux over all sites for a given ppm exposure and weighting regression by estimates of the numbers of cells at each of five sites.

$$\alpha_N = Exp\{-5.488 - 2.755 \times Exp[-7.808\times10^{-5} \times flux + (2.349\times10^{-4} \times flux)^2 - (2.166\times10^{-4} \times flux)^3]\} \quad \text{(E-10)}$$

N6: Linear-quadratic-cubic; initially increasing slightly with increasing flux, then decreasing slightly, and finally increasing, derived from fit to all Monticello et al. (1996) ULLI data, using weeks of exposure as a covariate. In this model, time was a regression (continuous) predictor, not a class variable, and its coefficient represents the decrease in $\log_{10} \alpha_N$ per week of exposure time.

$$\alpha_N = Exp\{7.785\times10^{-3} \times (weeks) - 5.722 - 2.501 \times Exp[1.103\times10^{-4} \times flux - (7.223\times10^{-5} \times flux)^2 - (1.575\times10^{-4} \times flux)^3]\} \quad \text{(E-11)}$$

E.3.3. Uncertainty in Model Specification of Initiated Cell Replication and Death

E.3.3.1. *Biological Implications of Assumptions in Conolly et al. (2003)*

The results of a two-stage MVK model are extremely sensitive to the values for initiated cell division (α_I) and death (β_I) rates, particularly in the case of a sharply rising dose-response curve as observed of formaldehyde. The pool of cells used for obtaining the available LI data (Monticello et al., 1996, 1991) consists of largely normal cells with perhaps increasing numbers of initiated cells at higher exposure concentrations. As such there is no way of inferring the division rates of initiated cells in the nasal epithelium, either spontaneous (baseline) or induced by exposure to formaldehyde, from the available empirical data. Conolly et al. (2003) considered α_I(flux) as a function of α_N(flux) as given by eq D-2 in Appendix D. As shown in Figure D-1 (Appendix D), α_I is estimated in Conolly et al. (2003) to be very similar to α_N. That is, with eq D-2 assumed to relate α_I(flux) to α_N(flux), a J- or hockey-shaped dose-response curve for α_N(flux) necessarily results in a J or hockey shape for α_I(flux).

The J shape for the TWA α_N(flux) in Conolly et al. (2003) could plausibly be explained, as suggested by the examples in Conolly and Lutz (2004), by a mathematical superposition of dose-response curves describing the effects of the inhibition of cell replication by the formation of DPXs (Heck and Casanova, 1999) and cytotoxicity-induced regenerative replication (Conolly, 2002). However, as explained earlier, there is considerable uncertainty and variability, both qualitative and quantitative, in the interpretation of the LI data and in the derivation of *normal* cell replication rates from the ULLI data. While the TWA values of ULLI indicate a J-shaped dose response for some sites, as also concluded by Gaylor et al. (2004), this is not consistently the case for all exposure times and sites as discussed earlier. Notwithstanding this uncertainty and variability, and in the absence of data, the following essential questions have a significant impact on risk predictions and need resolution if the model structure in eq D-2 is to be used in a biologically based (or motivated) sense:

- Should mechanisms that might explain a J-shaped dose response for normal cell replication be expected to prevail also for initiated cells? An identical question can be posed for the hockey-stick-shaped curve which indicates a cytotoxicity-driven threshold in dose response.
- Would the formaldehyde flux at which the cell replication dose-response curve rises above its baseline be similar in value for both normal and initiated cells as inferred by the CIIT model in Figure D-1?

The next critical assumption in Conolly et al. (2003) was that made for β_I (the death rate of initiated cells), namely, $\beta_I(\text{flux}) = \alpha_N(\text{flux})$ (see eq D-3). The rationale for this assumption is explained by assuming formaldehyde to be equally cytotoxic to initiated and normal cells since the mechanism is presumed to be via its general chemical reactivity (Subramaniam et al. 2008). In essence, this assumption brings the cytotoxic action of formaldehyde to bear strongly on the parameterization of the CIIT model.

There are no data to evaluate the strength of these assumptions, so Subramaniam et al. (2008) studied the plausibility of various inferences that arise as a result of these assumptions. These inferences are only briefly listed here (see the paper for further discussion).

- For flux <27,975 pmol/mm^2-hour, $\alpha_I > \alpha_N$ (see Figures D-1 and D-2 of Appendix D). Qualitatively, this concept of a growth advantage is in line with data on epithelial and other tissue types with or without exposure to specific chemicals.
- For higher flux levels, however, the model indicates $\alpha_I < \alpha_N$ (see Figure D-2). There are no data to shed further light on this inference.
- At these higher flux levels, initiated cells in the model die at a faster rate than they divide, indicating the extinction of initiated cell clones in regions subject to these flux levels. There are no data indicating formaldehyde to have this effect.

In evaluating these inferences, Subramaniam et al. (2008) point to various data that indicate that initiated cells represent distinctly different cell populations from that of normal cells with regard to proliferation response (Ceder et al., 2007; Bull, 2000; Schulte-Hermann et al., 1997; Coste et al., 1996; Dragan et al., 1995), have excess capacity to clear formaldehyde and, in general, are considerably more resistant to cytotoxicity, and may already have altered cell cycle control. The resistance to toxicity is manifested variably as decreased ability of the toxicant to induce cell death or to inhibit cell proliferation compared to corresponding effects in normal cells. Therefore, the influence of formaldehyde on apoptosis likely differs between normal and initiated cells.

As concluded in Subramaniam et al. (2008), taken together, there is much data to suggest that inferring $\alpha_I < \alpha_N$ at cytotoxic formaldehyde flux levels is problematic and that death rates of initiated cells are likely to be very different from those of normal cells.

In the absence of data to indicate that eq D-2 and eq D-3 (in Appendix D) are biologically reasonable approaches to link the kinetics of initiated cells with those of normal cells, alternate model structures other than those represented by these relationships considered by Conolly et al. (2003) need to be explored, given that the two-stage model is extremely sensitive to α_I and β_I. Such an evaluation needs to primarily explore if the assumptions in eq D-2 and eq

D-3 significantly impact the intended use of the model, namely extrapolation to low-dose human cancer risk and the calculation of an upper bound on human risk. Any such alternate model structure needs to provide a good fit to the time-to-tumor data.

E.3.3.2. *Plausible Alternative Assumptions for α_I and β_I*

Therefore, in the additional sensitivity analysis presented here,

a) Initiated cell kinetics are considered to be independent of normal cells,
b) Initiated cell replication dose-response cannot take a J shape; this is motivated by the consideration that lower-than-baseline turnover rate represents an increased amount of DNA repair taking place, which may not be consistent with impaired DNA repair in initiated cells.

Thus, two alternatives were considered to eq D-2 for α_I(flux):

I1: $$\alpha_I = \gamma_1 \times [1 + exp(\gamma_2 / \gamma_3)] / \{1 + exp[-(flux - \gamma_2) / \gamma_3]\} \quad \text{(E-12)}$$

I2: $$\alpha_I = max[\alpha_I(\text{I1}), \alpha_{NBasal}] \quad \text{(E-13)}$$

Here γ_1, γ_2, and γ_3 are parameters estimated by fitting the cancer model to the rat bioassay data. In eq E-12, α_I increases monotonically with flux from a background level of γ_1 asymptotically up to a maximum value of $\gamma_1 \times [1 + Exp(\gamma_2 / \gamma_3)]$. The choice of this functional form in eq E-12 and eq E-13 was considered in order to be parsimonious while at the same time allowing for a flexible shape to the dose-response curve. The sigmoidal curve allows for the possibility of a slow rise in the curve at low dose and an asymptote.

Equation E-13 is a modification of eq E-12 that restricts the rate of division of initiated cells to be at least as large as the spontaneous division rate of unexposed normal cells. There is evidence to suggest (e.g., in the case of liver foci) that initiated cells have a growth advantage over normal cells, with or without exposure to specific chemicals (Ceder et al., 2007; Grasl-Kraupp et al., 2000; Schulte-Hermann et al., 1999; Coste et al., 1996; Dragan et al., 1995).

In addition, in most runs, an upper bound (α_{high}) is selected for both α_N and α_I. This value is assumed to represent the largest biologically plausible rate of cell division. Following Conolly et al. (2003), in most cases α_{high} is set equal to 0.045 hours^{-1}. If a value of α_I or α_N computed using one of the above formulas exceeded α_{high}, the value of α_{high} was used in the computation rather than the value obtained by using the formula.

As noted above, Conolly et al. (2003) set the rate of death for intermediate cells, β_I, equal to the division rate of normal cells, $\beta_I = \alpha_N$. On the other hand, apoptotic rates and cell

proliferation rates are thought to be coupled (Schulte-Hermann, 1999; Moolgavkar, 1994), so that death rates of initiated cells would rise concomitantly with an increase in their division rates (Grasl-Kraupp et al., 2000; Schulte-Hermann et al., 1999). Therefore, as an alternative to the Conolly et al. (2003) formulation, it is assumed that the death rate of intermediate cells is proportional to the division rate of intermediate cells.

$$\beta_I = \kappa_\beta \times \alpha_I \qquad \text{(E-14)}$$

where the constant of proportionality, κ_β, is an additional parameter to be estimated by optimization against the tumor incidence data. Such an assumption has also been made by other authors (Luebeck et al., 2000, 1995; Moolgavkar et al., 1993).

E.3.4. Results of Sensitivity Analyses on α_N, α_I, and β_I

E.3.4.1. *Further Constraints*

The number of models that might be constructed if all the possibilities listed above for α_N, α_I, *and* β_I are to be tried in a systematic manner clearly become exponential and daunting. (Optimally, it would have been desirable to elucidate the role of a specific modification while keeping others unchanged to determine risk.) Therefore, in order to carry out a viable sensitivity analysis while at the same time examining the plausible range of risks resulting from variations in parameters and model structures, various uncertainties were combined in any given simulation. By using the constraints described above (see eqs E-6 through E-13 and associated text) for α_I , β_I, and α_N, 19 models were obtained that provided similarly good fits to the time-to-tumor data (which in some cases contained only five dose groups).

However, for many of these models, the optimal α_I(flux) displayed a threshold in flux even when the model utilized for α_N(flux) was a monotonic increasing curve without a threshold (i.e., model N4 for α_N in Figure E-4). Indeed, if a thresholded dose-response curve was plausible for α_I based on arguments of cytotoxicity, then a threshold is all the more plausible for α_N, and such models are removed from consideration.

Secondly, the basal value of α_I was required to be at least as large as the basal value of α_N. Another constraint was placed on the baseline initiated cell replication rate. In the absence of formaldehyde exposure, α_I was not allowed to be greater than two or four times α_N, even if such models described the tumor data, including the control data, very well. There are some data that suggest that baseline initiated cells have a small growth advantage over normal cells, so a huge advantage was thought to be biologically less plausible.

Finally, since most of the SCCs in the rat bioassays occurred in rats exposed to the highest formaldehyde concentration (15 ppm), the data from this exposure level have a big impact on the estimated model parameters. In most runs that incorporated the 15 ppm data, the model appeared, based on inspection of the KM plots, to fit the 15 ppm data quite well but to fit the lower exposure data less well. Because of the high level of necrosis occurring at 15 ppm, it is possible that the data at this exposure may not be particularly relevant to modeling the sharp upward rise in the dose response at 6 ppm. Furthermore, the principal interest is in the predictions of the model at lower levels to which human populations may be exposed. Consequently, in order to improve the fit of the model at lower exposures, some of the alternative models were constructed with the 15 ppm data omitted.

