



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).

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

14 **4.1.1.1.1. *Epidemiologic literature.***

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

25 **4.1.1.1.1.1. Residential epidemiology.**

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

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

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

1 (1984) showed approximately 17.5% predicted prevalence of burning eyes. The prevalence of
2 burning eyes rose linearly to approximately 65% prevalence at 0.5 ppm, with some diminishment
3 in the rate of rise up to approximately 80% prevalence at 0.8 ppm. While only 65 out of 208
4 randomly selected homes volunteered to complete the health questionnaires, the investigators
5 were able to complete home formaldehyde vapor measurements on all the homes and reported
6 nearly an identical distribution of formaldehyde concentrations in participating and
7 nonparticipating homes. Demographic characteristics of some of the nonrespondents were
8 available and were reported as nearly identical to those of participants. There was no indication
9 of selection bias. Confounding is unlikely to explain such a strong concentration response.

10 These findings of associations of sensory irritation with residential exposures to
11 formaldehyde are further supported by studies that did not examine concentration response but
12 nonetheless assessed the association of formaldehyde with sensory irritation. Similar findings to
13 those of Ritchie and Lehnen (1987) and Hanrahan et al. (1984) have been reported in other
14 residential studies of increased symptoms in association with formaldehyde exposure (Liu et al.,
15 1991; Thun et al., 1982; Dally et al., 1981). Dally et al. (1981) collected data in 100 “complaint
16 structures” (65% mobile homes, 27% conventional homes, 2% travel trailers, 2% office
17 buildings, etc). Of these, 60% were from home owners contacting the health department and
18 30% from physician referrals. Twenty percent of the buildings had concentrations below the
19 limit of detection (0.1 ppm), 20% had levels at or above 0.81 ppm, and overall the concentrations
20 ranged from below detection to above 3 ppm with an overall median of 0.35 ppm. The median
21 levels were 0.47 and 0.10 ppm for mobile and conventional homes, respectively. No other
22 contaminants were measured. Eye, nose, and throat irritation were reported in a high percentage
23 of occupants (eye irritation 68%, burning eyes 60%, runny nose 60%, dry or sore throat 57%,
24 cough 51%), but these were not reported as a function of dose or home type. Thus, there was no
25 control group to which rates of irritation could be compared. However, symptoms reportedly
26 stopped in 89% of occupants when they left the “complaint structure.” The most recent
27 residential study was performed on over 1,000 mobile homes with 1,394 participants (Liu et al.,
28 1991). Home formaldehyde concentration ranged from below 0.01 to 0.46 ppm. Analyses used
29 logistic regression to control for potential confounders. Eye irritation was positively associated
30 with formaldehyde with a clear concentration response demonstrated with cumulative exposure.
31 During the summer and winter months, formaldehyde exposure was associated with burning
32 eyes. In the winter months, formaldehyde exposure was associated with sore throat. There was
33 no association of formaldehyde exposure with cough or running nose during either season. Liu
34 et al. (1991) also report a synergistic effect on irritation by formaldehyde exposure and chronic
35 disease prevalence. Thun et al. (1982) reported increased symptoms of itchy skin and “wheezing

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

4 While not strictly a residential epidemiology study, Olsen and Dossing (1982) studied
5 occupational exposures within mobile and nonmobile daycare centers. They reported the mean
6 concentration in mobile and nonmobile day care centers were 350 (200–450) ppb and 65
7 (40–90) ppb, respectively. Adverse eye, nose, and throat irritation were significantly elevated in
8 the workers ($n = 70$) in the mobile units as compared with those in nonmobile units ($n = 34$).
9 The authors also state that a high percentage of workers in the mobile day cares reported that the
10 symptoms disappeared after working hours; however, the authors did not report any such
11 percentages among those working in nonmobile units.

13 **4.1.1.1.1.2. Occupational epidemiology.**

14 Horvath et al. (1988) compared irritation symptoms between 109 workers at a
15 particleboard manufacturing plant and 264 workers at food plants as a control group. The mean
16 8-hour time-weighted average (TWA) formaldehyde concentrations between these two groups
17 were 0.69 ppm (range 0.17–2.93) and 0.05 ppm (range 0.03–0.12), respectively. Eye, nose, and
18 throat irritation were more common among the former group (prevalence of symptoms during a
19 work shift: throat sore or burning—test 22.0%, controls 3.9%; cough—test 34.9%, controls
20 18.9%; burning of nose—test 28.4%, controls 2.0%; stuffy nose—test 33.9%, controls 14.2%;
21 itching of nose—test 21.1%, controls 7.9%; eyes burning or watering—test 39.5%, controls
22 9.1%; eyes itching—test 19.3%, controls 7.1%).

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

31 A Swedish study conducted at a chemical plant found that nasal and eye discomfort were
32 reported by 64 and 24%, respectively, of workers ($n = 70$) exposed to formaldehyde (range
33 $0.05\text{--}0.50 \text{ mg/m}^3$ with a mean of 0.26 mg/m^3) versus 25 and 6%, respectively, of the control
34 group made up of clerks from the local government ($n = 36$). In addition, the majority of
35 workers exposed to formaldehyde reported that their symptoms were relieved during weekends

1 and vacations (Holmström and Wilhelmsson, 1988). Another study by the same authors
2 (Wilhelmsson and Holmström, 1992) reported similar results. In this study irritation prevalent
3 among 66 workers from a formaldehyde-producing plant was compared with that seen among
4 36 community clerks. The workers were exposed to 0.26 mg/m³ of formaldehyde (range
5 0.05–0.6 mg/m³). The clerks were exposed to an average of 0.09 mg/m³. Nasal and eye
6 discomfort were reported at rates of 53 and 24%, respectively, among the workers. Among the
7 community clerks, 3 and 6%, respectively, reported discomfort.

8 Holness and Nethercott (1989) reported significant increases in eye irritation (42 vs.
9 21%) and nose irritation (44 versus 16%) among 84 funeral service workers as compared with
10 38 controls (students and individuals from a service organization). The former group had been
11 actively embalming for approximately 10 years and had nearly twice the pack-years smoked as
12 the controls. The exposure concentration in both groups was 0.36 and 0.02 ppm, respectively.
13

14 **4.1.1.1.1.3. Epidemiology on laboratory students.**

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

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

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

1 2.12 ppm (range 1.7–2.4), while the gas samplers worn on the students’ chests averaged 2.4 ppm
2 (range 1.8–3.8). Another study of students in an anatomy laboratory class in Japan (Takigawa et
3 al., 2005) measured formaldehyde concentrations and irritation symptoms before and after the
4 installation of a ventilation system. This system reduced the median personal formaldehyde
5 exposure concentration from 2.7 to 0.72 ppm. Before installation of the ventilation system, the
6 students complained about exacerbation of all the sensory irritation symptoms on average. The
7 increase in 8 out of 25 symptoms was significantly reduced after installing general ventilation
8 ($p < 0.05$). After installation of the ventilation system, a dose-dependent relationship with
9 formaldehyde was seen for irritated eyes but not for itchy nose.

10 Akbar-Khanzadeh et al. (1994) detected mean personal area levels of formaldehyde at
11 1.24 ppm and a range of 0.1–2.94 ppm from personal air sampling devices. Almost 90% of the
12 students in this study reported eye irritation, 74% reported nose irritation, and close to 30%
13 reported throat irritation during or after exposure to formaldehyde during the laboratory period
14 after having completed at least 6 weeks of laboratory sessions with formaldehyde exposure. In
15 addition, Uba et al. (1989) demonstrated that symptoms of eye, nose, and throat irritation were
16 correlated with formaldehyde exposure among medical students by comparing students’
17 responses on a questionnaire completed after a lab with formaldehyde exposure to a
18 questionnaire completed after a lab with no formaldehyde exposure. The authors compared
19 questionnaires completed prior to students’ first anatomy lab to a questionnaire completed
20 7 months later. Reports of cough were more frequent after the 7 months. These students were
21 exposed to a mean level of 1.9 ppm (range 0.1–5.0) while dissecting (measured using portable
22 infrared spectrophotometer), and a TWA from all laboratory activities ranged from below limits
23 of detection to 0.93 (measured using personal sampling devices in the students’ breathing zones).

24 25 **4.1.1.1.2. Acute studies: controlled chamber exposures.**

26 Results from controlled human studies demonstrate eye, nose, and throat irritation in
27 association with formaldehyde exposure (Lang et al., 2008; Yang et al., 2001; Krakowiak et al.,
28 1998; Kulle, 1993; Green et al., 1989, 1987; Kulle et al., 1987; Sauder et al., 1987, 1986;
29 Schachter et al., 1987, 1986; Witek et al., 1987; Day et al., 1984; Bender et al., 1983; Anderson
30 et al., 1983; Weber-Tschopp et al., 1977; Andersen, 1979; Schuck et al., 1966). A key advantage
31 of chamber studies is the ability to monitor and closely control formaldehyde concentrations
32 during exposure. However, chamber studies may also be limited by other aspects of the study
33 design, including small number of participants, use of healthy volunteers, short exposure
34 durations (a few minutes), and often studies were conducted with only one exposure group and at
35 relatively high concentrations (>1 ppm). The lack of multiple exposure levels in many studies

1 limits the understanding of exposure-response relationships. Additionally, numerous reports that
2 demonstrate multiple symptoms of eye, nose, and throat irritation at levels at or above 1 ppm did
3 not explore lower levels of exposure and can only be used for primary hazard identification
4 (Yang et al., 2001; Green et al., 1989, 1987; Sauder et al., 1987, 1986; Schachter et al., 1987,
5 1986; Witek et al., 1987; Day et al., 1984).