E.3.4.2. *Sensitivity of Risk Estimates for the F344 Rat*

Figure E-5 contains plots of the MLE of additional risk computed for the F344 rat at formaldehyde exposures of 0.001, 0.01, 0.1, and 1 ppm for eight models. Two log-log plots are provided. For those models for which the estimates of additional risk are all positive, the additional risks are plotted (panel A), and, for those for which estimates of additional risk are negative, the negatives of additional risks are plotted (panel B). Only five dose groups were considered (i.e., 15 ppm data omitted) for models 8, 5, 15, and 16. Figure E-6 shows the dose-response curves for α_N and α_I for these eight cases (panels A and B corresponding to those in Figure E-5). The specification and estimated values of the parameters for these models are provided in Tables E-4 and E-5. The primary results are as follows:

1. Among the models considered, negative values for additional risk can arise only in models in which the dose response for normal cells is J shaped. Thus, all of the models with negative dose responses for risk have J-shaped dose responses for normal cells. However, the converse is not necessarily true as may be noted from model 8. This model has both a positive dose response for risk and a J-shaped dose response for normal cells. In this case, the strong positive increase in response of initiated cells at low dose was sufficient to counteract the negative response of normal cells.
2. For doses below which no tumors were observed, the risk estimates predicted by the different models span a very large range. This result points to large uncertainties in model specification (how to relate the kinetics of normal and initiated cells) as well as in parameter values. As mentioned above, the analysis does not attempt to separate the influence of the different sources of uncertainty, so this range also incorporates the uncertainty arising from the use of different control data and that due to α_{max}.

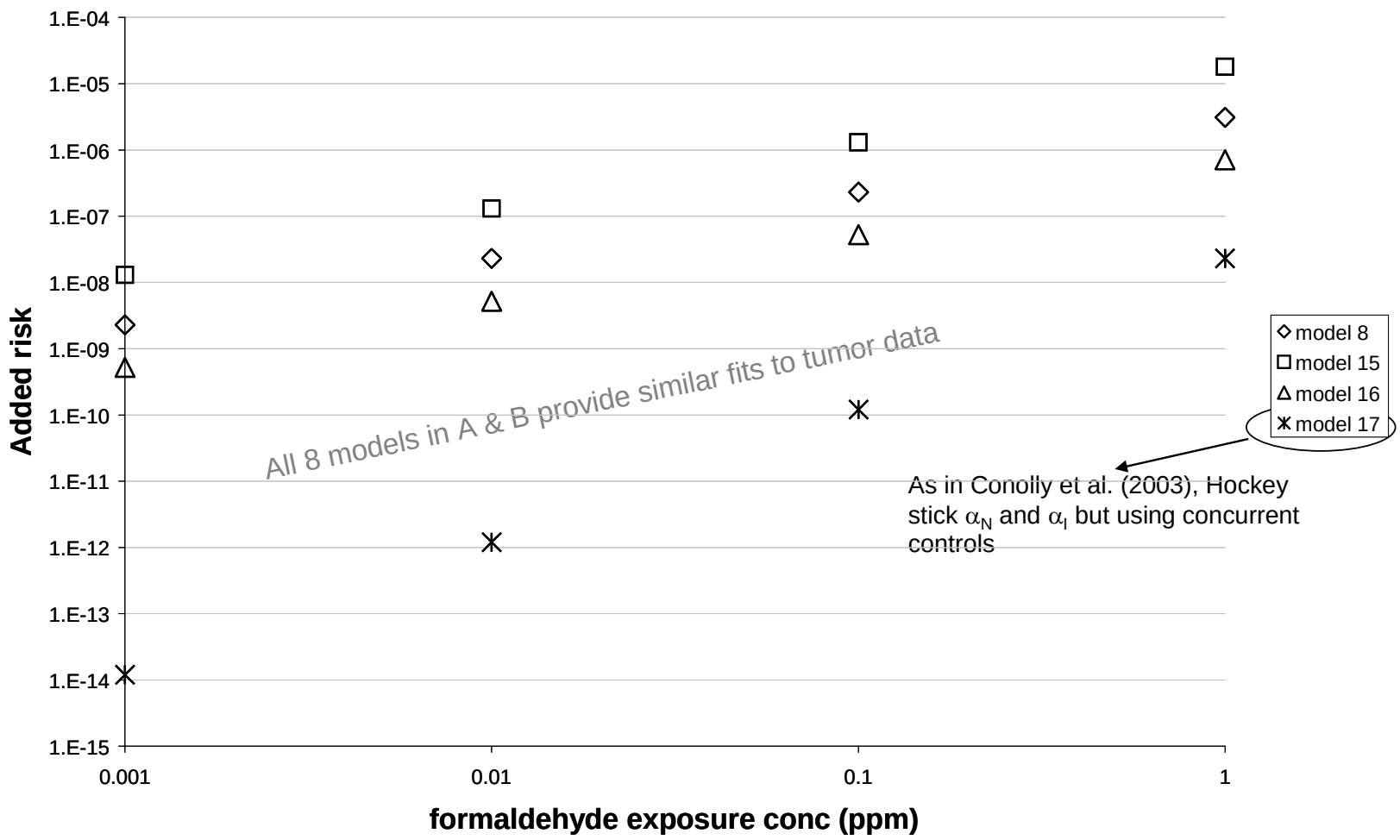

Figure E-5A. BBDR models for the rat—models with positive added risk.

Note: All four models provide "similar" fits to tumor data (see text).

Figure E-5B. BBDR rat models resulting in negative added risk.

Note: All four models provide "similar" fits to tumor data (see text).

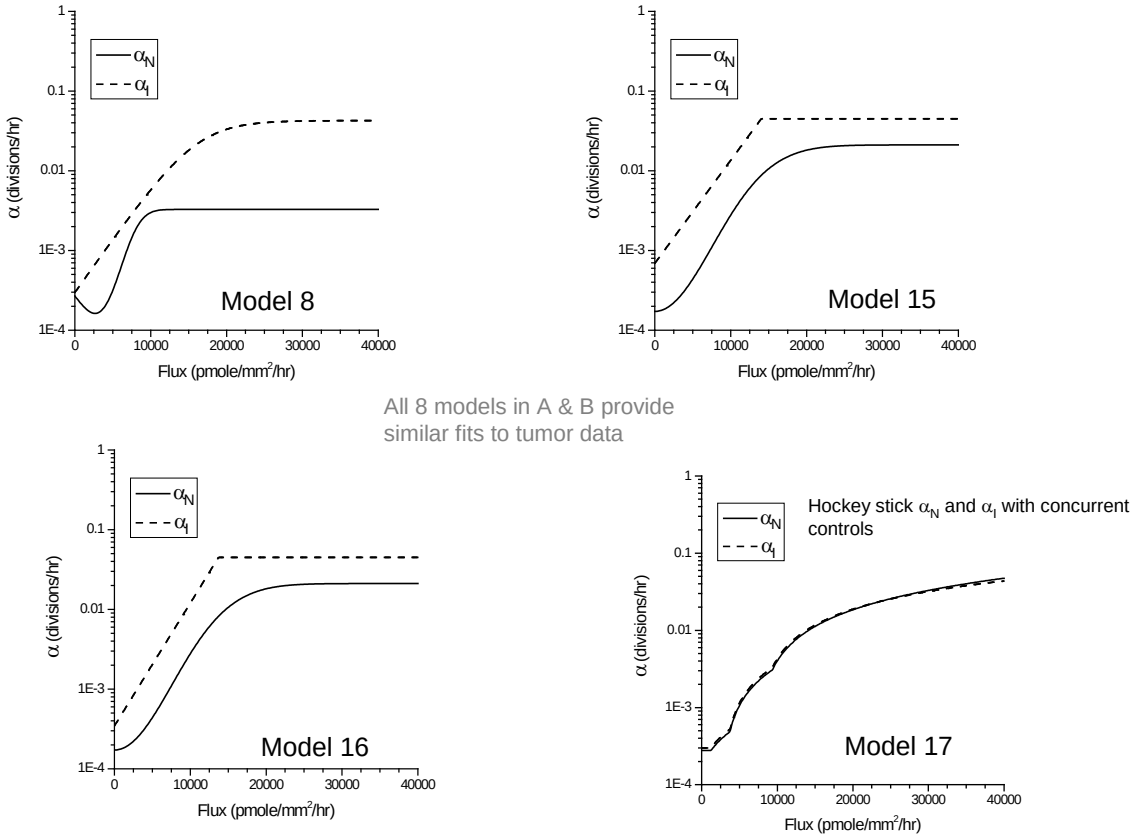

Figure E-6A. Models resulting in positive added rat risk: Dose-response for normal and initiated cell replication.

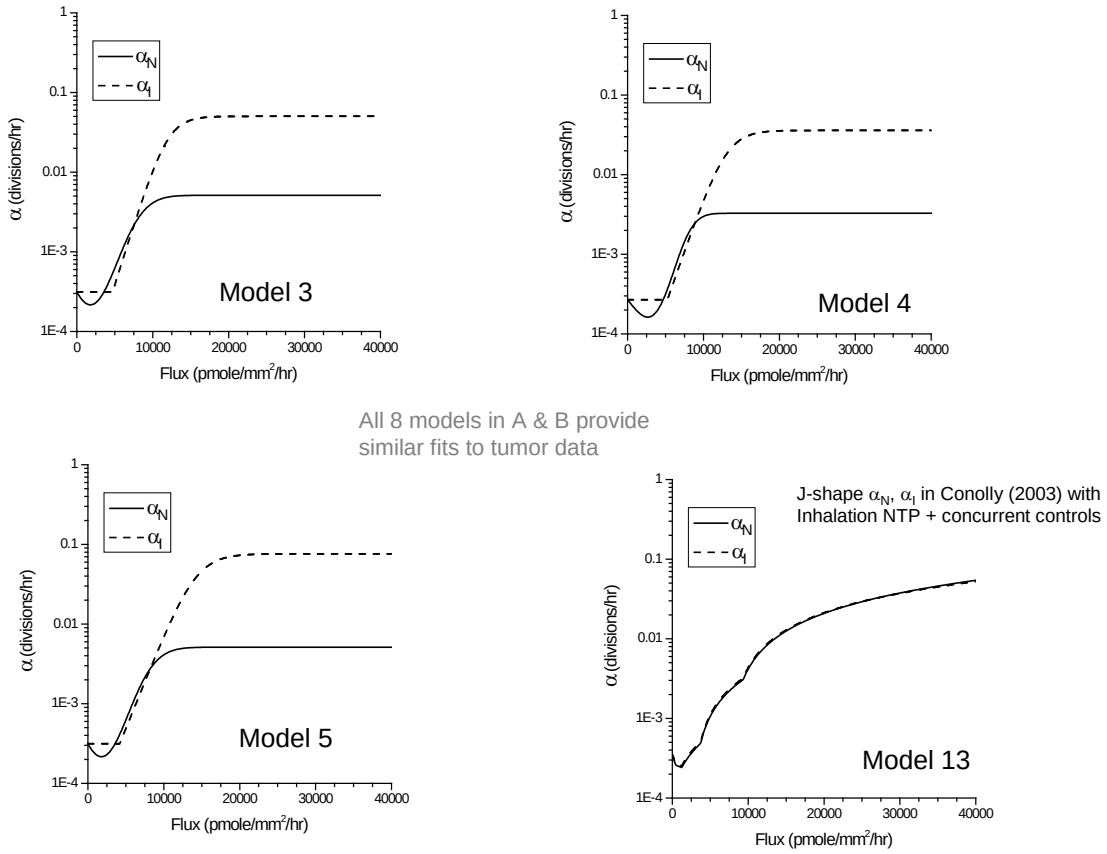

Figure E-6B. Models resulting in negative added rat risk: Dose-response for normal and initiated cell replication.