6 The National Aeronautical and Space Administration conducted experiments in closed-
7 environment living, including environmental monitoring and air quality. James et al. (2002)
8 quantified air pollutants, including formaldehyde, during 30, 60, and 90-day tests in a closed
9 chamber study of a Lunar-Mars life support chamber. Unfortunately, the detection methods used
10 during the 30-day test were not sensitive enough to detect formaldehyde at levels below 2
11 mg/m³. Thus, badge samples were obtained in the 60-day and 90-day tests and provided greater
12 detection sensitivity (to 0.02 mg/m³). Measured values of formaldehyde increased over time. In
13 the 60-day test, formaldehyde levels were well above accepted limits (data not shown). Health
14 effects data are limited since there were only four crew members. One crew member reported
15 eye and upper airway irritation at formaldehyde concentrations of 0.25 mg/m³ (203 ppb) on day
16 15. It should also be noted that astronauts are exceptionally healthy individuals, and these data
17 should be interpreted carefully when determining expected health effects in the general
18 population. The experimenters determined that formaldehyde levels increased as temperature
19 increased. Formaldehyde was also linked to murals lining the chamber and was subsequently
20 removed before executing the 90-day study. Between days 0 and 60, formaldehyde levels
21 remained between 0.02 and 0.04 mg/m³, with one sharp peak that occurred at day three to 0.07
22 mg/m³. Between days 60 and 90, formaldehyde concentrations increased to 0.07 to 0.09 mg/m³.
23 The increase was attributed to an incomplete oxidation of methanol in a catalytic bed rather than
24 in excessive off-gassing of formaldehyde. No crew members reported any adverse effects in the
25 90-day study.

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

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

4 Kulle (1993) and Kulle et al. (1987) enrolled 19 healthy volunteers and exposed them to a
5 range of formaldehyde concentrations. At 2 ppm, 53% reported mild or moderate eye irritation
6 (32% mild, 21% moderate). At 3 ppm, 100% of subjects exposed at this level ($n = 9$) reported
7 irritation. The reported increase in irritation was shown to correspond with increasing
8 formaldehyde concentration in a linear fashion. Mild nose/throat irritation was present among
9 37% of those exposed to 2 ppm of formaldehyde. Odor detection was very similar to the
10 distribution seen for eye irritation. Nineteen subjects performed light to moderate exercise while
11 exposed to 2 ppm; there was no increase in report of eye irritation, but nose/throat irritation did
12 increase. The data were reanalyzed (Kulle, 1993), and thresholds for irritation were found to be
13 0.5–1 ppm for eye irritation and 1 ppm for nose/throat irritation.

14 Yang et al. (2001) reported that eight individuals exposed to varying levels of
15 formaldehyde (1.65, 2.99, and 4.31 ppm) had mild to moderate eye irritation during the 5-minute
16 exposures. The increase in irritation was detected at 30 seconds with exposure to 1.65 ppm of
17 formaldehyde. The highest severity ratings at this concentration occurred between 60 and
18 90 seconds. Frequency of eye blinking was also measured. The peak in blinking rate occurred
19 after about 1 minute of exposure and then decreased almost back to a normal rate after 5 minutes
20 of exposure. Higher formaldehyde concentrations were associated with increased frequency of
21 blinking compared with the 1.65 ppm exposure.

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

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

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

3 Assessment of sensory irritation for pain and discomfort often relies on self-reporting,
4 using symptom questionnaires and severity ratings (e.g., mild, moderate, severe). In the case of
5 formaldehyde, subjective ratings of eye irritation correlate positively with eye-blinking
6 frequency (Lang et al., 2008). Lang et al. (2008) saw an increase in eye blinking after
7 195 minutes of exposure to formaldehyde at 0.5 ppm with four peak exposures of 1 ppm. After
8 this amount of time and formaldehyde exposure, there was also an increase in moderate eye
9 redness. Weber-Tschopp et al. (1977) reported that, among concentrations ranging from 0.03 to
10 3.2 ppm, eye-blinking frequency was increased at 1.7 ppm; similarly Yang et al. (2001) reported
11 increased blinking at >1.5 ppm (the lowest concentration examined). There are studies that
12 suggest that psychological factors (e.g., anxiety) can impact the perception of irritation—and
13 perhaps more so at lower concentrations (Lang et al., 2008; Ihrig et al., 2006; Dalton, 2003).
14 However, when Lang et al. (2008) controlled for mood prior to exposure, subjective symptoms
15 of eye, nasal, and olfactory irritation were significantly related to exposure (0.5 ppm)

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

22 Andersen (1979) and Anderson and Mølhave (1983) reported on a controlled experiment
23 in which 16 individuals were exposed to varying levels of formaldehyde for five hours and rated
24 their level of discomfort over the exposure period. Discomfort occurred within 1 hour at
25 formaldehyde exposure levels of 1 and 2 mg/m³ (Andersen and Mølhave, 1983) After 2 hours,
26 increasing discomfort was reported among the groups exposed to 0.3 and 0.5 mg/m³. Subject
27 reported that discomfort was mainly conjunctival irritation and dryness in the nose and throat.
28 Subjects complained at all four concentrations of formaldehyde: 0.3, 0.5, 1.0, and 2.0 mg/m³ and
29 of 16 subjects, 3, 5, 15, and 15 subjects complained at each respective exposure concentration
30 (Andersen and Mølhave, 1983).

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

1 performed at the start of the study found that these individuals had pulmonary function similar to
2 that seen in healthy individuals. The workers in this study reported subjective measures of eye,
3 nose, and throat irritation after 40 minutes of exposure to 2 ppm of formaldehyde. However, the
4 2 ppm acute exposure in this study may be sufficiently high to induce significant irritation in
5 most individuals. Krakowiak et al. (1998) reported that 10 asthmatics with occupational
6 exposure to formaldehyde (via formaldehyde solutions or pure gaseous formaldehyde) exhibited
7 similar symptom scores to healthy controls (never exposed to formaldehyde in the workplace)
8 exposed to 0.4 ppm formaldehyde for 2 hours. The mean symptom scores and standard
9 deviation (SD), which included information on sneezing, rhinorrhea, mucosal edema, and
10 itching, were 4.6 ± 1.6 (mean \pm SD) for asthmatics and 4.3 ± 1.2 for healthy subjects
11 immediately after inhalation. These dropped to 1.8 ± 1.2 and 1.2 ± 1.3 , respectively, 4 hours
12 after the exposure. It is unclear if sensitive individuals may not be represented in either of these
13 groups, as the workers were tolerating their exposures during the work shift “healthy worker”
14 effect. However, residents ($n = 9$) exposed to formaldehyde in their homes, who complained
15 about adverse effects from the material, but with no occupational exposure reported eye, nose,
16 and throat irritation at a similar rate as controls (individuals in homes without formaldehyde or
17 individuals in homes with formaldehyde but not reporting adverse effects [$n = 9$]) after a
18 90-minute exposure to 1 ppm (Day et al., 1984). The number of individuals reporting eye
19 irritation, nasal congestion, and throat irritation were seven, three, and two among sensitive
20 individuals and eight, four, and three among controls, respectively. These individuals may be
21 considered a sensitive population since they had “previously complained of various
22 nonrespiratory effects from the UFFI in their homes” (household concentrations unknown).

23

24 **4.1.1.2. Pulmonary Function**

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

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

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

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

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

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

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

16 17 **4.1.1.2.1. *Epidemiologic literature.***

18 The potential adverse effects of formaldehyde exposure on pulmonary function in
19 humans can be examined on several time scales of interest. The epidemiologic literature
20 supports the assessment of exposures among exposed anatomy medical students where all
21 participants have well-defined and similar duration of exposure (i.e., a semester-long class)
22 (Kriebel et al., 2001, 1993; Akbar-Khanzadeh and Mlynek, 1997; Akbar-Khanzadeh et al., 1994;
23 Uba et al., 1989; Fleisher, 1987); among individuals living or working in buildings with
24 formaldehyde exposure (Franklin et al., 2000; Krzyzanowski et al., 1990; Main and Hogan,
25 1983); and among workers (industrial, manufacturing, mortuary, hospital staff, etc.) (Ostojic et
26 al., 2006; Herbert et al., 1994; Khamgaonkar and Fulare, 1991; Malaka and Kodama, 1990;
27 Nunn et al., 1990; Alexandersson and Hedenstierna, 1989; Holness and Nethercott, 1989;
28 Holmström and Wilhelmsson, 1988; Horvath et al., 1988; Kilburn et al., 1985; Alexandersson et
29 al., 1982). These studies are summarized in Table 4-1.

30 Studies of anatomy students provided information about acute effects related to
31 formaldehyde exposures experienced in the laboratory (Kriebel et al., 2001, 1993) as well as
32 special insight into the intermediate stages of possible sensitization (Kriebel et al., 1993).
33 Kriebel and colleagues (1993) examined the prelaboratory and postlaboratory PEF using Mini-
34 Wright peak flowmeters in 24 students attending 3-hour anatomy classes to dissect cadavers
35 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, <i>p</i> < 0.001 ^b Day 2 vs Day 1, <i>p</i> = 0.03) ^c Day 3 vs Day 1 <i>p</i> = 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) (<i>N</i> = 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					
				$\beta = -2.7 \pm 1.1$ L/min per week; <i>p</i> = 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 1 st lab)				
				β (SE)	<i>p</i> 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			
				Mean percent cross-lab change over 3 hours (SD)		
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 were 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.		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	β (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)		
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, <i>p</i> = 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 <i>p</i> < 0.001, ^b <i>p</i> < 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/m ³ (0.34 ppm) in 1980 and 0.50 ± 0.12 mg/m ³ (0.4 ppm) in 1984.		Annual change (1980-1984), Mean (SD)			
				Smokers (<i>N</i> = 10)	Nonsmokers (<i>N</i> = 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 <i>p</i> < 0.001				
				Cross-shift change (All <i>N</i> = 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 <i>p</i> < 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 (<i>N</i> = 35)	Nonsmokers (<i>N</i> = 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 <i>p</i> < 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	4.979 ^a	4.929 ^a	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 (<i>N</i> = 100) were randomly selected with stratification by smoking status and years of occupation (< 5 and ≥5 years). The unexposed group (<i>N</i> = 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, <i>p</i> < 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 <i>p</i> < 0.001				
			Multiple regression model of pulmonary function ^a					
				β (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 <i>p</i> < 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 <i>p</i> < 0.002, ^b <i>p</i> < 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 <i>p</i> < 0.001, ^b <i>p</i> < 0.04							

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

Reference	Study design	Exposure assessment	Results, effect estimate, statistical significance				
				Lung function (percent of expected, SD)			
Ostojic 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 and stature. All were nonsmokers. The timing of the spirometric tests was not stated.	Assessment of formaldehyde exposure was not described.		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)		

1 ranged between 0.49–0.93 ppm with a geometric average concentration of 0.73 (SD 1.22) ppm.
2 Students, trained in the use of the flowmeters, took measurements at the beginning and end of
3 each session, and an additional 1 to 3 times during the sessions. The average of the 3 largest
4 values in a set was used in the analyses. The strongest pulmonary response was observed for the
5 average cross-laboratory decrement in PEF in the first 2 weeks of the study. Overall, the
6 students exhibited a 2% decrement in PEF during the laboratory session in the first 2 weeks,
7 while 5 students with any history of asthma showed a 7.3% decrement in PEF. The numbers in
8 the two groups were too small to evaluate differences in response statistically. The Kriebel et al.
9 (1993) study also shows how the acute effects of formaldehyde exposure were altered following
10 several weeks of weekly episodic exposure. By the fifth week of class, the pre- and
11 postlaboratory measurements of PEF were no longer reflecting a clearly demonstrated acute
12 effect, but, following the seventh week of episodic exposure, both pre- and postlaboratory PEF
13 continued to drop steadily until the class adjourned after 10 weeks. While the acute effects of
14 formaldehyde exposure appeared to diminish after several weeks of exposure, the intermediate
15 effect across 10 weeks was a 2.7 ± 1.1 L/minute per week drop in PEF that was statistically
16 significant ($p < 0.01$) in a model adjusting for random person effects, asthma, interaction
17 between time and asthma, and eye as well as nose symptoms of irritation. Prevalence of eye and
18 nose symptoms was associated with decreased PEF ($p < 0.02$).

19 These findings were partially corroborated in a subsequent study, which used a similar
20 study design and more thorough exposure assessment (Kriebel et al., 2001). This study,
21 involving 38 students with data for at least one week, measured formaldehyde in 6 zones in the
22 laboratory and linked students locations, recorded every 12 minutes, to the zone measurements to
23 estimate a mean formaldehyde exposure over the entire 2.5 hour lab. The geometric mean
24 formaldehyde concentration was 0.70 (GSD 2.13) ppm and the highest 12 minute exposure was
25 10.91 ppm. Generalized estimating equation models included an interaction term for recent
26 exposure (mean formaldehyde concentration during the lab) and time (natural log weeks) which
27 indicated an association with post lab PEF (percent of prelab PEF at first session) and recent
28 exposure that attenuated over several weeks. Past average exposure (average exposure over all
29 previous weeks) was associated with prelab PEF. The model indicated an approximate overall
30 decrease in maximum PEF of 1% per ppm formaldehyde associated with formaldehyde levels
31 during the laboratory in the early weeks of the course and an additional 0.7% (SE 0.3, $p = 0.02$)
32 decrease per ppm associated with past average formaldehyde levels during the weeks previous to
33 the current week. The addition of having a cold on the day of the lab reduced the coefficient for
34 past average exposure somewhat ($\beta = -0.52 \pm 0.3$, $p = 0.08$). Spirometry measurements (FEV₁
35 and FVC) before and after the course were not related to formaldehyde exposure. Class

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

5 A study of 103 medical students was performed over a period of 7 months in which the
6 students were exposed to formaldehyde in the breathing zone at a time weighted average that
7 ranged from 0.93 ppm to below the limit of detection (0.05 ppm) during anatomy laboratory
8 sessions meeting twice a week (Uba et al., 1989). Mean concentrations during dissection were
9 1.9 ppm (0.1–5 ppm). Monthly average formaldehyde concentrations were highest during the
10 first two months of the course (0.6 and 0.8 ppm) and declined over the next 5 months to 0.1 ppm.
11 Twelve students were asthmatics. Pulmonary function tests were conducted according to criteria
12 published by the American Thoracic Society before the start and at the end of the anatomy
13 laboratory on three occasions, before the laboratory began, at 2 weeks and after 7 months. The
14 largest of all acceptable spirometry values were used. Unlike the studies by Kriebel et al. (2001,
15 1993), these researchers did not find a prelab decrement in pulmonary function over the course
16 of 7 months. However, mean change for pulmonary function before and after the exposure,
17 expressed as percent of the prelab value, showed a similar pattern; at 2 weeks, all parameters
18 were decreased and, except for FEV₁/FVC, the changes were larger compared to the first test
19 day when students were not exposed to formaldehyde (FVC -0.80 versus -0.23%,
20 p value < 0.001; FEV₁ -1.04 versus -0.71%, p value 0.03; FEF₂₅₋₇₅ -1.99 versus -1.76%,
21 p value > 0.05; FEV₁/FVC -0.36 versus -.48%, p value > 0.05). At 7 months, the changes were
22 less marked, with measures showing decreases in function at the end of the laboratory session
23 (measurements taken at the 7-month time point: FVC -0.79% (change greater than day 1,
24 p > 0.001), FEV₁ -0.48% (change less than day 1, p < 0.01), FEF_{25-75%} 0.07%, FEV₁/FVC
25 0.24%). Symptoms of eye, nose and throat irritation, sneezing, rhinorrhea and chest tightness
26 (p = 0.05) were more frequent during the exposure (p < 0.01). While formaldehyde
27 concentrations appear to be comparable to those measured by Kriebel et al., Uba et al., used
28 spirometry to assess lung function at 3 separate points in time while Kriebel et al. measured peak
29 expiratory flow among subjects using multiple measures during each laboratory session
30 providing greater statistical power to detect a response to formaldehyde. Also, the lung function
31 parameters used by the two studies may have measured effects in different parts of the lung.

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

1 (approximately 3 hours) over five consecutive weeks (Akbar-Khanzadeh et al., 1994). The
2 medical students had been exposed to formaldehyde for at least 6 weeks prior to the study. The
3 time-weighted average exposure from personal sampling ranged from 0.07-2.94 ppm. More than
4 94% of the subjects were exposed to >0.3 ppm and 31.7% were exposed to an 8-hour time-
5 weighted average of >0.5 ppm. The best of 4 spirometric measurements were used in the
6 analysis. Comparing pre- and postexposures among the exposed students, on average FVC
7 decreased by 1.4%, FEV₃ decreased by 1.2%, FEV₁/FVC increased by 1.6%, and FVC_{25-75%}
8 increased 2.5%. The average percent changes in the unexposed group were -0.3, 1.30, 2.31, and
9 0.6%, respectively. The researchers also calculated correlation coefficients by examining the
10 relationship between lung function and formaldehyde concentration, but no association was
11 found. However, the pulmonary function changes were not evaluated with statistical models and
12 baseline parameters were not adjusted for height, gender, or age.. Further, age and race
13 composition varied between exposed and unexposed. Therefore, it is not clear that the results of
14 this study are informative. Akbar-Khanzadeh and Mlynek (1997) performed another study with
15 50 exposed first-year medical students and 36 unexposed second-year physiotherapy students to
16 evaluate pulmonary function changes during a 3-hour anatomy lab that met during the morning.
17 Average formaldehyde concentrations measured in the breathing zone of the students was 1.88
18 (SD = 0.96) ppm with a range of 0.30-4.45 ppm. At least 2 exposed and 2 unexposed students,
19 close in age to the exposed, were evaluated on each test day for the duration of the course. Lung
20 function over one 3-hour lab session was evaluated for each participant. Spirometric
21 measurements were conducted according to criteria published by the American Thoracic Society.
22 Prelab spirometric variables, expressed as a percentage of reference values accounting for height,
23 weight, age, sex, and race, were comparable between the two exposure groups. Although lung
24 function (percent change from prelab value) increased in both groups during the first hour, the
25 authors reported a larger increase among the unexposed students when compared with exposed
26 students during the second two hours of exposure (FVC 3.0 versus 0.9%, FEV₁ 4.1 versus 1.2%,
27 FEV₃ 3.3 versus 0.8%, forced expiratory flow during the middle of the FVC [FEF_{25-75%}] 6.1
28 versus 0.7%). These differences between exposed and unexposed remained for FEV₁, FEV₃, and
29 FEF_{25-75%} after 3 hours. Since lung function has been shown to have diurnal variation,
30 increasing during the morning, the authors suggested that formaldehyde exposure may have
31 inhibited a normal increase in respiratory function over the 3 hour period.

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

1 ppm formaldehyde. Of the 38 students who completed the questionnaire after both courses, over
2 8% reported experiencing shortness of breath during the laboratory with formaldehyde exposure,
3 but none of the students reported shortness of breath in the laboratory session with no exposure.
4 No objective measurements of formaldehyde exposure were used and spirometric tests were not
5 performed. A higher proportion of students reported other symptoms after the anatomy class
6 compared to the pathology/microbiology class including eye and nose irritation, sneezing,
7 headache, nausea, cough, throat irritation and sinus problems (p value ≤ 0.01).