Table E-4. Parameter specifications and estimates for clonal growth models of nasal SCC in the F344 rat using alternative characterization of cell replication and death rates

Parameters	Model 3	Model 4	Model 5	Model 8	Model 15	Model 16
Historical controls added to concurrent	Inhalation NTP	Inhalation NTP	Inhalation NTP	Inhalation NTP	Inhalation NTP	Inhalation NTP
Number of dose groups	6	6	5	5	5	5
DPX concentration	Subramaniam et al. (2007)	Subramaniam et al. (2007)	Subramaniam et al. (2007)	Subramaniam et al. (2007)	Subramaniam et al. (2007)	Subramaniam et al. (2007)
α_N definition	N3	N6	N3	N6	N4	N4
α_I definition	I2	I2	I2	I1	I1	I1
α_{high}	--	0.045	--	0.045	0.045	0.045
β_I definition	$\beta_I = K_\beta \times \alpha_I$	$\beta_I = K_\beta \times \alpha_I$	$\beta_I = K_\beta \times \alpha_I$	$\beta_I = K_\beta \times \alpha_I$	$\beta_I = K_\beta \times \alpha_I$	$\beta_I = K_\beta \times \alpha_I$
					$\gamma_1 \leq 4\ \alpha_{NBasal}$	$\gamma_1 \leq 2\ \alpha_{NBasal}$
Log-likelihood	-1495.34	-1495.61	-184.02	-184.22	-182.75	-186.37
μ_{NBasal}	7.518E-7	1.664E-6	8.684E-7	9.230E-7	1.037E-6	1.662E-7
KMU	3.884E-7	3.471E-7	0.0	0.0 (0.0, 2.093E-6)	4.582E-6 (1.8E-6,1.86E-5)	0.0
KMX (*KMU* / μ_{NBasal})	0.5166	0.2086	0.0	0.0 (0.0, 4.696)	4.420 (1.53, 17.67)	0.0
D_0[§]	214.3	199.7	261.8	254.2	423.2	245.1
D_{0F}[§]	75.26	79.81	119.7	101.1	100.8	98.83
γ_1	1.164E-5	1.006E-5	3.168E-5	2.967E-4	6.888E-4	3.441E-4
γ_2	1427	1591	1825	3223	4652	2818
γ_3	11944	13017	14207	15989	54334	37896
K_β	0.9893	0.9848	0.9804	0.9504	1.006	0.9660

[§]See Subramaniam et al. (2007) for an explanation of the time delay constants D_0 and D_{0F}.

Table E-5. Parameter specifications and estimates for clonal growth models of nasal SCC in the F344 rat using cell replication and death rates as characterized in Conolly et al. (2003)

Parameters	Model 13	Model 17
Historical controls added to concurrent	All NTP	NO historical controls
Number of dose groups	6	6
DPX concentration	Conolly et al. (2000)	Subramaniam et al. (2007)
α_N definition	J-shape (TWA, Conolly et al. 2003)	Hockey (TWA, Conolly et al., 2003)
α_I definition	eq. D-1	eq. D-1
α_{high}	--	--
β_I definition	$\beta_I = \alpha_N$	$\beta_I = \alpha_N$
Log-likelihood	−1692.68	−1474.29
μ_{NBasal}	1.731E-6	0.0
KMU	0.0	1.203E-6 (1.0E-6,1.427E-6)
KMX (*KMU*/μ_{NBasal})	0.0	Infinite (0.4097,infinite)
D_0[§]	239.5	243.13
D_{0F}[§]	66.31	68.83
multib	1.047	1.078E+0
multic	1.510	3.347
α_{max}	5.153E-2	0.045

[§]See Subramaniam et al. (2007) for an explanation of the time delay constants D_0 and D_{0F}.

3. At the 10 ppb (0.01 ppm) concentration, MLE risks range from -4.0×10^{-6} to $+1.3 \times 10^{-7}$. At this dose, models that gave only positive risks resulted in a five orders of magnitude risk range from 1.2×10^{-12} to 1.3×10^{-7}, while narrowing to a four orders of magnitude risk range from 1.2×10^{-10} to 1.3×10^{-6} at the 0.1 ppm level. This narrowing continues as exposure concentration increases, and the curves coalesce to substantially similar values at 6 ppm and above (not shown). For all these 8 models, the rat added risk at 6.0 ppm ranged from 1.8×10^{-2} to 2.1×10^{-2}.

4. There does not seem to be any systematic effect on additional risk that depends on whether the 15 ppm data are included in the analysis.

5. For all of the models except models 13 and 17 in Figures E-5 and E-6, the additional risk varies substantially linearly with exposure at low exposures between 0.001 and 1.0 ppm (departing only to a small extent from linearity between 0.1 and 1.0 ppm). Models 13 and 17 show a quadratic dependence; these models employ the TWA J-shape and hockey stick dose response curves for α_N used in Conolly et al. (2003) and the same equations used by those authors to relate α_I and β_I to α_N (see eqs D-2 and D-3, Section D-6). However, the control data in Model 17 was different from those used by Conolly et al.; while all NTP controls were added to the concurrent controls in model 13, only concurrent controls were used in model 17.

The various model choices presented in Figure E-5 all provided equally good fits to the time-to-tumor data although within the context of a significant qualification. It was not possible to simply use the maximized log-likelihood values as a means of comparing the goodness-of-fit to the tumor incidence data across all these model choices. This is because many of the model choices differed in the number of doses or in the number of control animals that were used, so the fits were compared across such models only visually.

Wherever results from the BBDR modeling are discussed, values of added risk, as opposed to extra risk, are reported. This is purely for convenience in interpretation. Because of the low background incidence, these values are only negligibly different from the corresponding extra risk estimate. The final risk (or unit risk) estimates provided in this document are based on extra risk estimates.

E.3.4.3. *MOA Inferences Revisited*

The ratio KMU/μ_{Nbasal} represents the added fractional probability of mutation per cell generation ($\mu_N - \mu_{Nbasal}$)/μ_{Nbasal} due to unit concentration of DPXs. As discussed in Sections E.3.1.2 and E.3.1.5 (see Appendix E), this parameter has a critical impact on the extrapolation as well as on inferring whether the mutagenic action of formaldehyde is relevant to explaining the observed tumor incidence or its carcinogenicity at lower concentrations. In that prior discussion, this ratio was found to be extremely sensitive to the choice of historical control data. The analysis indicates that, for a given set of control data that is used, uncertainties associated with α_N and α_I also have a large impact on this ratio.

As discussed in E.3.1.2, this ratio was infinite when concurrent controls were used because the MLE value for μ_{Nbasal} was found to be zero. The use of these concurrent controls, however, does not necessarily imply that μ_{Nbasal} will be determined to be zero. In one of the scenarios examined in the sensitivity analysis, where concurrent controls were used along with the combination of dose-response curves eq D-9 for α_N (see Figure E-4) and eq E-13 for α_I, the

optimal value of the ratio KMU/μ_{Nbasal} was equal to 0.25. For the models in Figure 5-13A, this ratio was 0 for all except model 17 for which it was infinite. For the models in Figure 5-13B with negative added risk, the ratio ranged from 0–4.5. For some of those models where KMU/μ_{Nbasal} was finite, the upper confidence bound on this ratio was found to increase by an order of magnitude from the MLE value.

Thus, we conclude that the modeling does not help resolve the debate as to the relevance of formaldehyde's mutagenic potential to its carcinogenicity.

E.3.4.4. *Confidence Bounds: Model Uncertainty Versus Statistical Uncertainty*

For models 15 and 17 in Figures E-5A and E-6A, 90% CIs for additional risk were calculated by using the profile likelihood method. Table E-6 compares the lower and upper confidence bounds for these models for 0.001 ppm, 0.1 ppm (doses well below the range where tumors were observed), and 6 ppm (the lowest dose where tumors were observed) with the MLE risk estimates at these doses. In both cases, these intervals were quite narrow compared with the differences in risk predicted by different models in Figure E-5. This suggests that model uncertainty is of more consequence in the formaldehyde animal model than is statistical uncertainty. We also estimated confidence bounds using the bootstrap method for select models, and determined that these estimates were in agreement with the bounds calculated using the profile likelihood method. These results are not presented here. We return to the calculation of confidence limits when determining points of departure (PODs).

Table E-6. Comparison of statistical confidence bounds on added risk for two models

Dose (ppm)	Model	Lower bound	MLE	Upper bound
0.001	Model 15	4.4×10^{-9}	1.3×10^{-8}	1.6×10^{-8}
	Model 17	1.2×10^{-14}	1.2×10^{-14}	1.3×10^{-14}
0.1	Model 15	4.5×10^{-7}	1.3×10^{-6}	1.7×10^{-6}
	Model 17	1.2×10^{-10}	1.2×10^{-10}	1.3×10^{-10}
6	Model 15	1.8×10^{-2}	2.1×10^{-2}	2.3×10^{-2}
	Model 17	1.3×10^{-2}	1.8×10^{-2}	3.0×10^{-2}

In conclusion, it is demonstrated that the different formaldehyde clonal growth models can fit the data about equally well and still produce considerable variation in additional risk and biological inferences at low exposures. However, even with these large variations, the highest

MLE added risk for the F344 rat is only of the order of 10^{-6} at 0.1 ppm. Thus, with regard to calculating a reasonable upper bound that includes model and statistical uncertainty, the relevant question is whether the range arising out of uncertainties in the rat model amplifies when extrapolated to the human. Thus, in Appendix F, the human model in Conolly et al. (2004) will be examined.

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Appendix F

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APPENDIX F

SENSITIVITY ANALYSIS OF BBDR MODEL FOR FORMALDEHYDE INDUCED RESPIRATORY CANCER IN HUMANS

F.1. MAJOR UNCERTAINTIES IN THE FORMALDEHYDE HUMAN BBDR MODEL

Subsequent to the BBDR model for modeling rat cancer, Conolly et al. (2004) developed a corresponding model for humans for the purpose of extrapolating the risk estimated by the rat model to humans. Also, rather than considering only nasal tumors, it is used to predict the risk of all human respiratory tumors. The human model for formaldehyde carcinogenicity (Conolly et al., 2004) is conceptually very similar to the rat model and follows the schematic in Figure 5-11 in Chapter 5. The model structure, notations, and calibration are described in Appendix D. Unlike the sensitivity analysis of the rat modeling where a number of issues were examined, a much more restricted analysis will be presented here for the sake of brevity. A more extensive analysis was carried out initially that carried forward several of the rat models from Appendix E to the human, and the lessons learned from those exercises are in agreement with the more restricted presentation that follows. Table F-1 lists the major uncertainties and assumptions in the human extrapolation model in Conolly et al. (2004).

Table F-1. Summary of evaluation of major assumptions and results in CIIT human BBDR model

Assumptions[a]	Rationale in Conolly et al. (2003) or CIIT (1999)	EPA evaluation	Further elaboration
Cell division rates derived from rat labeling data were assumed applicable to human (except for assuming different fraction of cells with replicative potential).	There are no equivalent LI data for human or guidance for extrapolating cell division rate across species.	Enzymatic metabolism plays a role in mitosis. Therefore, we expect interspecies difference in cell division rate. Basal cell division rates in humans are expected to be much more variable than in laboratory animals.	Subramaniam et al. (2008)
Parameters for enzymatic metabolism of formaldehyde in human PBPK model for DPX concentrations: K_m varies by order of magnitude between rat and monkey but is same for monkey and human. V_{max}/K_m is similar for rat and monkey but 6-fold lower for human.	See text (Section 3.6.6.2)	See text (Section 3.6.6.2)	Section 3.6.6.2; Conolly et al. (2000); Subramaniam et al. (2008); Klein et al. (2010)
Anatomically realistic representation of nasal passages.	Reduces uncertainty (over default calculation carried out by averaging dose over entire nasal surface).	Computer representation pertains to that of one individual (Caucasian male adult). There is considerable interindividual variability in nasal anatomy. Susceptible individuals are even more variable.	Kimbell et al. (2001a, b); Subramaniam et al. (2008, 1998)
KMU/μ_{Nbasal} is species invariant (used to estimate human).	Human cells are more difficult to transform than rodent, both spontaneously and by exposure to formaldehyde.	μ_{Nbasal} is 0 when concurrent controls or inhalation NTP controls in time frame of concurrent bioassays are used. Leads to infinitely large KMU for human.	Subramaniam et al. (2007); Crump et al. (2009, 2008).
Conservative assumptions were made. Results are conservative in the face of model uncertainties.	1) Hockey-stick dose-response for α_N was included even though TWA indicated J-shape. 2) Overall respiratory tract cancer incidence data for human baseline rates were used. 3) Risk was evaluated at statistical upper bound of the proportionality parameter relating DPXs to the probability of mutation.	Results in Conolly et al. (2004) are not conservative in the face of model uncertainties: (a) Human risk estimates are very sensitive to use of historical controls in the analysis of the animal bioassay. (b) Human risk estimates are unboundedly large when concurrent controls are used in rat model. (c) Minor perturbations in model assumptions regarding division and death rates of initiated cells lead to upper bound risks that were more than 1,000-fold greater than the highest estimates in Conolly et al. (2004).	Conolly et al. (2004); Subramaniam et al. (2007); Crump et al. (2009, 2008).