8 Three studies have been performed that examine formaldehyde exposure from the
9 buildings in which individuals live or work. Krzyzanowski et al. (1990) studied the health
10 effects of formaldehyde exposure in a residential population in Arizona. A stratified random
11 sample of households of municipal employees was selected based on information about potential
12 exposure (age of housing) and potential susceptibility obtained from an initial screening
13 questionnaire. Households with children aged 5–15 years (613 adults and 298 children) were
14 eligible for inclusion in this cross-sectional study of home exposures. Residential exposures to
15 formaldehyde were based on two one-week samples from each individual’s kitchen, living area,
16 and bedroom using passive sampling tubes. The average formaldehyde concentration was
17 26 ppb, with a maximum sample value of 140 ppb. The majority of subjects (83%) lived in
18 homes with 2-week average concentrations below 40 ppb.

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

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

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

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

12 Franklin et al. (2000) studied children 6–13 years of age (median age = 9.5 years) and
13 measured the levels of formaldehyde in their homes. A total of 224 children (116 girls,
14 108 boys) with no current, or history of, upper or lower respiratory tract disease were included
15 from responses to a respiratory health questionnaire and household inventory distributed through
16 local primary schools. The length of time the children had lived in their homes was not reported.
17 Three to four-day passive samples were collected in the child’s bedroom and the main living area
18 of the house and formaldehyde levels were recorded as a time-weighted average. Clinical
19 respiratory measures were obtained at the children’s hospital. Exhaled nitric oxide was
20 measured during a single-breath exhalation and the average of three plateaus varying by less than
21 10% was used for the NO concentration value. Spirometry was conducted on all children
22 according to ATS guidelines and skin prick tests were measured for 7 common allergens. There
23 was no association between FVC or FEV1 and the indoor concentrations of formaldehyde,
24 although there were signs of lower airway inflammation as measured by levels of exhaled nitric
25 oxide (NO) in children exposed to average formaldehyde levels ≥ 0.05 ppm compared to < 0.05
26 ppm (Franklin et al., 2000). The multiple regression models controlled for age and atopic status.
27 Other housing factors evaluated in bivariate analyses, were not associated with eNOS or
28 spirometry measures, and therefore were not included in the regression models ($p > 0.1$);
29 however, the effect of environmental tobacco smoke was not evaluated. The measurement of
30 lung volume at one point in time by Franklin et al. may have been less sensitive to detect an early
31 change in small airways than the measurement of flow rate used by Krzyzanowski et al.

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

1 22% nonsmokers). There were no differences between the exposed and unexposed groups’
2 percent predicted FEV₁ or FVC regardless of smoking status (unadjusted group means compared
3 using t tests). Although pulmonary function measures were analyzed stratified by smoking status
4 in this small study, exposure to environmental tobacco smoke was more common among the
5 unexposed (44%) compared to the exposed group (29%). Since ETS is associated with lung
6 function, this parameter may have acted as a confounder.

7 Several studies allowed for the examination of potential chronic effects of formaldehyde
8 exposure. These included an occupational study at a plywood company by Malaka and Kodama
9 (1990) that reported preshift pulmonary function as a percentage of expected function among the
10 formaldehyde exposed compared with plant workers not exposed to formaldehyde, matched for
11 age, ethnicity, and smoking status. Exposed and unexposed workers were identified using area
12 formaldehyde measurements. Exposed workers ($N = 100$) were randomly selected with
13 stratification by smoking status and years of occupation (< 5 and ≥ 5 years). Formaldehyde levels
14 ranged between 0.22 and 3.48 ppm. The unexposed group ($N = 100$) worked in areas where
15 formaldehyde was not used (range 0.003–0.07 ppm) and had no previous or current exposure to
16 formaldehyde based on occupational histories. Among the 93 exposed and 93 unexposed
17 participants who completed the protocol (93% participation), an average formaldehyde exposure
18 of 1.13 ppm from area samples was associated with statistically significant decrements in FEV₁,
19 FEV₁/FVC, and FEF_{25–75%} using analysis of covariance adjusting for sampled dust levels. The
20 strongest response was for FEF_{25–75%}, which showed a 12% drop in observed function compared
21 with expected function in the unexposed. Workers in the unexposed group had been employed
22 slightly longer than those in the exposed group (6.7 ± 2.3 versus 6.2 ± 2.4 years, $p < 0.05$).
23 When the exposed group was categorized into groups of low and high exposure based on a
24 cumulative measure calculated from area concentrations and length of employment in the current
25 job, a decrease was reported for one measure, FEV₁/FVC (percent of expected 86.9 ± 4.9 ,
26 85.3 ± 6.4 , and 84.4 ± 6.5 among unexposed, low and high formaldehyde groups, respectively
27 (statistical tests not reported). In multiple regression models adjusting for age, height, weight,
28 cigarettes/day, and dust, formaldehyde as a continuous variable was a significant predictor for
29 FEV₁, FEV₁/FVC, and FEF_{25–75%}. Each unit increase in formaldehyde (ppm-years) was
30 associated with a decrease of 0.015 liters, 0.347%, and 0.043 l/s in FEV₁, FEV₁/FVC, and
31 FEF_{25–75%}, respectively. Changes in spirometric measures across the shift were not associated
32 with formaldehyde exposure in a subgroup of 55 exposed and 50 unexposed participants. A
33 higher prevalence of cough, phlegm, chronic bronchitis, asthma, occupational asthma, shortness
34 of breath and chest colds were reported by the exposed group ($p < 0.04$). This was a carefully

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

3 A cross-sectional study comparing 99 oriented strand board workers (exposed to
4 formaldehyde) (98% participation rate) with 165 oil/gas field plant workers (not exposed to
5 formaldehyde) from the same geographic area (82% participation rate) demonstrated a difference
6 in pulmonary function between the two groups (Herbert et al., 1994). Time weighted average
7 formaldehyde concentrations based on 21 hour continuous sampling in the breathing zone at 5
8 work sites on 2 separate days ranged between 0.07 and 0.27 ppm. The groups were similar in
9 regard to measured FVC and FEV₁ (controlled for age, height, and smoking), but the workers
10 exposed to formaldehyde had lower FEV₁/FVC (78.6% and 80.3% for oriented strand board and
11 oil workers, respectively ($p = 0.028$)). In addition, those exposed to formaldehyde showed a
12 decrease in FVC and FEV₁ after their shift, with an average pre- and postshift difference of
13 47 mL ($p = 0.022$) and 39 mL ($p = 0.044$) for FVC and FEV₁, respectively (however change
14 could not be compared with the controls of this study because no postshift measurements were
15 taken). Strand board workers were more likely to report respiratory symptoms in analyses
16 controlling for age and smoking status including cough, phlegm, shortness of breath, wheeze,
17 and chest tightness. Odds ratios were statistically significantly elevated.

18 Another occupational study of wood products workers found no differences in preshift
19 percent predicted pulmonary function between 109 exposed (workers at a particleboard and
20 molded products operation, 68.6% of all exposed) and 254 unexposed (workers from nearby
21 food processing facilities) (Horvath et al., 1988). Regression models controlled for height, sex,
22 age, and smoking. Formaldehyde measured using individual monitors averaged 0.69 ppm (
23 0.17–2.93 ppm) and 0.05 ppm (0.03–0.12 ppm) in the exposed and unexposed industries,
24 respectively. The exposed workers had been employed an average of 10.3 years (1–20 years).
25 Although there was no difference in preshift measurements, the authors found a cross-shift
26 decline in FEV₁, FEV₁/FVC, FEF_{25–75%}, FEF_{25%–} and FEF_{75%} among exposed workers and in
27 FVC and FEV₁ among the unexposed group (paired t-test, $p < 0.05$). Thus, while acute declines
28 in large airway function over the shift were observed in both groups, the exposed group also
29 demonstrated declines in small airway function. The authors also evaluated cross-shift changes
30 between the groups and reported a significant difference for FVC, FEV₁/FVC, FEF_{25–75%},
31 FEF_{25%–} and FEF_{75%} (data were not presented). When the investigators assigned all unexposed
32 workers a formaldehyde exposure value of 0.05 ppm and evaluated formaldehyde associations
33 among exposed and unexposed combined, a correlation was detected in pre- and postshift
34 pulmonary function changes and formaldehyde levels, though no specific details on regression

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

3 No association between formaldehyde and lung function was observed among funeral
4 workers and an unexposed control group (Holness and Nethercott, 1989). Funeral workers,
5 including 67 currently active embalmers and 17 who were no longer active, were recruited
6 through a list of funeral homes developed by the Metropolitan District Funeral Directors
7 Association in Toronto, Canada (86.6% participation). An unexposed group ($N = 38$) was
8 recruited from a large service organization and paid student volunteers. Information on
9 symptoms, past and family medical history, and work practices was collected by questionnaire,
10 and pulmonary function and skin tests were administered. The average formaldehyde
11 concentration, measured during embalming procedures lasting from 30 to 180 minutes, was 0.36
12 ± 0.19 ppm (0.08–0.81 ppm). Funeral workers had performed the embalming procedure for an
13 average of 10 years. Unexposed participants were stated to be exposed to an average
14 concentration of 0.02 ppm. The authors reported no difference in percent predicted pulmonary
15 function of the two groups at baseline, although 10% of the funeral workers compared to 3% of
16 the unexposed group had an FVC or FEV₁ value less than 80% of predicted. Exposed and
17 unexposed were of similar age, height and years worked, although embalmers had higher
18 weights, a higher proportion of current smokers, and a higher number of pack-years smoked.
19 The 17 funeral workers who were no longer active had lower FVC (95.8 ± 12.0 versus $101.7 \pm$
20 12.2 , $p = 0.04$) and FEV₁ (93.1 ± 14.1 versus 100.8 ± 12.1 , $p = 0.07$) compared to the 67 active
21 embalmers, when corrected for age, height, and pack-years smoked. The authors reported that
22 active workers were younger and had worked less time in the industry. After exposure, there
23 was no change in lung function for the exposed or unexposed when comparing lung function
24 tests done immediately before and after an embalming procedure (for controls the repeat
25 measures were taken approximately 2–3 hours after the first measure) (changes in percentage
26 predicted FVC and FEV₁ were 0.88 ± 2.95 and -0.03 ± 2.40 for exposed and 1.13 ± 3.98 and
27 1.45 ± 4.43 for unexposed). Further analysis showed no association between formaldehyde
28 levels and changes in lung function during the embalming procedure. Although this study did
29 not find lung function decrements among embalmers compared to an unexposed group,
30 decrements in FVC and FEV₁ among those who had stopped embalming suggests a possible
31 response among more sensitive individuals. Sample sizes in the exposure group were small, and
32 the null association with formaldehyde exposure may have been influenced by differences
33 between a healthy occupational population and the service organization volunteers.