[a]Assumptions in this table are in addition to those listed for the BBDR model for the F344 rat.

F.2. SENSITIVITY ANALYSIS OF HUMAN BBDR MODELING

Crump et al. (2008) carried out a limited sensitivity analysis of the Conolly et al. (2004) human model. This analysis was limited to evaluating the effect on the human model of the following. These evaluations have been the subject of some debate in the literature and at various conferences (Conolly, 2009; Conolly et al., 2009, 2008; Crump et al., 2009).

1. The use of the alternative sets of control data for the rat bioassay data that were considered in the sensitivity analysis of the rat model in Appendix E.
2. Minor perturbations in model assumptions regarding the effect of formaldehyde on the division and death rates of initiated cells (α_I, β_I).
    - As mentioned in Section D.7 one (of the two) adjustable parameter in the expression for the human α_I in Conolly et al. (2004) was determined from the model fit to the rat tumor incidence data while the second parameter was determined from background rates of cancer incidence in the human. Therefore, variations considered in α_I were constrained to only those that (a) did not meaningfully degrade the fit of the model to the rat tumor incidence data and (b) were in concordance with background rates in the human.
    - Crump et al. (2008) also evaluated these variations with respect to their biological plausibility. The sensitivity analysis on assumed initiated cell kinetics was thought to be particularly important since there were no data to even crudely inform the kinetics of initiated cells for use in the models, even in rats, and the two-stage clonal expansion model is very sensitive to initiated cell kinetics (Gaylor and Zheng, 1996; Crump, 1994a, b).

Crump et al. (2008) note that, since the purpose of their analysis was to carry out a sensitivity analysis, in order to illustrate certain points, only risks to the general U.S. population from constant lifetime exposure to various levels of formaldehyde under the Conolly et al. (2004) environmental scenario (8 hours/day sleeping, 8 hours/day sitting, and 8 hours/day engaged in light activity) are considered. Fits based on the hockey-stick and J-shape models were identical, and, of the three estimated parameters (μbasal, multb, and D), only the estimate of μbasal differed between the two models.

F.2.1. Effect of Background Rates of Nasal Tumors in Rats on Human Risk Estimates

Crump et al. (2008) quantitatively evaluated the impact of different control groups on estimates of additional human risk as follows:

1. Concurrent controls plus all NTP controls:, the same as used by Conolly et al. (2004);
2. Concurrent controls plus controls from NTP inhalation studies;
3. Only concurrent controls;
4. Each set of control data was applied with both the J shape and hockey-stick models in Conolly et al. (2004) for α_N(flux) and α_I(flux) for a total of six analyses;.
5. Uncertainties associated with α_N or α_I are not addressed. Parameters α_{max}, multfc, and KMU were estimated in exactly the same manner as in Conolly et al. (2004).

Crump et al. (2008) present the following dose-response predictions of additional risk in humans from constant lifetime exposure to various levels of formaldehyde arising from exercising the above six cases. Their plots are reproduced in Figure F-1**,** where the corresponding curves based on Conolly et al. (2004) are also shown for comparison.

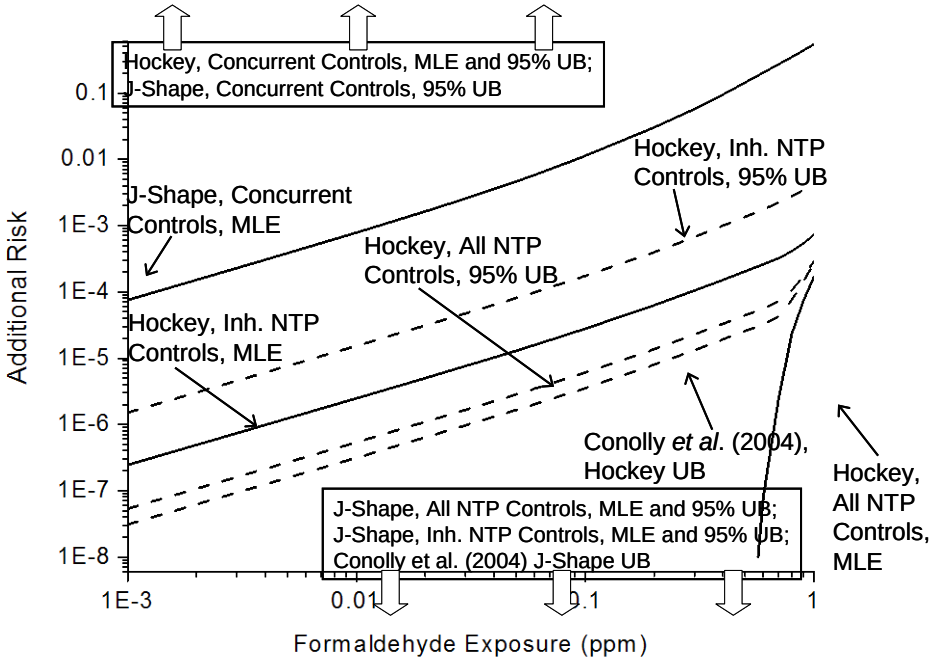

Figure F-1. Effect of choice of NTP bioassays for historical controls on human risk.

Note: Estimates of additional human risk of respiratory cancer by age 80 from lifetime exposure to formaldehyde are obtained by using different control groups of rats.

Source: Crump et al. (2008).

The lowest dotted curve in Figure F-1 represents the highest estimates of human risk developed by Conolly et al. (2004). This resulted from use of the hockey-stick model for cell division rates in conjunction with the statistical upper bound for the parameter KMU. As indicated by the downward block arrows in the figure, their corresponding estimates based on the J-shape model were all negative for exposures below 1 ppm.

Consider next the solid curves in the figure, which show predicted MLE added risks that were positive and less than 0.5. Crump et al. (2008) next examined the added risk obtained when the MLE estimate of (KMU/μ_{basal}) in these cases is replaced by the 95% upper bound of this parameter ratio. The upper bound risk estimates in Conolly et al. (2004) were calculated in a similar manner (but using all NTP historical controls). Except for minor differences, risk estimates corresponding to such an upper bound and using all NTP controls were very similar in the two efforts (Crump et al., 2008; Conolly et al., 2004).

Figure F-1 shows that the choice of controls to include in the rat model can make an enormous difference in estimates of additional human risk. For the J-shaped model for cell replication rate both estimates based on the MLE and those based on the 95% upper bound on KMU/μ_{basal} are negative for formaldehyde exposures below 1 ppm. However, when only concurrent controls are used in the model in Crump et al. (2008), the MLE from the J-shape model is positive and is more than three orders of magnitude higher than the highest estimates obtained by Conolly et al. (2004). Using only concurrent controls, estimates based on the 95% upper bound on KMU/μ_{basal} are unboundedly large (block arrows at the top of the figure). For the hockey-stick shaped model for cell replication rate, when all NTP controls are used, the estimates based on the MLEs are zero for exposures less than about 0.5 ppm. If only inhalation controls are added, the MLEs are about seven times larger than the Conolly et al. (2004) upper bound estimates, and the estimates based on the 95% upper bound on KMU/μ_{basal} are about 50 times larger than the Conolly et al. (2004) estimates. If only concurrent controls are used, both the MLE estimates and those based on the 95% upper bound on KMU/μ_{basal} are unboundedly large.

F.2.2. Alternative Assumptions Regarding the Rate of Replication of Initiated Cells

For the human model, Conolly et al. (2004) made the same assumptions for relating α_I(flux) and β_I(flux) to α_N(flux) as in their rat model (Conolly et al., 2003). That is, these quantities were related by using eqs D-2 and D-3 (see Appendix D). As discussed in the context of the rat modeling, by extending the shape of these curves to humans, the authors' model brings the cytotoxic action of formaldehyde to bear strongly on the parameterization of the human model as well.

In the sensitivity analyses of the rat modeling in Appendix E, it was concluded that other biologically plausible assumptions for α_I and β_I resulted in several orders of magnitude variations in the low dose risk relative to those obtained by models based on the assumptions in Conolly et al. (2003) but that the highest risks were nonetheless of the order of 10^{-6} at the 10 ppb level. This section examines how these uncertainties in the rat model propagate to the human model.

Crump et al. (2008) made minor modifications to the assumed division rates of initiated cells in Conolly et al. (2004), while all other aspects of the model and input data were kept unchanged. Two alternatives were considered for each of the J-shape and hockey-stick models. Figure F-2 shows the hockey-stick model for initiated cells in rats. In the first modification to the hockey-stick model (hockey-stick Mod 1), rather than having a threshold at a flux of 1,240 pmol/m^2-hour, the division rate increases linearly with increasing flux until the graph intersects the original curve at 4,500 pmol/m^2-hour, where it then assumes the same value as in the original curve for larger values of flux. The second modification (hockey-stick Mod 2) is similar, except the modified curve intersects the original curve at a flux of 3,000 pmol/m^2-hour.

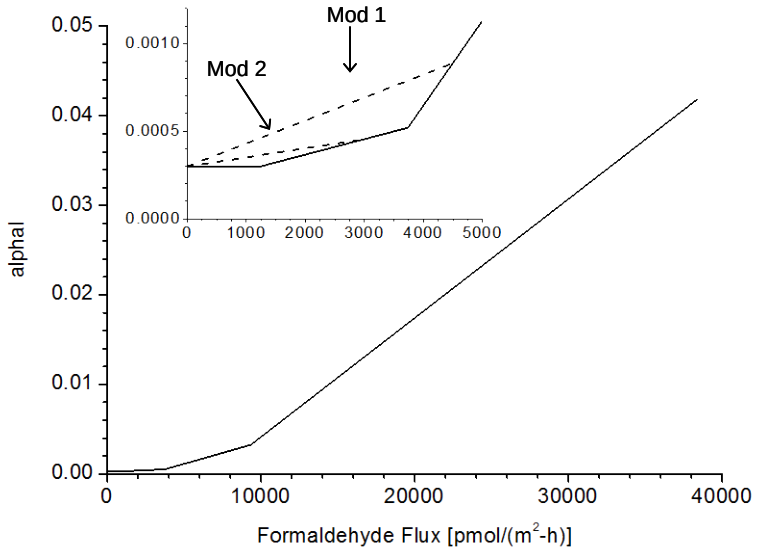

Figure F-2. Conolly et al. (2003) hockey-stick model for division rates of initiated cells in rats and two modified models.

Source: Crump et al. (2008).

Figure F-3 shows the rat J-shape model for initiated cells. In the first modification to this dose response (J-shape Mod 1), rather than having a J shape, the division rate of initiated cells remains constant at the basal value until the original curve rises above the basal value and has the same value as the original curve for larger values of flux. In the second modification (J-shape Mod 2), the J shape is retained but somewhat mitigated. In this modification, the division rate initially decreases in a linear manner similar to that of the original model but with a less negative slope until it intersects the original curve at a flux of 1,240 μm/m^2-hour, where it then follows the original curve for higher values of flux.

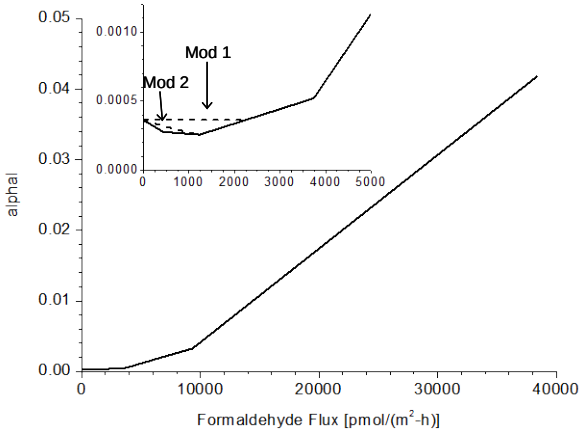

Figure F-3. Conolly et al. (2003) J-shape model for division rates of initiated cells in rats and two modified models.

Source: Crump et al. (2008).

Since the first constraint on the variation in α_I was in concordance with the rat time-to-tumor incidence data, Crump et al. (2008) applied each of the modified models in Figures F-2 and F-3 to the version of the formaldehyde models in Subramaniam et al. (2007) that employed all NTP controls and the hockey-stick curve for α_N. These authors restricted their analysis to this case since their stated purpose was only a sensitivity analysis as opposed to developing alternate credible risk estimates. Figure F-4 reproduces (from Crump et al. [2008]) curves of the cumulative probability of a rat dying from a nasal SCC by a given age for bioassay exposure groups of 6, 10, and 15 ppm. For comparison purposes, the corresponding KM (nonparametric) estimates of the probability of death from a nasal tumor are also shown. Three sets of probabilities are graphed: the original unmodified one and the ones obtained by using hockey-

stick Mod 1 and Mod 2. Crump et al. (2008) state that the changes in the tumor probability resulting from these modifications are so slight that the three models cannot be readily distinguished in this graph.[4] Thus, the modifications considered to the models for the division rates of initiated cells caused an inconsequential change in the fit of the model-predicted tumor incidence to the animal tumor data.

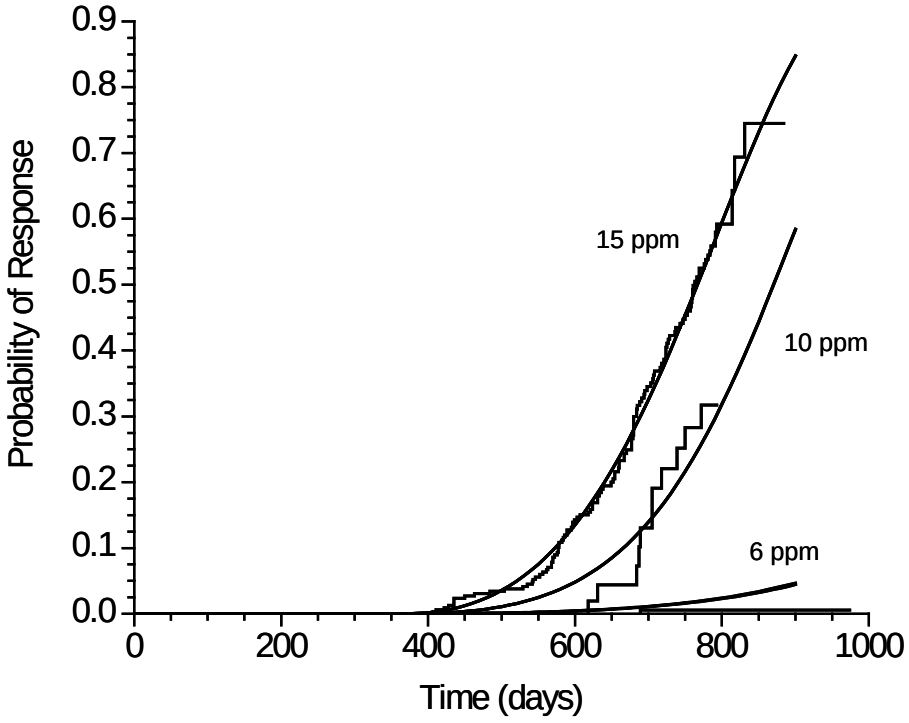

Figure F-4. Very similar model estimates of probability of fatal tumor in rats for three models in Figure F-2.

Note: The differences are visually indistinguishable. Models were derived from the implementation of Conolly et al. (2003) with the hockey-stick curves for α_I(flux) and α_N(flux) and variants derived from modifications (Mod 1 and Mod 2, Figure F-2) to α_I(flux). Model probabilities are compared to KM estimates. The three sets of model estimates are so similar that they cannot be distinguished on this graph.

Source: Crump et al. (2008).

[4] The largest change in the tumor probability resulting from this modification for any dose group and any age up through 900 days was found to be less than 0.002, a change so small that it would be impossible to detect, even in the largest bioassays ever conducted. The changes in tumor probability resulting from the other modifications described earlier were found to be even smaller. These comparisons were made in Crump et al. (2008) without re-optimizing the likelihood. The authors note that re-optimization of the model subsequent to the variations would have made the fit of modified models even better.

The above modifications did not affect the basal rate of cell division in the model and likewise had no effect on the fit to the human background data (Crump et al., 2008).

Crump et al. (2008) noted that, although the threshold model for initiated cells in Conolly et al. (2003) was replaced with a model that had a small positive slope at the origin, the resulting curves, hockey-stick Mod 1 and hockey-stick Mod 2, could have been shifted slightly to the right along the flux axis in order to introduce a threshold for α_I without materially affecting the risk estimates resulting from these modified curves. Thus, "the assumption of a linear no-threshold response is not an essential feature of the modifications to the hockey-stick model; clearly threshold models exist that would produce essentially the same effect" (Crump et al. 2008).

F.2.3. Biological Plausibility of Alternate Assumptions

These very small variations made to the α_I in Conolly et al. (2003) are seen to be

- consistent with the tumor-incidence data (see Figure F-4);
- small compared with the variability and uncertainty in the cell replication rates characterized from the available empirical data (at the formaldehyde flux where α_I was varied);
- supported (qualitatively) by limited data, suggesting increased cell proliferation at doses below cytotoxic;
- perturbations that one should expect on any dose response derived from laboratory animal data because of human population variability in cell replication;
- and biologically plausible because cell cycle control in initiated cells is likely to be disrupted.

The averaged cell replication rate constants as tabulated in Table 1 of Conolly et al. (2003) and shown by the red curve in Figure E-2 of Appendix E (for various exposure concentrations and corresponding average formaldehyde flux values in the F344 rat nose) demonstrate an increase over baseline values only at exposure concentrations of 6 ppm and higher. Increased cell proliferation at these concentrations of formaldehyde, whether transient or sustained, have been associated in the literature with epithelial response to the cytotoxic properties of formaldehyde (Conolly, 2002; Monticello and Morgan, 1997; Monticello et al., 1996, 1991). The labeling data are considered to show a lack of cytotoxicity and regenerative cell proliferation in the F344 rat at exposures of 2 ppm and below (Conolly, 2002). In the Conolly et al. (2003) modeling, it is further assumed that the formaldehyde flux levels at which cell replication exceeds baseline rates remain essentially unchanged when extrapolated to the human and for initiated cells for the rat as well as the human. These assumptions need to be first

viewed in the context of the uncertainty and variability in the data on normal cells discussed in Appendix E.

Arguments for a hockey-stick or J shape over the background have been made in the literature for sustained and chronic cell replication rates. However, the analyses of the cell replication data show that the data are not consistently (over each site and time) indicative of a hockey-stick or J shape as the best representation of the data (see Appendix E). This uncertainty is particularly prominent when examining the cell replication data at the 13-week exposure time and the pooled data from the PLM nasal site from Monticello et al. (1996) (see Figures E-1 [dotted curve], E-3B, and E-4 of Appendix E). The earliest exposure time in this experiment was at 13 weeks, and the 13-week cell replication data appear to be more representative of a monotonic increasing dose response without a threshold; it is possible that early times are of more relevance to the carcinogenesis as well as for considering typical (frequent short duration) human exposures.

Recently, Meng et al. (2010) measured cell replication in the anterior lateral meatus of the F344 rat using continuous labeling on rats exposed to all the concentration levels in the Monticello et al. (1996) experiment. Labeling index (i.e., LI, as opposed to ULLI in the Monticello experiment) was measured as the percentage of BrdU-labeled cells among the total number of cells counted at the nasal site. Their data are reproduced below in Figure F-5, where the asterick denotes the observation of a statistically significant difference from the control group (Dunnett's test, $p < 0.01$). These data appear to be consistent with a monotonically increasing dose-response shape for cell replication. Linear regression provided good fits to all of the data ($R^2 = 0.97$) as well as to the subset of the data obtained by deleting the higher dose data at 10 ppm and 15 ppm exposures ($R^2 = 0.84$). We cite these data in support of considering the modifications carried out in Figure F-2.

For initiated cells, there are no data on which to evaluate the modifications made in Section F.2.2 to these rates. However, some perspective can be gained by comparing them to the variability in the division rates obtained from the data on normal cells used to construct the formaldehyde model. As shown in Figure E-2 and discussed further in Subramaniam et al. (2008), these data show roughly an order of magnitude variation in the cell replication rate at a given flux. As part of a statistical evaluation of these data, a standard deviation of 0.32 was calculated for the log-transforms of individual measurements of division rates of normal cells (Crump et al., 2008). By comparison, the maximum change in the log-transform division rate of initiated cells resulting from hockey-stick Mod 2 was only 0.20, and the average change would be considerably smaller. Thus, although there are no data for initiated cells, it can be said that

the modifications introduced in Crump et al. (2008) for initiated cells are extremely small in comparison to the dispersion in the data for normal cells.

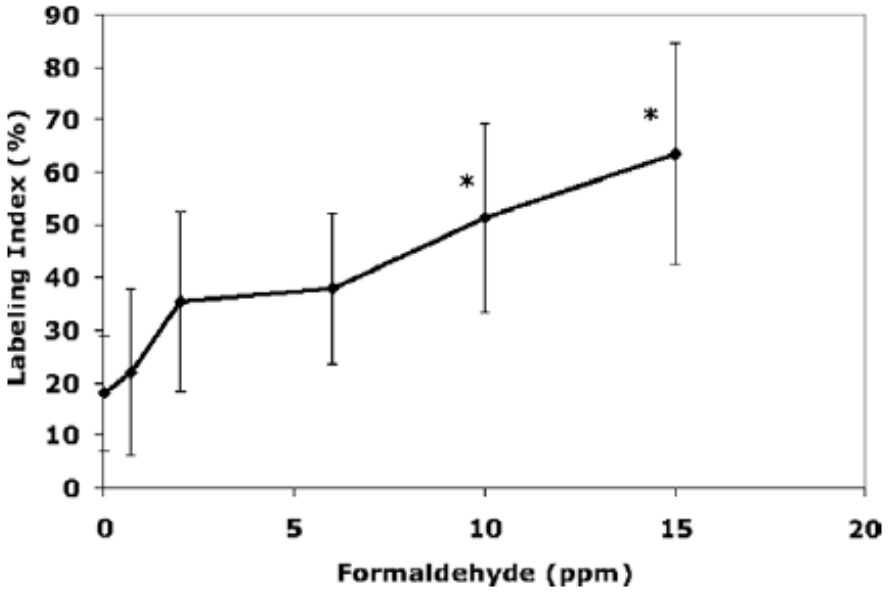

Figure F-5. Cell proliferation data from Meng et al. (2010). The Y-axis shows the percentage of BrdU-labeled cells among the total number of cells counted in the ALM section of the rat nose.

Reproduced with permission from Meng et al. (2010).

Subramaniam et al. (2008) also point to some additional, albeit limited, data, suggesting that exposure to formaldehyde could result in increased cell replication at doses far below those that are considered to be cytotoxic. Tyihak et al. (2001) treated different human cell lines in culture to various doses (0.1–10 mM) of formaldehyde and found that the mitotic index increased at the lowest dose of 0.1 mM. These findings considered along with human population variability and susceptibility (for example, polymorphisms in ADH3 [Hedberg et al., 2001]) indicate that it is necessary to consider the possibility of small increases in the human α_I over baseline levels at exposures well below those at which cytotoxicity-driven proliferative response is thought to occur.

Heck and Casanova (1999) have provided arguments to explain that the formation of DPXs by formaldehyde leads to inhibition of cell replication (i.e., if this effect alone is considered, normal cell replication rate of the exposed cells would be less than the baseline rate). However, this hypothesis was posed for normal cells. Subramaniam et al. (2008) argue that if an initiated cell is created by a specific mutation that impairs cell cycle control, the effect would be

to mitigate the DPX-induced inhibition in cell replication, either partially or fully, depending on the extent to which the cell cycle control has been disrupted. In the absence of data on initiated cells, the above argument provided biological motivation to the modification applied to the J-shape model for cell division (Crump et al. 2008).

Thus, the previous paragraphs suggest that the changes made in the analysis in Crump et al. (2008) to the assumption by Conolly et al. (2003) regarding the dose response for the division rate of initiated cells are plausible.

F.2.4. Effect of Alternate Assumptions for Initiated Cell Kinetics on Human Risk Estimates

Figure F-6 contains graphs of the additional human risks estimated (in Crump et al. [2008]) by applying these modified models for α_I and using all NTP controls, compared with those obtained by using the original Conolly et al. (2004) model. Each of the four modified models presents a very different picture from that of Conolly et al. (2004). At low exposures, these risks are three to four orders of magnitude larger than the largest estimates obtained by Conolly et al. (2004).