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

1 differences in spirometric measures related to formaldehyde (Pourmahabadian et al., 2006).
2 Lung function testing was conducted for 124 workers from the pathology labs (38), surgery (65),
3 endoscopy (21) departments and 56 administrative affairs staff during February 2002 to February
4 2003. Formaldehyde concentrations were highest in the pathology lab (8-hour average [SD]:
5 0.96 [0.74] ppm) compared to the surgery rooms (0.25 [0.18] ppm) and endoscopy departments
6 (0.25 [0.22] ppm). Highest one-hour spot samples recorded concentrations of 6.5 ppm, 2.7 ppm
7 and 0.6 ppm in pathology, surgery and endoscopy, respectively. Formaldehyde levels in
8 administrative offices were not reported. Pulmonary function measured before the work shift
9 was lower in the exposed group compared to the administrative staff. Average VC (liters, SD),
10 FVC, FEV₁, and FEF₂₅₋₇₅ were 3.34 (1.07), 3.46 (1.04), 2.45 (1.02) and 2.79 (1.4) in the exposed
11 groups and 3.92 (1.26), 4.03 (1.23), 1.21 (2.59) and 1.7 (2.5) among the unexposed group
12 ($p < 0.01$). FEV₁/FC and FEV₁/FVC were not significantly different between exposed and
13 unexposed. In addition, the change in pulmonary function across the shift was significantly
14 greater among the exposed workers compared to the unexposed ($p < 0.04$). Over 80% of the
15 exposed staff reported eye irritation and the prevalence of nose and throat irritation ranged
16 between 57 and 81%. The differences between the exposure groups is difficult to interpret
17 because the pulmonary function measures were not adjusted for age, gender, and height while
18 distribution by age, sex and smoking status varied between the groups.

19 A study performed in India (Khamgaonkar and Fulare, 1991) examined 74 individuals
20 working in anatomy and histopathology departments at 3 colleges in India and exposed to
21 formaldehyde (mean 1.00 ppm, range 0.036–2.27). A comparison group matched by age and sex
22 ($N = 74$) (individuals not working in laboratories with formaldehyde) was exposed to an average
23 of 0.102 ppm formaldehyde (range 0–0.52). Persons with a history of lung disease before their
24 present occupation were excluded. Multiple thirty minute air samples were collected in the
25 breathing zone in both the exposed ($N = 43$) and unexposed ($N = 18$) areas for formaldehyde
26 analysis. Lung function tests were performed on a subset of 37 exposed and 37 comparison
27 individuals on a Monday morning after days of no exposure in order to examine chronic effects.
28 The FVC, FEV₁%, and maximum mid-expiratory flow rate of the exposed group, respectively,
29 were 17.12 ($p < 0.01$), 22.94 ($p < 0.01$), and 42.81% ($p < 0.05$) lower compared with the
30 unexposed. Mean height was comparable in the two groups. The exposed group also had a
31 higher prevalence of symptoms including productive cough, breathlessness and tightness of chest
32 ($p < 0.01$). However, while the pool of exposed and unexposed were matched on age and
33 gender, there was no mention by the investigators of normalizing the pulmonary function metrics
34 by gender and height, which would have made for more appropriate comparisons. Kilburn et al.
35 (1985) also demonstrated reduced pulmonary function (lower percent predicted FVC, FEV₁, and

1 FEF_{25-75%}) among 45 male fiberglass bat makers exposed to formaldehyde when compared with
2 26 hospital employees, including respiratory therapists, gardeners, and attendants, without
3 formaldehyde exposure. However, formaldehyde levels in work areas were not measured and
4 group differences were not analyzed statistically in this small study.

5 Two occupational studies found no association between formaldehyde exposure and
6 deficits in pulmonary function (Ostojic et al., 2006; Holmström and Wilhelmsson, 1988).
7 Ostojic et al. (2006) examined nonsmoking male health service professionals working in
8 pathoanatomic laboratories with 8 hours of formaldehyde exposure per day at an unspecified
9 concentration for at least 4 years ($n = 16$). The source of the comparison group, comprised of
10 sixteen age- and stature-matched nonsmoking male controls, was not described. There was no
11 difference in mean percent predicted FVC or FEV₁ between exposed and unexposed. The
12 researchers also examined values for diffusing lung capacity for carbon monoxide and membrane
13 diffusion capacity, which were similar between the exposed and control groups. However, blood
14 volume of pulmonary capillaries was found to be higher in the exposed group. Holmström and
15 Wilhelmsson (1988) recruited individuals from a chemical plant where formaldehyde and
16 formaldehyde products were made ($n = 70$). Exposure levels varied from 0.05–0.5 mg/m³. A
17 comparison group, mostly comprised of clerks for the local government worked in an office with
18 mean formaldehyde concentrations of 0.09 mg/m³ ($n = 36$). FEV% was not different from the
19 predicted value among either the exposed or comparison groups. Mean FVC was lower than
20 expected among the exposed group (expected values were based on age, sex, smoking habits,
21 height, and weight) but not among the comparison group. Spirometric measures between the
22 exposure groups were not statistically compared. The comparison group was older (39.9 versus
23 36.9 years of age) and contained more women (44% versus 13%). The investigators measured
24 changes in pulmonary function for those employed more than 5 years and reported no signs of
25 increasing restrictivity after 5 years. Cumulative exposure to formaldehyde was estimated using
26 mean annual formaldehyde concentrations based on sampling conducted between 1979 and 1985
27 summed over the number of years employed. There was no correlation between pulmonary
28 function and cumulative dose of formaldehyde (Holmström and Wilhelmsson, 1988). The
29 studies by Ostojic et al. and Holmström and Wilhelmsson are less informative because the
30 adequacy of the comparison group cannot be evaluated, sample sizes were small, and analytic
31 methods were not adequate. There have been two studies that have reported on the longitudinal
32 follow-up of workers exposed to formaldehyde (Nunn et al., 1990; Alexandersson and
33 Hedenstierna, 1989). The Alexandersson and Hedenstierna (1989) investigation examined
34 Monday morning preshift lung function and the acute effects of exposure across shift at a
35 carpentry works in central Sweden in 1980 and then again in 1984 (Alexandersson et al., 1982).

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

15 For comparison with the 12% drop in the same pulmonary metric reported by Malaka and
16 Kodama (1990) over an estimated 6.5 years, the extrapolated percentage decrease in FEF_{25-75%}
17 was computed for the Alexandersson and Hedenstierna (1989) study by using the reported yearly
18 decrement applied to the preshift values at the time of the initial study period. From the
19 predicted value of 4.26 L/second, a decrease of 0.168 L/second for each year of exposure
20 regardless of smoking status was calculated. For 6.5 years of exposure, this would result in a
21 24% drop in FEF_{25-75%}. Significant across shift decreases in FEV1/FVC and FEF_{25-75%} were
22 reported in 1980 and cross-shift decreases in FEV1, FVC%, and MMF were report in 1984,
23 particularly for nonsmokers.

24 The study by Nunn et al. (1990) assessed the decrease in FEV₁ among workers, aged 25
25 or older, at a chemical factory in Duxford, England manufacturing urea formaldehyde resin
26 followed over a 6 year period from 1980. A total of 164 workers exposed to free formaldehyde
27 in 1980 and a group of 129 workers from the bonded structures division at the same factory in
28 1980 were included. Data on formaldehyde concentrations from area samples (1–6 hours sample
29 collections) taken periodically between 1979 and 1985, and from personal samplers attached to
30 representative exposed workers from 1985 to 1987 were used to categorize the workers'
31 employment experience into low, medium and high formaldehyde groups. Exposure
32 assessments prior to 1976 were based on subjective determinations and knowledge of process
33 changes and industrial hygiene measures. FEV1 and FVC measurements (highest of two
34 readings within 5% of each other) were obtained during annual health screenings by the same
35 nurse throughout the study. Follow-up was complete for 76% of the exposed and 74% of the

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

15 To summarize, the epidemiologic literature contains studies of formaldehyde exposure
16 and both acute and chronic effects on pulmonary function. Several studies with adequate
17 analytical methods and reporting have assessed acute exposures among naïvely exposed anatomy
18 graduate students (Kriebel et al., 1993; 2001), anatomy graduate students with several weeks of
19 episodic exposure (Kriebel et al., 1993; Uba et al., 1989), and postshift versus preshift worker
20 pulmonary function among those with regular occupational exposure (Malaka and Kodama,
21 1990; Herbert et al., 1994; Alexandersson et al., 1982; Alexandersson and Hedenstierna, 1989;
22 Holness and Nethercott, 1989; Horvath et al., 1988). Depending on whether the exposures are
23 naïve or not, the epidemiologic studies that assessed the pulmonary effects after acute exposures
24 to formaldehyde are assessing different biological responses, namely, the acute effect alone or
25 the acute effect(s) in people who may have already been sensitized to different and unknown
26 degrees. Generally, these studies found that intermittent laboratory or occupational shift
27 exposures to high formaldehyde concentrations (time weighted averages between 0.3–3 ppm)
28 were associated with deficits in lung function across the shift or laboratory session and, in some
29 studies, over time (Kriebel et al., 1993; 2001; Uba et al., 1989; Herbert et al., 1994; Horvath et
30 al., 1988; Alexandersson et al., 1982; Alexandersson and Hedenstierna, 1989. Pourmahadadian
31 et al. (2006) reported cross-shift reductions in pulmonary function but results were not adjusted
32 for age, height or gender. Acute exposure associated with eye, nose and throat irritation and
33 other respiratory symptoms including chest tightness and cough also was reported by some
34 studies (Kriebel et al., 1993; Uba et al., 1989; Fleisher, 1987; Horvath et al., 1988;
35 Pourmahadadian et al., 2006).