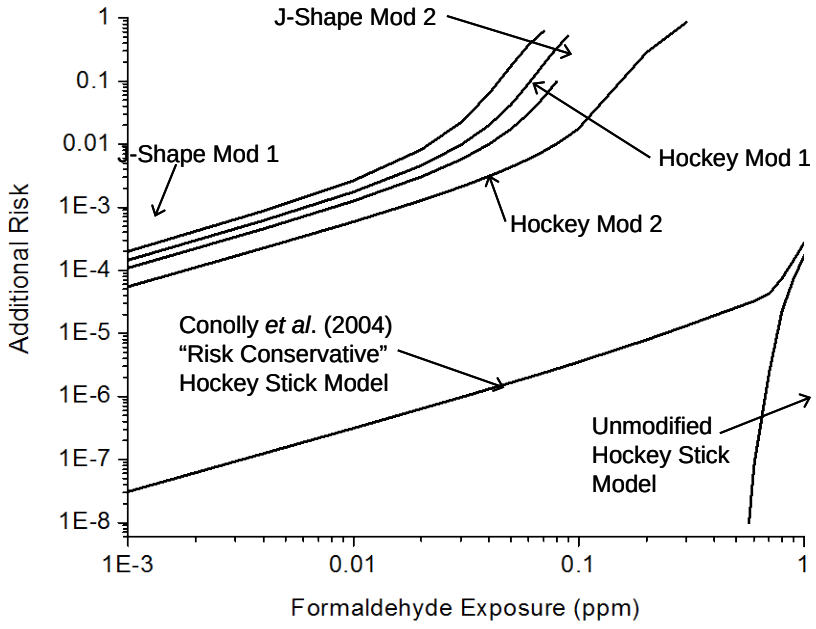

Figure F-6. Graphs of the additional human risks estimated by applying these modified models for α_I, using all NTP controls, compared to those obtained using the original Conolly et al. (2004) model.
Source: Crump et al. (2008).

These results have been criticized by Conolly et al. (2009) as being unrealistically large and above the realm of any epidemiologic estimate for formaldehyde SCC. Thus, they argue that the parameter adjustments made in Crump et al. (2008) are inappropriate. Crump et al. (2009) rebutted these points by arguing that the purpose of their work was not to provide a more reliable or plausible model but to carry out a sensitivity analysis. They argued that the changes made to the model (in their analyses) were reasonable since they did not violate any biological constraints or the available data. Further, they pointed out that "by appropriately mitigating the small modifications [they] made to the division rates of initiated cells, the model [would] provide any desired risk ranging from that estimated by the original model up to risks 1,000-fold larger than the conservative estimate in Conolly et al. (2004)."

Crump et al. (2008) also evaluated the assumption in eq D-3 of the CIIT modeling pertaining to initiated cell death rates (β_I) by making small changes to β_I. They report that they obtained similarly large values for estimates of additional human risk at low exposures. Obtaining reliable data on cell death rates in the nasal epithelium appears to be an unusually difficult proposition (Hester et al., 2003; Monticello and Morgan, 1997), and, even if data are obtained, they are likely to be extremely variable.

Appendix G

1

APPENDIX G

EVALUATION OF THE CANCER DOSE-RESPONSE MODELING OF GENOMIC DATA FOR FORMALDEHYDE RISK ASSESSMENT

G.1. MAJOR CONCLUSIONS IN ANDERSEN ET AL. (2008)

In Chapter 4, the gene microarray data from animal studies on formaldehyde (Andersen et al., 2008; Thomas et al., 2007) were described. The analysis of these animal high throughput data and the conclusions reached in these two groundbreaking papers were closely examined for use in this assessment. Studies on high throughput animal data provide a wealth of information that helps further understanding of the relevant mechanisms. However, such studies have generally not made quantitative bottom-line inferences that inform low dose human risk. The above-mentioned studies are a notable exception due to the breadth of their conclusions on low dose MOAs, their pioneering application of the benchmark dose (BMD) methodology to genomic data, their use of BMD-response analysis that identified dose estimates at which specific cellular processes were significantly altered, and the fact that they were accompanied by recommendation in the literature urging use of these results in setting exposure standards for formaldehyde (Daston, 2008).

We focus here on the conclusions in these papers with regard to modeling the cancer dose-response for formaldehyde. In addition to supporting our disposition of these analyses for this assessment, this write-up serves the purpose of exemplifying critical issues that need to be considered for the future.

The overall BMD determined in Andersen et al. (2008) for all genes with significant dose-response averaged 6.4 ppm. These analyses indicated a general progression with the lowest BMD values (i.e., the most sensitive epithelial responses) for extracellular and cell membrane components and higher BMD values for intracellular processes. Overall, these authors concluded that

- Genomic changes, including those suggestive of mutagenic effects, did not temporally precede or occur at lower doses than phenotypic changes in the tissue
- Genomic changes were no more sensitive than tissue responses
- Formaldehyde, being an endogenous chemical, is well handled until some threshold is achieved. Above these doses, toxicity rapidly ensues with concomitant genomic and histologic changes.
- Linear extrapolations, or extrapolations that specify similar MOAs at high and low doses would be inappropriate.

These findings were judged to have significant implications on the debated MOA for formaldehyde carcinogenicity, confirming results from earlier bioassays and dose-response modeling that the mutagenicity of formaldehyde was too weak to be of relevance to its carcinogenicity. Daston (2008) judged the method in these efforts to be extremely sensitive and therefore suited to examining whether responses at the molecular level take place at doses below which frank adverse effects occur. Daston (2008) argued that "… if there are pleiotropic effects at lower exposure levels that would elicit a different profile of gene expression, those genes would not go unnoticed" and thus concluded that "the gene expression data confirm that the responses are not linear at low doses."

In the analyses that follow, we point to some significant quantitative factors that impact on these conclusions.

G.2. USE OF MULTIPLE FILTERS ON THE DATA

The analyses in these papers involved the following sequence of data filters.

1. Gene probe sets that differed in expression in response to treatment were identified by one-way analysis of variance. Probability values were adjusted for multiple comparisons by using a false discovery rate of 5%.
2. Next, in addition to the above statistical filter, the output was further screened by selecting only those genes that exhibited a change from the control group that was greater than or equal to 1.5-fold (logarithmic).
3. The gene probe sets that demonstrated significant dose-response behavior were then matched to their corresponding biological process and molecular function gene ontology (GO) categories (considering only those involving more than three genes) and grouped into process categories such as cell division, DNA repair, cellular proliferation, apoptosis, and related molecular function categories.

A large number of genes are expressed in these studies; therefore, clearly some appropriate filter needs to be used for meaningful interpretation of the vast database. Tissue pathology served as a phenotypic anchor for the interpretation of microarray results, and the genomic study confirmed (and improved on) the qualitative and quantitative understanding derived from the histopathology and observation of frank effects. It is possible that the combination of filters used by these authors is adequate for an inquiry into some mechanisms associated with the specific phenotypic effects. However, the studies reached bottom-line conclusions with regard to the low-dose MOA and approach to be considered for quantitative extrapolation. These conclusions necessarily involve questions as to whether there were gene

expression changes at low dose and at early exposure times that may be relevant to initiating carcinogenesis and finally as to whether there is a threshold in dose associated with formaldehyde carcinogenesis. However, collectively, the three filters employed in these studies likely constitute overly stringent criteria, taking away the resolution needed to observe critical gene changes needed to delineate low dose effects. An indication that this may indeed be the case can be seen by examining the correlations in their findings with the observed trend in the data on DPXs formed by formaldehyde. This is detailed in the following section.

G.3. DATA FOR LOW-DOSE CANCER RESPONSE

A significant finding in Thomas et al. (2007) is that BMD estimates for the GO categories applicable to cell proliferation and DNA damage were similar to values obtained for cell labeling indices and DPXs in earlier studies and to BMD estimates obtained for the onset of nasal tumors. The mean BMD for the GO category of "positive regulation of cell proliferation" was 5.7 ppm; in comparison, Schlosser et al. (2003) obtained a 10% BMD of 4.9 ppm for the cell labeling index. The GO category associated with "response to DNA damage stimulus," seen as a genomic correlate to a mutagenic effect, had a mean BMD of 6.31 ppm. Thomas et al. (2007) compare this finding with significant increase at 6 ppm of DPXs following a 3-hour exposure in the study by Casanova et al. (1994). The formation and repair of DPXs have been considered to be one of the potential mechanisms associated with the genotoxic action of formaldehyde (Conolly et al., 2003, 2000). Based on earlier work in the same laboratory (Conolly et al., 2004, 2003; Conolly, 2002), Slikker et al. (2004) concluded that there is a dose threshold (at about 6 ppm) to formaldehyde carcinogenicity and that the putative mutagenic action of formaldehyde is not relevant to its carcinogenicity. Therefore, the finding that a significant genomic response (e.g., induction of DNA repair genes) is not observed at doses lower than those that induce tumors in rodent bioassays is seen by these authors (Andersen et al., 2008; Daston, 2008; Thomas et al., 2007) to further buttress the above conclusions related to the mode of action for formaldehyde-induced respiratory cancer.

However, phenotypic anchoring to the DPX data drawn only from Casanova et al. (1994) misses critical low-dose data that informs mode of action. In an earlier study, Casanova et al. (1989) observed statistically significantly elevated (over controls) levels of DPXs at 2 ppm and a trend towards elevated DPXs at 0.7 ppm. In analysis of low-dose data, the trend in the dose-response is critically important because data inherently lack the power to establish statistical significance. Furthermore, the two studies by Casanova and coworkers are different in some respects. The earlier study was a 6-hour exposure, while the later study was a 3-hour study; thus, on this account alone, it appears more relevant to compare with the older study. Exposures in

the earlier study were additionally at 0.3 and 10 ppm, thus affording a lower exposure concentration. In the earlier study, tissue from the whole nose was analyzed, whereas in the later study tissue from two specific regions was obtained from the "high" tumor (Level II) and "low" tumor regions. Together, these data suggest that DPXs occur at exposure concentrations considerably lower than those that elicited transcriptional changes. One possible explanation is that the increase in DPXs was not sufficient to induce DNA repair genes. Alternatively, these discrepancies may be due to the stringent filters and the low statistical power of the Andersen et al. (2008) study. These disparities between the gene array study and the DPXs question the ability of the studies in Andersen et al. (2008) and Thomas et al. (2007) to inform the presence or absence of a mutational MOA for formaldehyde, and in essence, to inform the low-dose response curve for formaldehyde-induced cancer.

In another instance, Andersen et al. (2008) clearly stated that no genes were significantly altered by exposure to 0.7 ppm, yet they state that there was "a trend toward altered expression at 0.7 ppm" in some genes with U and inverted U shape dose-responses (Figures 4 and 5 of their paper). While these changes may not be statistically significant, they could be biologically significant.

G.4. DIFFICULTIES IN INTERPRETING THE BENCHMARK MODELING

The benchmark analyses are summarized in Thomas et al. (2007) as average BMD estimates for genes in a given GO that were statistically significantly dose related. The benchmark modeling was then used by the authors to identify that the dose below individual cellular processes was judged to be "not altered."

The BMD definition used by these authors is quite stringent: it defines an effect so that only 0.005 of controls will be considered affected and sets the BMR corresponding to this dose at 0.105. The net effect is that the BMD is the air level, such that the increase in the mean response is 1.349 × standard deviation. This is essentially an arbitrary definition. For comparison, if 0.05 of controls are considered affected and the BMR is set at 0.1 (common values that are applied to whole animal data), the BMD is the air level such that the increase in the mean response is 0.608 × standard deviation. Thus, if this definition had been used (as is traditionally the case), the BMD estimates would all be 2.2 times smaller than those obtained by Schlosser et al. (2003). Furthermore, the analysis assumes equal variance in all dose groups. Thus, further consideration of these issues with regard to interpretation of the BMR obtained from these studies is needed before it can be used in regulatory exposure setting. Secondly, lower confidence limits on the BMDs need to be derived for the data in Andersen et al. (2008).