1 Several adequately conducted and reported studies evaluated chronic effects of
2 occupational (Malada and Kodama, 1990; Herbert et al., 1994; Horvath et al., 1988; Holness and
3 Nethercott, 1989; Alexandersson et al., 1982; Alexandersson and Hedenstierna, 1989) or
4 residential exposure to formaldehyde (Krzyzanowski et al., 1990; Franklin et al., 2000).
5 Decreased preshift lung function relative to unexposed groups was reported in several different
6 occupational groups including plywood, oriented strand board and carpentry (Malaka and
7 Kodama, 1990; Herbert et al., 1994; Alexandersson et al., 1982; Alexandersson and
8 Hedenstierna, 1989). The studies included appropriate comparison groups, formaldehyde
9 measurements, adequate reporting of methods, and adjustment for potential confounding
10 variables including age and smoking status (in some studies weight and ethnicity). All studies
11 presented lung function values as percent of expected based on age, height, and sex or adjusted
12 for these variables in the analysis. Some studies reported lower mean values for several preshift
13 measures of lung function among formaldehyde exposed workers (university laboratories,
14 fiberglass bat makers) suggestive of chronic decrements in pulmonary function but results were
15 not adjusted for age, height or gender, or statistical analyses were not reported complicating their
16 interpretation (Khamgaonkar and Fulare, 1991; Pourmahadadian et al. (2006); Kilburn et al.,
17 1985). Studies of long-term exposure also reported increased respiratory symptoms such as
18 cough, increased phlegm, asthma, chest tightness and chest colds in exposed workers (Malaka et
19 al., 1990; Herbert et al., 1994; Pourmahadadian et al., 2006, Alexandersson et al., 1982;
20 Alexandersson and Hedenstierna 1989). Some of the studies reporting null findings suffered from
21 small sample size, methodological, analytical or reporting deficiencies complicating the
22 interpretation of the findings (Main and Hogan, 1983; Ostojic et al., 2006; Holmström and
23 Wilhelmsson, 1988; Nunn et al., 1990). Also, occupationally exposed groups compared to
24 nonoccupational referent groups may have exhibited a healthy worker effect masking any
25 formaldehyde related lung function decrements (Holness and Nethercott, 1989).

26 The longitudinal study by Alexandersson and Hedenstierna (1989) provides support for
27 the association of lung function decrements over time with occupational formaldehyde
28 concentrations of 0.42–0.5 mg/m³ (340–400 ppb) over 5 years. Kryzanowski et al. (1990), a
29 well-designed and executed cross-sectional study of residential formaldehyde exposure in a
30 large, representative sample, provides clear evidence of a linear relationship between increased
31 formaldehyde exposure and decreased peak expiratory flow rate among children. The average
32 formaldehyde concentration was 26 ppb, with a maximum sample value of 140 ppb. Decrements
33 in PEFR associated with increasing formaldehyde concentrations were observed among adults
34 beginning at an average concentration of 40 ppb. While Franklin et al. (2000) did not observe an
35 association between a one-time measurement of FVC or FEV₁ among children aged 6–13 years

1 and indoor concentrations of formaldehyde, levels of exhaled nitric oxide (NO) were higher in
2 children exposed to average formaldehyde levels ≥ 0.05 ppm compared to < 0.05 ppm. These
3 findings indicate that formaldehyde may increase lower airway inflammation at concentrations
4 associated with effects on pulmonary function. Malaka and Kodama (1990) also observed an
5 exposure-response pattern for a cumulative formaldehyde measure and declines in FEV₁/FVC.

6 The pulmonary function measures associated with formaldehyde exposure are consistent
7 with bronchial constriction, inflammation, or chronic obstructive lung disease. Decreases in
8 spirometric values, including vital capacity (VC), forced expiratory volume (FEV), forced vital
9 capacity (FVC) and FEV/FVC have been documented. Decreases in lung volume (FEV₁, FVC)
10 indicate possible pulmonary obstruction (narrowing of the airways during exhalation) (Pellegrino
11 et al., 2005). Early changes in small airways are observed as reductions in expiratory flow in the
12 terminal portion of the spirogram (PEF, FEF_{75%}, MEF_{25%-75%}). These changes may be observed
13 even if FEV₁ is not affected.

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

21 22 **4.1.1.2.2. Acute studies: controlled chamber exposures.**

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

28 Anderson and Mølhave (1983) described a 5 hour controlled exposure study with
29 16 healthy students (5 female and 11 male). The students were an average of 23 years (20-33)
30 and included 5 smokers. After a 2 hour exposure to clean air, subjects in groups of four, were
31 exposed to four 5-hour formaldehyde exposures (0.3, 0.5, 1.0, and 2.0 mg/m³) on 4 consecutive
32 days in an order determined by a Latin square design. Measurements were taken prior to, after
33 2–3 hours, and 4-5 hours of exposure to formaldehyde. Only average standard deviations from
34 the mean were presented for vital capacity (0.28), FEF_{25-75%}, (0.28), and FEV_{1.0} (0.24). The
35 authors concluded that no significant changes were measured in the airway resistance measures.

1 Day et al. (1984) exposed 18 volunteers to formaldehyde (1.0 ppm for 90 minutes)
2 generated either from formalin in chambers or offgassing from urea formaldehyde foam insulation
3 (1.2 ppm for 30 minutes) in individual hoods. Pulmonary function tests were conducted before
4 formaldehyde exposures in room air (0.02 ppm formaldehyde), immediately after exposures, and
5 8 hours after the beginning of the exposure. No differences in absolute values for FVC, FEV1 or
6 FEF_{25-75%} before and after formaldehyde exposures, analyzed using paired t-tests, were observed
7 among 9 subjects who claimed to experience adverse effects to UFFI and 9 who had not. For
8 example, FEV1 before, immediately after, and 6.5 hours after exposure was 3.31 ± 0.79 ,
9 3.32 ± 0.81 , and 3.41 ± 0.77 L among those with complaints and 3.81 ± 1.0 , 3.75 ± 1.04 , and
10 3.71 ± 1.02 L among those with no complaints or not living in homes with UFFI. Mean change
11 in FEV1 after methacholine challenge was -3.2% (-13–7.4%) and 0.04% (-6.0–5.0%) among
12 those with prior complaints and those with no prior complaints, respectively. Since demographic
13 and other characteristics of the subjects were not presented, and only absolute values were
14 analyzed, the null findings in this small study cannot be adequately interpreted.

15 A study of healthy nonsmokers evaluated a dose-response relation using 2 groups (mean
16 age 26.3 ± 4.7 years, 10 males and 9 females) exposed at rest and during exercise (Kulle et al.,
17 1987). Subjects had no history of allergy, asthma, hay fever, or upper respiratory infection in the
18 6 weeks before the study began. One group of 10 subjects were exposed to formaldehyde
19 concentrations of 0.0, 0.5, 1.0, and 2.0 ppm in random order and the other group was exposed to
20 0.0, 1.0, 2.0, and 3.0 ppm in random order. Both groups were also exposed to 2.0 ppm with
21 exercise. Each session was separated by 1 week. Spirometric measurements were taken at the
22 beginning and end of each exposure, at several points during the 3 hour session, and 24 hours
23 post exposure. Airway resistance and thoracic gas volume were measured at the beginning and
24 end of each exposure. Nonspecific airway reactivity, measured by methacholine challenge, was
25 assessed at the end of the exposure and after 24 hours. No differences between dose levels and
26 no trend with increasing dose was found in analyses using lung function values at each time
27 point as a ratio of the value measured before exposure began. Lung function was not expressed
28 as a percent of predicted according to age, sex or height and dose level means were presented by
29 time point without standard deviations to characterize individual variability. In addition, no
30 differences in bronchial reactivity were observed.

31 A study of sensory irritation and subjective symptoms among 21 healthy volunteers with
32 4 hour exposures to 0, 0.15, 0.3 and 0.5 ppm formaldehyde over 2 weeks did not observe
33 differences in pulmonary function measured at baseline, before the first exposure and after the
34 last exposure (Lang et al., 2007). The differences between an individual's postexposure and
35 pre-exposure values were analyzed in relation to formaldehyde. The data were not presented.