G.5. STATISTICAL SENSITIVITY OF THE DATA FOR DOSE-RESPONSE

Another cautionary note pertains to the qualification of gene array studies as being extremely sensitive. Such a qualification should actually refer to the fact that only tiny amounts of mRNA are needed, that is, the sensitivity of the assay per se for measuring gene expression. However, this should not be confused with the sensitivity needed to identify the very small dose-related changes at low dose. Andersen et al. (2008) reports on results of studies that involve small numbers of animals in each dose group (five or eight). Despite the limited power in such studies, the paper equates the absence of a statistically significant effect with no effect. This limitation is generally true of studies of the dose responses of changes in gene expression conducted to date; they have generally relied on very few animals (≤10 per dose group). Since there will likely always be background amounts of gene expression, quantifying the dose response requires statistically significant changes in gene expression as a function of dose. If the genomic data involve even fewer animals per group than the histopathological data, they have even less power to delineate the dose response; in particular, whether there is a threshold at low exposures. This is illustrated by the example in Figure G-1 of the dose responses for epithelial hyperplasia. The data in this figure are from lesion 2 in Andersen et al. (2008); the linear regressions and confidence limits were determined by EPA These appear equally consistent with both a threshold at around 1 ppm and a linear response down to zero.

G.6. LENGTH OF THE STUDY AND STOCHASTIC EVENTS

Another significant consideration with regard to MOA conclusions that are pertinent to the disease process is the length of the study, 15 days. If formaldehyde-induced tumor formation is a stochastic process (e.g., genotoxicity), then exposure of a small number of animals to low concentrations for 15 days may not be long enough to detect changes that might occur under long-term exposure scenarios.

Relatedly, it has been suggested that gene (and protein) expression is a stochastic process whereby steady state gene expression obeys Poisson statistics (i.e., distribution of rare events), and that events of interest may occur in a single cell or small number of cells in which larger tissue samples can average out such stochastic events and prevent the detection of nonaverage behavior (Quakenbush, 2007). Given the implied difficulty in such an analysis, duration of exposure may be one of the most tenable ways of addressing whether a chemical increases the probability of an adverse response.

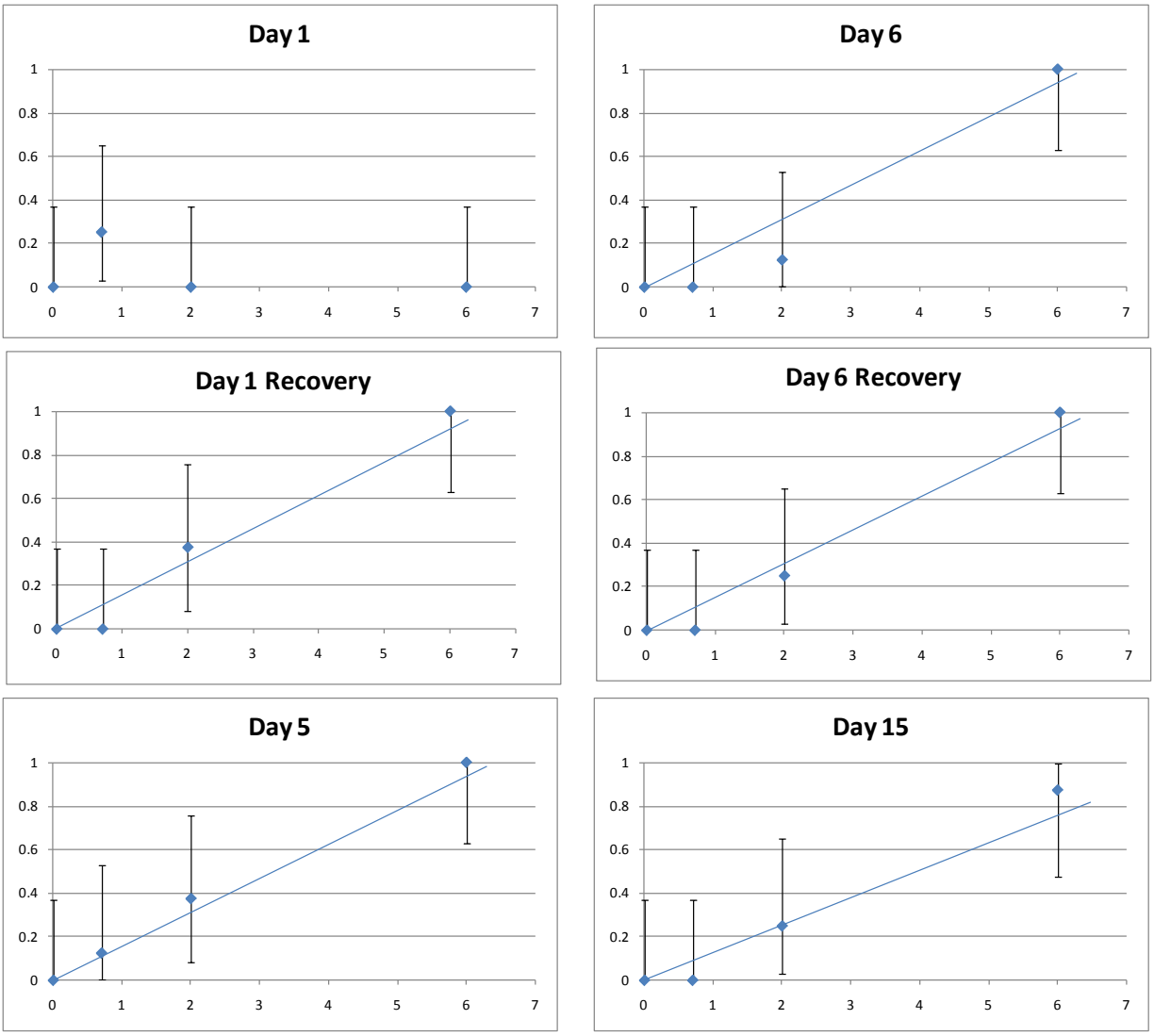

Figure G-1. Graphs of epithelial hyperplasia (Lesion 2) versus formaldehyde concentration (ppm) with 95% confidence intervals (with linear fit by eye).

Source: Fit to data from Andersen et al. (2008).

G.7. OVERALL CONCLUSION

We believe our analyses of the presentations in Andersen et al. (2008) and Daston (2008) are generally useful with regard to future developments in quantitative analyses of genomic data if they are to be of relevance to risk assessment. For risk assessment, rather than focusing on what responses are statistically significant, an analysis should focus on (1) what range of values of critical parameters (e.g., gene expression) are consistent with the data, and (2) what these values imply for whole animal risk. This is of course, an extremely difficult proposition because

we do not know nearly enough about how changes in genes quantitatively affect whole animal risk, or even which genes are important.

Appendix H

1

APPENDIX H

EXPERT PANEL CONSULTATION ON QUANTITATIVE EVALUATION OF ANIMAL TOXICOLOGY DATA FOR ANALYZING CANCER RISK DUE TO INHALED FORMALDEHYDE

The National Center for Environmental Assessment convened an expert panel of scientists for advice on evaluating available approaches for incorporating biological information in analyzing animal tumor data for assessing cancer risk due to inhaled formaldehyde. This Appendix pertains to the major deliberations and results of that meeting and is divided into three sections.

A. Scope and Agenda of Meeting on Quantitative Evaluation of Animal Toxicology Data for Analyzing Cancer Risk due to Inhaled Formaldehyde. October 28 & 29, 2004.

B. Summary of Consultative Meeting on CIIT Formaldehyde Model. October 28 & 29, 2004.

C. Meeting Report from Dr. Rory B. Conolly

A. Scope and Agenda of Meeting on Quantitative Evaluation of Animal Toxicology Data for Analyzing Cancer Risk due to Inhaled Formaldehyde

October 28 & 29, 2004. Washington, DC.

This meeting is to assist EPA in evaluating available approaches for incorporating biological information in analyzing animal tumor data for assessing cancer risk due to inhaled formaldehyde. The CIIT Centers for Health Research (CIIT) has published a novel risk assessment that links site-specific predictions of flux using computational fluid dynamics (CFD) modeling with a two-stage clonal growth model of cancer to analyze nasal tumor incidence in two rodent bioassays. The rodent models are used with corresponding human models for low-dose extrapolation of cancer risk to people.

Key predictions of the CIIT effort are a zero maximum likelihood estimate of the probability of formaldehyde-induced mutation per cell generation in the rat and a *de minimus* additional lifetime risk in nonsmokers due to continuous environmental exposure below 0.2 ppm. The National Center for Environmental Assessment is carrying out sensitivity analyses and examining variations of the CIIT model in order to understand the implications of the model structure and parameters on model predictions. In this meeting, we wish to focus on the strengths and key uncertainties of this model, the extent to which assumptions in the CIIT model are supported by biological data, and examine the impact of uncertainty and variability on the overall quantitative risk characterization.

Broadly, the discussions will focus on the following areas:

- Impact of uncertainties in dosimetry on human risk estimates
- Uncertainties in the use of experimental data on labeling index
- The model structure related to initiated cells and DNA protein cross-links
- Considerations of time-to-tumor in the clonal growth modeling
- Inferences and information on the role of mutation and cytotoxicity in estimating human risk
- Relative merits of benchmark dose modeling vs. the 2-stage clonal growth model

Discussions on Mode of Action are expected to be an integral part of several of the sessions. Therefore a specific time-slot is not set aside for this purpose.

The meeting will have a panel discussion format. There will be no formal presentations unless necessary to elucidate an issue. Various attachments referred to in the Agenda below, as well as the relevant manuscripts will be sent separately.

Specifically, we suggest the following issues upon which to focus the discussion in the above areas, and approximate time frames and discussion leads, although discussants should feel free to bring up other critical issues.

I. Introduction and purpose of discussion

Peter Preuss.

9:00 AM, Oct 28

II. Impact of uncertainties in dosimetry on risk estimates

Lead discussant: Linda Hanna

9:15 - 11 AM Oct 28

Boundary conditions

The CFD modeling specified a mass transfer coefficient as a boundary condition on the nasal lining, adjusting the value of this coefficient on the "absorbing" portion of the lining so as to match simulated overall uptake in the rat nose to the experimentally determined average overall uptake. This value was then used for the corresponding human nasal lining. Are these boundary conditions appropriate surrogates for the underlying pharmacokinetics, including saturation in metabolism and mucociliary clearance, particularly with reference to humans?

Turbulence

Turbulent flow has been seen to occur in experimental models of the human nose at some of the higher flow rates at which the CFD models were used in CIIT's assessment. It is not likely that the CIIT CFD model can reliably identify signatures of transition to turbulent behavior. Turbulent flow can significantly alter regional uptake patterns. Additionally, significant mass balance errors were seen at the higher flow rates in the human flow models. Discuss if these are likely to impact significantly on risk estimates.

Interindividual variability

The CIIT assessment has focused on the nasal anatomy of a single individual. Discuss the implications of interindividual variations in nasal anatomy on the population distribution in risk.

III. Uncertainties in the use of experimental data on labeling index

Lead discussant: George Lucier

11AM – 11:45 AM, 1:00 - 3:15 PM Oct 28

Cell-replication rate and its relationship to flux is a critical determinant of risk. Therefore uncertainties and variability in measurement of the unit length labeling index and its use in the CIIT clonal growth modeling need to be characterized.

1. Discuss the strengths, uncertainties and limitations associated with estimating cell replication rates from the unit length labeling index (ULLI).
    a. For example, a constant ratio of the measured ULLI to the labeling index (LI) that is used in the model is assumed. Is it valid to assume this ratio to be constant across nasal sites, dose and exposure time.
    b. How uncertain is this ratio?

2. Considering the large patterns of variability in the ULLI data, discuss the validity of using ULLI averaged over site and exposure times.
    a. The averaging loses information on the sequential effect of change with time, and on significant differences among sites.
    b. How sensitive is the clonal growth modeling result to these variations in the dose-response function for cell replication rates vs. flux to the tissue? A discussion of this question in this session is intended to serve as input to later deliberations on the issue.

3. Discuss the validity of combining data collected in different experiments using different labeling methods, and the validity of estimating cell replication rates from LI or ULLI measured in a single pulse labeling experiment.