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

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

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

31 Other acute controlled studies including asthmatics also reported no changes in
32 pulmonary function associated with formaldehyde exposure (Sheppard et al., 1984, Ezratty et al.,
33 2007; Harving et al., 1990; Green et al., 1987; Sauder et al., 1987; Witek et al., 1987, 1986),
34 including studies of individuals thought to have formaldehyde-induced bronchial asthma
35 (Krakowiak et al., 1998). Specific airway resistance was not significantly increased among

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

18 Harving et al. (1990) evaluated pulmonary function and bronchial reactivity among
19 15 nonsmoking volunteers with asthma (8 female and 7 male) exposed to filtered air (0.008
20 mg/m^3 formaldehyde), 0.12 mg/m^3 and 0.85 mg/m^3 formaldehyde for 90 minutes. The subjects,
21 15 to 36 years of age, were assigned at random to 1 of 3 groups and each group (5 subjects per
22 group) was exposed to formaldehyde or filtered air in random order over a 3 week period. FEV1
23 as a percent of baseline was not significantly changed after a 90 minute exposure. Values were
24 104%, 103% and 103% for formaldehyde levels of 0.85, 0.12, and 0 mg/m^3 , respectively. In
25 addition, no significant changes in the concentration of inhaled histamine required for a 20% fall
26 in peak expiratory flow measured immediately after exposures were noted in relation to
27 formaldehyde levels. The PC_{20} for subjects exposed to 0, 0.12, and 0.85 mg/m^3 formaldehyde
28 was 0.29 ± 0.3 , 0.36 ± 0.53 , and 0.26 ± 0.31 , respectively.

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

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

3 Krakowiak et al. (1998) exposed 10 subjects exposed to formaldehyde occupationally
4 (aged 23-52 years) and 10 volunteers with no occupational exposure to formaldehyde (aged
5 19–49 years) to 0.5 mg/m³ formaldehyde for 2 hours. The group with formaldehyde exposure
6 had bronchial asthma diagnosed by the doctor in the workplace and included 7 males and 3
7 females, some of whom smoked. The nonasthmatic group included only nonsmoking males.
8 Exposure to formaldehyde did not result in changes in FEV₁, PEF or the dose of histamine
9 causing a 20% fall in FEV₁ measured before, immediately after and up to 24 hours after the
10 exposure period in either group.

11 Small but statistically significant deficits in pulmonary function due to acute
12 formaldehyde exposure (2 or 3 ppm) have been reported in healthy volunteers during exercise
13 (Green et al., 1987, 1989; Sauder et al., 1986). Although changes in lung function parameters
14 averaged over experimental groups were generally small, some individuals exhibited clinically
15 significant deficits, even after only 2 hours of exposure (Green et al., 1987). Nine healthy
16 nonsmoking subjects, aged 26 ± 3.6 years, were exposed to clean air for 3 hours on day 1 and
17 then 3 ppm formaldehyde for 3 hours on day 2 (Sauder et al., 1986). In addition, exercise for
18 8 minutes on a bicycle was included prior to each spirometry measurement during the exposure
19 at 30, 60, 90, 120, 150, and 180 minutes. Deficits in FEV₁ and FEF_{25–75%} after the first 30
20 minutes of formaldehyde exposure compared to clean air were 2% ($p < 0.05$) and 7% ($p < 0.01$),
21 respectively. Spirometric measures were not different after 60 and 180 minutes of exposure,
22 even when assessed as absolute rather than relative measurements. The authors reported that the
23 range of individual responses was -5% to +1% for FEV₁ and -14% to +2% for FEF_{25–75%}.

24 Another study compared the responses of healthy and asthmatic subjects exposed to clean
25 air and 3 ppm formaldehyde for 1 hour with 15 minute exercise segments at 15 and 45 minutes
26 using the same analytical methods as described by Sauder et al. (1986, 1987) (Green et al.,
27 1987). Among the 22 healthy subjects, small but statistically significant decrements were
28 observed during 3 ppm formaldehyde exposures, compared to the clean air exposures, at 47 and
29 55 minutes. Decreases of 2.1 to 3.8% were observed for FEV₁, FVC, and FEV₃. Decreases in
30 FEF_{25–75%} also were observed (5.7 to 10.9%), but the changes were not statistically significant.
31 No changes in mean specific conductance or airway reactivity measured by methacholine
32 challenge were observed in either the healthy subjects or 16 subjects with a clinical history of
33 asthma. No changes in pulmonary function measures were observed in the asthmatic group.
34 Individual variability in responsiveness was noted by the authors. Thirteen percent (5 of

1 38 subjects) demonstrated formaldehyde-induced clinically significant deficits when exposed at
2 3 ppm during exercise (defined by Green et al. (1987) as a decrease in FEV₁ > 10% of control).

3 The authors followed up with an additional study to evaluate the effects of formaldehyde
4 alone or in combination with respirable carbon particles (mass median aerodynamic diameter
5 [GSD] 1.4 μm [1.8 μm]). A total of 29 healthy subjects were exposed according to a randomized
6 block design for 2 hours each to clean air, 3.0 ppm formaldehyde, 0.5 mg/m³ activated carbon
7 aerosol and a mixture of 3 ppm formaldehyde and 0.5 mg/m³ activated carbon aerosol.
8 Exposures occurred at the same time of day and were separated by one week. Spirometric
9 measurements were obtained prior to the beginning of exposure, and at 20, 50, 80, and
10 110 minutes during the exposure. In addition, a 15 minute bicycle ergometer exercise was
11 completed at 15, 45, 75, and 105 minutes. The subject also measured peak flow at the end of the
12 2 hour exposure, on the hour for 8 hours, and 12 and 16 hours post exposure using Wright peak
13 flow meters. A statistically significant decrease in FEF_{25-75%} (< 6% mean decrease) related to
14 formaldehyde was observed among the 24 subjects who were able to complete the exercise
15 protocol at 50 ($p < 0.05$) and 80 minutes ($p < 0.01$), and a decrease in peak flow was evident at
16 110 minutes ($p < 0.03$). Formaldehyde exposure also resulted in decreased specific airways
17 conductance at 120 minutes ($p < 0.01$). No formaldehyde effects on FVC, FEV₁, or FEV₃ were
18 observed for formaldehyde alone, however statistically significant decreases in FVC (mean
19 2.5–4.5% decrease) and FEV₃ (mean decrease 3%) were reported at 20 and 50 minutes, and peak
20 flow at 110 minutes. The combined exposure also significantly increased coughing at 20 and
21 80 minutes ($p < 0.05$). Formaldehyde alone did not have an effect on cough but statistically
22 significant increases in headache, eye, nose and throat irritation, and chest discomfort occurred at
23 all time points. The authors concluded that formaldehyde exposure during exercise resulted in
24 an acute, transient effect on both large and small airways among healthy individuals. In addition,
25 a combined exposure to formaldehyde and carbon particles increased coughing and small
26 decrements in pulmonary function, suggesting that adhesion to particles increased delivery of
27 formaldehyde to the lower respiratory tract.

28 Casset et al. (2006) evaluated the effect of formaldehyde exposure on the bronchial
29 response to dust mite allergen in sensitized asthma patients. The study included 19 nonsmoking
30 subjects (12 women and 7 men) ages 19-35 years with mild asthma. Subjects had positive skin
31 prick tests and specific IgE to Dermatophagoides pteronyssinus (dust mites). Subjects had not
32 had a respiratory tract infection in the two weeks prior to the testing. Individuals sensitized to
33 pollen were studied outside the relevant pollen season and people sensitive to pet allergens did
34 not have pets at home. The 30 minute crossover exposures to 100 μg/m³ formaldehyde and air
35 were randomly assigned with a three-week washout period in between. Subjects underwent mite

1 allergen challenge immediately after exposure to formaldehyde or air to determine the dose of
2 allergen that resulted in a 20% reduction in FEV₁ (PD₂₀). The dose which induced early-phase
3 bronchial response was significantly lower in the subjects exposed to formaldehyde (34.3 ng vs.
4 45.4 ng: $p < 0.05$). Late-phase bronchial response was measured over the 6 hours following the
5 dust mite challenge by comparing FEV₁. The maximum percentage FEV₁ reduction observed
6 was significantly higher after exposure to formaldehyde 15% vs. 11% ($p < 0.05$) (Casset et al.,
7 2006).

8 While the study by Casset et al. (2006) clearly showed that acute formaldehyde exposure
9 (100 µg/m³) enhanced both early-phase and late-phase bronchial responsiveness to mite allergen
10 in mite-allergen sensitized people with asthma, a subsequent study with a similar protocol did
11 not duplicate this finding. Ezratty et al. (2007) evaluated the response of asthmatics to inhaled
12 allergen after a 60 minute exposure to 500 µg/m³ (0.4 ppm) formaldehyde. The 12 subjects
13 (7 men and 5 women) were 18–44 years old, were nonsmokers, and were diagnosed with
14 intermittent asthma and allergy to pollen. No subjects had contracted an upper respiratory
15 infection for at least 4 weeks before the study. The crossover exposures (60 minutes) to filtered
16 air and 500 µg/m³ formaldehyde occurred 2 weeks apart in random order. Allergen inhalation
17 challenge, using an extract of 5 grass pollen allergens, was conducted immediately after each
18 exposure and the dose producing a 15% decrease in FEV₁ was determined (PD₁₅FEV₁).

19 Responsiveness to methacholine was determined 8 hours after the allergen inhalation
20 challenge ended. Lung function measurements were taken using a spirometer before, during and
21 8 hours after the end of the allergen challenge. In addition, PEF and FEV₁ were measured with a
22 portable spirometer every 15 minutes during exposures to filtered air or formaldehyde and every
23 hour until the methacholine challenge. The authors reported that pulmonary function, expressed
24 as percent predicted, was not affected by the formaldehyde exposure, although the data were not
25 presented. The median PD₁₅FEV₁ for the allergen challenge was 0.80 (0.15-2.0) index of
26 reactivity (IR) after formaldehyde exposure and 0.25 (0.10-2.0) IR after the filtered air exposure
27 ($p = 0.06$). The ratio of response after formaldehyde exposure compared to filtered air exposure
28 was 1 in 7 subjects and higher in 5 subjects. The PD₂₀ for methacholine challenge was 0.23
29 (0.01-3.6) and 0.17 (0.03-4) ($p = 0.42$). The PD₂₀ for methacholine for formaldehyde compared
30 to filtered air was lower in 3 subjects, higher in 4 subjects, and not changed in 5 subjects.