    See attachment C: "ULLI Dose-Response Modeling and Statistical Analysis" for a discussion of these issues, and Moolgavkar and Luebeck (1992).

IV. **Model Structure: Birth and death rates for Initiated cells, Role of DPX**

Lead discussant: Kenny Crump

3:30 - 6:00 PM Oct 28.

Parameters for initiated cells

1. The CIIT analysis of ULLI data allows for a virtual threshold in dose in the replication rate of normal cells. Discuss the validity of ascribing such a behavior to initiated cells considering the sensitivity of 2-stage model results to the initiated cell replication rates.

2. Discuss the treatment of death rate for initiated cells in the model (set equal to birth rate of normal cells in Conolly et al., 2003) and implications for confidence in model predictions.

    Also see Attachment A (memo from Rory Conolly) and Attachment D (EPA discussion of CIIT clonal growth modeling and some sensitivity analyses. . .)

Treatment of DNA protein cross-links (DPX) in clonal expansion model

3. Formaldehyde-induced mutation is modeled as taking place only while DPX are in place with DPX undergoing rapid repair. Discuss the possibility of persistent genetic damage that extends beyond the DPX half-life and enhances mutation. How might this issue be included in the model structure?

V. Considerations of time-to-tumor in the CIIT clonal growth modeling

Lead discussant: Christopher Portier

8:30 – 11:00 AM, Oct 29.

1. A number of issues affect likelihood values and the model fit to the time-to-tumor data. Discuss assumptions in the treatment of time-to-tumor in the CIIT clonal expansion model, and their impact on parameter estimates. For example,
    a. Results in Conolly et al. (2003, 2004) are derived considering all tumors to be fatal. Note in this context that serially sacrificed animals have been combined with those experiencing mortality—the effect of this is visible as irregularities in the time-to-tumor curve.
    b. How is the time variability in ULLI likely to impact on the time-to-tumor predictions?

2. Long delay times are predicted by the model for observation of detectable tumor. Is this compatible with the assumption of rapidly fatal tumors?

3. Discuss the weight to be given to differences in likelihood when comparing with variations on the Conolly et al. (2003) model structure such as in Attachment A or D.

VI. Inferences on the role of formaldehyde-induced mutation and cell proliferation

Lead discussant: Dale Hattis

11:15 – 12:00 PM, 1:00 – 4:00 PM, Oct 29.

1. The model structure in Conolly et al. (2003) predicts a zero maximum likelihood estimate for the constant of proportionality (KMU) linking DPX to the probability of formaldehyde-induced mutation per cell generation. Examine the strength of this conclusion, and the extent to which an insignificant probability of formaldehyde-induced mutation per cell generation is supported by data.

2. Discuss the biological relevance and validity of model-estimated parameters, particularly in the context of low-dose predictions.
    a. Discuss possible avenues to validate CIIT cancer model predictions.

3. Discuss the validity of using cell replication rates determined for the rat to predict human risk in a population.

4. In the face of uncertainties, are the results in Conolly et al. (2003, 2004) conservative in the sense of overpredicting risk?
    a. Discuss the extent to which sensitivity analyses have addressed this issue and the extent to which sensitivity analyses can speak to the strength of the model. *[See Attachments A: Memo from Conolly, and D: EPA discussion of CIIT clonal growth modeling and some sensitivity analyses….]*

VII. Benchmark Dose Modeling

Lead discussant: Kenny Crump
4:15 – 5:30 PM, Oct 29.

Discuss the relative merits of using a benchmark dose approach that incorporates biological modeling (such as estimating flux to tissue or DPX levels) as compared with the CIIT 2-stage model for cancer. (*See attachment E and Schlosser et al., 2003.)*

B. Summary of Consultative Meeting on CIIT Formaldehyde Model October 28 & 29 2004, NCEA, Washington, DC

Date: November 10, 2004

Ravi P. Subramaniam, Ph.D.
Quantitative Risk Methods Group
National Center for Environmental Assessment, ORD, US EPA

This is a broad summary of the most important issues at the formaldehyde meeting.

It was generally felt by consultants that the broad framework of the approach adopted by CIIT, namely the use of a two-stage model for cancer, the linking of localized flux to cell replication rates and DPX concentration, and the expression of formaldehyde-induced mutation as a linear function of DPX, was reasonable.

Potential errors in the dosimetry modeling were seen not to have a significant effect on risk estimates. The boundary conditions used were discussed to be a reasonable representation of the pharmacokinetics for both rats and humans. The discussion on the impact of interindividual variability of nasal anatomy was not particularly conclusive. It was determined that there was likely to be much less variability in reactive gas uptake than that seen in particulates.

Crucial errors were however identified on several fronts in the manner in which the clonal growth model had been implemented in the CIIT effort. Dr. Portier felt that the calculation of probability was seriously flawed on account of lumping serially-sacrificed animals and animals that died of tumor together, while at the same time assuming rapid fatality of all tumors. This was seen to significantly alter the calculation of tumor probability (the shape of the dose-response curve), and his insight was that a correction was likely to allow for a substantially higher value for the probability of formaldehyde-induced mutation at low-dose. The best estimate for this probability is now zero in the model. Drs. Crump, Portier and Hattis argued that replacing this estimate by an upper confidence bound on KMU (the coefficient determining the role of DPX in the probability of mutation per cell generation), keeping other structural problems in the model unexplored, or other parameters fixed, would not be enough. There was a discussion on the need to provide confidence bounds on risk determined by allowing all the parameters to vary. Drs. Crump and Hattis (and Portier?) felt such an estimate would be very different from that calculated based on individual parameters.

Drs. Crump, Hattis and Portier urged us not to be constrained by the optimal likelihood values of a single plausible model, and underscored the need to explore a variety of biologically reasonable model structures as a requisite for utilizing such a model in risk assessment.

Likelihood was seen to be an inadequate expression of what is to be considered an optimal model (okay only for comparing models that were nested, etc.). These models should allow the expression of variability and uncertainty in the data, as well as in underlying assumptions in model specification. Dr. Crump (and Hattis also?) felt that alternate model structures, if explored, could potentially lead to risk estimates, for the range below the observed data, that were higher by several thousands.

Dr. Crump cautioned that extrapolating to human using the hockey or J-shaped cell replication curve used in the rodent carried with it a large uncertainty that had not been characterized in the Conolly modeling.

Dr. Portier expressed concern over the manner in which historical and concurrent controls were lumped together. The thrust of Portier's comments was that such a combination of controls was generally not done. The large number of historical controls was likely to significantly bias the impact of the bioassay data in determining the time-to-tumor fits.

There were various discussions about the pros and cons of constructing a joint likelihood of the cell replication data and the tumor data, and the weights to be assigned to the separate likelihoods. This was considered to be problematic by Dr. Portier.

Dr. Crump's opinion was that the Conolly model, and those explored by EPA, fit the tumor data poorly, and that an improved description of the tumor data was needed before the model could be used for low-dose and interspecies extrapolation.

Drs. Lucier and Hattis placed emphasis on including the early-time cell replication data instead of constructing a time-weighted average. It was felt that the two Monticello experiments could not be combined together as in Conolly et al. Dr. Lucier felt that the early-time data would have a greater impact in the progression of carcinogenesis. In general, the effect of "time" was considered to have significant effects on the time-to-tumor modeling, and they urged us to incorporate time-dependent terms in the modeling. CIIT expressed willingness to provide the original cell replication data to us for further analysis. (Further discussion on this matter did not take place in the open forum.)

Preliminary indications are, particularly based on Dr. Portier's insight, that the currently-held "de-minimus" picture of low-dose risk, as expressed in Conolly et al. (2004), is not likely to be the case if these various suggestions are incorporated in the modeling.

C. Meeting Report from Dr. Rory B. Conolly

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July 24, 2005

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Dear Dr. Nourse,

The following is my final written report on the formaldehyde review meeting held at the U.S. EPA in Washington, D.C. on 28-29 October, 2004.

EPA provided no guiding philosophical statement about the criteria being used to evaluate the CIIT assessment. The new Guidelines for Carcinogen Assessment state that the preferred default approach is to use a biologically based model. Since the key components of the CIIT assessment have been published in the peer-reviewed literature and have undergone several peer reviews other than the current NCEA effort, one has to wonder just how high the bar is set for acceptance of biologically based assessments. Given the time and resources expended on the CIIT assessment and the richness of the supporting data base, I find it difficult to imagine what an acceptable biologically-based assessment might look like if in the end the CIIT assessment is deemed not acceptable by NCEA. If this is in fact the outcome it will have major implications for the likelihood that anyone will be willing to commit the significant resources needed to develop of these kinds of risk assessment models.

The documents provided in advance of the October 2004 review meeting were collectively a discussion of uncertainty about the CIIT work. With respect to the clonal growth model, however, no new risk predictions were provided, so there was no way to judge how the uncertainties that NCEA identified might impact predicted risk. Evaluation of the significance of "uncertainties" when the impact of the uncertainties on the predicted risk is not known is itself an uncertain process.

A related concern is that there did not seem to be any consideration of the historical context of the CIIT assessment. EPA developed formaldehyde assessments in 1987 and 1991. The 1987 assessment used ppm as the input and the LMS model for the dose-response prediction. The 1991 assessment used DPX as a dosimeter and the LMS model. BMD assessments have since become available from other sources such as Paul Schlosser's work. The risk predictions of the BMD models are similar to the 1991 LMS assessment. Both the DPX-LMS and BMD assessments predicted somewhat less risk than the 1987 assessment, establishing the trend of less risk with increased incorporation of relevant data. I have always argued

(probably initially at the 1998 Ottawa review) that the historical context is the appropriate context for evaluating the CIIT clonal growth model. For a "level playing field" the uncertainties of the 1987 and 1991 assessments, and of the more recent BMD models, should be analyzed to the same degree as the clonal growth model. Does NCEA think that, because the LMS and BMD approaches used structurally simpler dose-response models and much more limited data inputs, they are less uncertain? The NCEA analysis seemed to be implying that use of more data and of a biologically more realistic model structure actually makes the CIIT approach more uncertain than the LMS and BMD approaches. I encourage NCEA to consider how uncertainties that can be evaluated explicitly in the structurally rich CIIT model compare to hidden uncertainties in the simpler models, where the hidden uncertainties encompass, for example:

1. Missing or incomplete descriptions of the regional dosimetry of formaldehyde.
2. Lack of simultaneous incorporation of the directly mutagenic and cytolethal/regenerative proliferation modes of action.
3. Lack of explicit consideration of the multistage nature of cancer.
4. Lack of consideration of the growth kinetics of initiated cell populations
5. Lack of evaluation of the measured J-shaped dose response for regenerative cellular proliferation.

A careful, balanced comparison of the CIIT assessment with the previous assessments along these lines would be informative with respect to the suitability of the CIIT assessment as the basis for a new IRIS listing for formaldehyde.

A further concern involves the peer-review of the CIIT formaldehyde assessment held in Ottawa in 1998. This review was sponsored by the U.S. EPA and Health Canada and involved what was arguably a world-class review panel. The CIIT assessment was not in its final form at that time, though we did provide a detailed description of the overall approach and the specific methods we were using to generate dose-response predictions. The 1999 CIIT document and the subsequent peer-reviewed publications are responsive to the comments and suggestions raised by the reviewers. My concern is that no information was provided on the role that Ottawa review plays in the ongoing review of the CIIT formaldehyde assessment by NCEA. Should the October 2004 review be viewed as standing on the shoulders of the 1998 review or as being in parallel to it? It was not at all clear to me that the October 2004 review in any way utilized the judgments of the 1998 review. It seems that the 2004 review was more of a parallel effort and that the 1998 review was ignored and was effectively a waste of time and money. I would like to have some clear understanding of how the 2004 review effort should be viewed relative to that of 1998.

In closing, let me reiterate that while the detailed examination of the CIIT formaldehyde assessment is laudable, this examination should be conduced with an eye to the historical context of formaldehyde risk assessment on the one had and, on the other hand, to a concern for encouraging, and not discouraging, development of biologically based risk assessment models.

Sincerely yours,

Rory B. Conolly, Sc.D., D.A.B.T.

- End of Volume IV -