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

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

8 In a study unrelated to formaldehyde, Praml et al. (2005) compared the physical and
9 biologic doses of methacholine for different nebulizers including the type used by Ezratty et al.
10 (2007). Praml et al. (2005) compared the airway responsiveness of 34 subjects using two types
11 of nebulizers and found that in 17 subjects, neither system caused a 20% decrease in FEV₁,
12 while among 8 subjects, both systems were able to provoke a 20% decrease. The remaining
13 9 subjects responded to only one type of nebulizer. Using the same protocol, the same
14 methacholine agent produced different results based on the type of nebulizer. It may be that the
15 results of Casset et al. (2006) and Ezratty et al. (2007) which did have other differences can be
16 explained thusly. It may also be that a difference in particle size plays a role if the pollen
17 allergens used in Ezratty et al. (2007) were smaller and penetrated the lung beyond the upper
18 lung where formaldehyde exposures are greater.

19 In general, acute formaldehyde exposures (0.5–3 ppm) have not induced significant
20 pulmonary deficits in healthy, nonexercising volunteers (Kulle et al., 1987; Schachter et al.,
21 1986; Schachter et al., 1987; Witek et al., 1986; Day et al., 1984; Andersen and Molhave, 1983).
22 However, it is unclear whether the data analysis in these reports had the statistical power to
23 substantiate the small deficits reported in occupational and student studies. All five reports had
24 relatively small study groups of healthy individuals ($n = 19$ [Kulle et al., 1987], $n = 16$
25 [Andersen and Molhave, 1983], $n = 15$ [Schachter et al., 1986], $n = 15$ [Schachter et al., 1987],
26 $n = 15$ [Witek et al., 1986], and $n = 9$ [Day et al., 1984]). The studies exposed a small number of
27 diverse individuals, often including males and females of varying age, and some included current
28 smokers. Three studies report the absolute values of the lung function parameters without
29 adjustment to individual expected function or the unexposed baseline for each individual (Kulle
30 et al., 1987; Andersen and Molhave, 1983; Day et al., 1984). As discussed, this decreases the
31 power of the study to detect formaldehyde-induced changes in pulmonary function. In contrast,
32 Witek et al. (1986) and Schachter et al. (1986, 1987) report lung function as a percent of baseline
33 (although not normalized for age, gender and height). Each study showed an increase in FEV₁ in
34 formaldehyde-exposed individuals at rest and increases in maximal expiratory flow (MEF) at
35 50% of expired vital capacity (MEF50%) (Witek et al., 1986; Schachter et al., 1986). However,

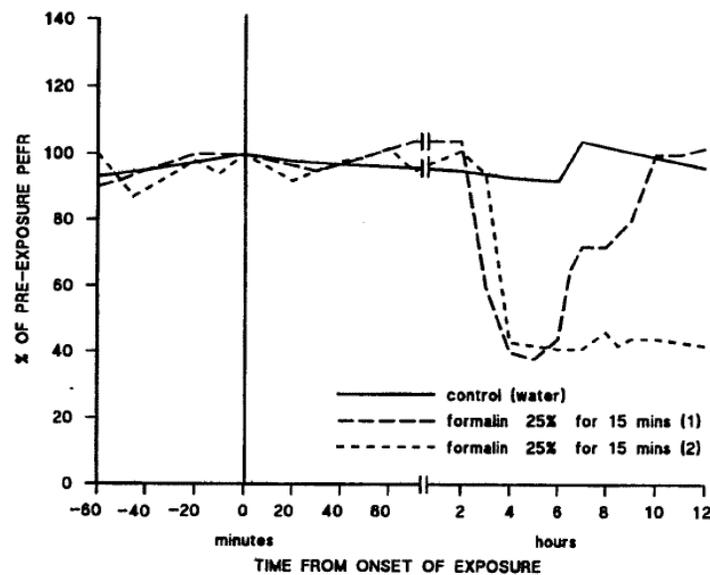
1 in both reports the SDs of changes in lung function parameters are quite large, nearly equaling
2 the reported value and exceeding it in several cases. The absence of normalized raw data,
3 combined with large individual variation, limit the interpretation of these studies.

4 Small but statistically significant deficits in pulmonary function (e.g., decreased FEV₁,
5 FVC₁, FEV₃, specific airways conductance) due to formaldehyde exposure (2 or 3 ppm) have
6 been reported in healthy volunteers in controlled human exposure studies using exercise (Green
7 et al., 1987, 1989; Sauder et al., 1986;). Although changes in lung function parameters averaged
8 over experimental groups were generally small, some individuals exhibited clinically significant
9 deficits, even after only 2 hours of exposure (Green et al., 1987). This differential response
10 suggests susceptibility in certain subjects (Green et al., 1987). Other studies that included an
11 exercise component did not report a difference in response among healthy volunteers (Schachter
12 et al., 1986; Kulle et al., 1987). Acute controlled studies that evaluated responses among
13 asthmatics reported no changes in pulmonary function associated with formaldehyde exposure
14 (Sheppard et al., 1984, Ezratty et al., 2007; Harving et al., 1990; Green et al., 1987, 1989; Sauder
15 et al., 1987; Witek et al., 1987, 1986; Krakowiak et al., 1998). These findings suggest that a
16 brief exposure to formaldehyde may not trigger a response in the airways of asthmatic
17 individuals in the absence of allergen. However, the large variation in pulmonary response
18 among the individuals (healthy and asthmatic) that participated in the experimental exposure
19 studies suggests that some individuals may be more sensitive to formaldehyde.

21 **4.1.1.3. Asthma**

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

1 The National Research Council concluded in its report on Formaldehyde that,
2 “Formaldehyde has been shown to cause bronchial asthma in humans” (NRC, 1981), citing
3 numerous studies demonstrating the induction of asthma following exposure to formaldehyde
4 (Hendrick and Lane, 1975, 1977; Laffont and Noceto, 1961; Nova and Touraine, 1957; Paliard et
5 al., 1949; Popa et al., 1969; Sakula, 1975; Schoenberg and Mitchell, 1975; Turiar, 1952;
6 Vaughan, 1939). In a subsequent review article on formaldehyde and the health effects that have
7 been associated with it, Stenton and Hendrick (1994) reported on formaldehyde and asthma in
8 occupational settings and starkly describe the “...first detailed case report of formaldehyde
9 asthma confirmed by specific inhalation challenge test occurring in a nursing sister on a renal
10 dialysis unit. Her symptoms were suggestive of late asthmatic reactions occurring 4 to 5 hours
11 after heavy exposures. The occurrence of late reactions was confirmed in a series of challenge
12 tests” (Stenton and Hendrick, 1994; Hendrick 1997). The results of the challenge tests are
13 illustrated in Figure 4-1.
14



15
16
17 **Figure 4-1. Delayed asthmatic reaction following the inhalation of**
18 **formaldehyde after “painting” 100% formalin for 20 minutes.** Challenge 2
19 was premedicated with inhaled betamethasone 200 µg.
20

21 Source: Stenton and Hendrick (1994).
22
23

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

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

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

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

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

6 Categorical analysis of the data indicates the ORs for asthma were increased in the two
7 highest formaldehyde exposure groups, reaching statistical significance for household exposures
8 $> 60 \mu\text{g}/\text{m}^3$ (49 ppb) (OR of 1.39) (Rumchev et al., 2002). Analysis of the data with
9 formaldehyde as a continuous variable provides a statistically significant increase in the risk of
10 asthma (3% increase in risk per every $10 \mu\text{g}/\text{m}^3$ increase in formaldehyde level. The paper states
11 this effect as OR 1.003 (95% CI 1.002-1.004) which appears at odds with a 3% increased in risk
12 per every $10 \mu\text{g}/\text{m}^3$ but this must be the effect per $1 \mu\text{g}/\text{m}^3$ and can be confirmed by comparing
13 the per unit effect to the plotted results¹. All analyses controlled for other indoor air pollutants,
14 allergen levels, relative humidity, and indoor temperature as well as other risk factors.

15 While the study by Rumchev et al. (2002) focused on formaldehyde controlling for other
16 indoor air pollutants, a subsequent report described the specific effects of those indoor air
17 pollutants (Rumchev et al., 2004). This paper evaluated the risk of asthma incidence with
18 10 VOCs. The highest odds ratios were increased risks of asthma diagnosis associated with
19 benzene, toluene, and ethylbenzene and were statistically significant associations. Compared to
20 the effects observed for formaldehyde, the strength of the associations appear to be stronger on a
21 per $10 \mu\text{g}/\text{m}^3$ basis. The strength of these effects is an important consideration as the relative
22 strength of the VOC effects appears to be larger than that attributable to formaldehyde if the
23 effects of the measured indoor air pollutants had not been controlled for in the formaldehyde
24 analysis (Rumchev et al., 2002). However, as these indoor air pollutants had been controlled for,
25 the reported effect of formaldehyde should be independent of the effect of benzene and other
26 VOCs in the absence of residual confounding. If two factors both cause the same outcome and
27 are statistically associated, then they may mutually confound. In Rumchev et al. (2004) on page
28 750, the investigators assessed whether the effect of the VOCs were confounded by
29 formaldehyde and stated that the results showed that exposure to VOCs still had a highly
30 significant effect on asthma even when formaldehyde was controlled for. This finding further
31 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 $60 \mu\text{g}/\text{m}^3$, the extrapolated linear effect would be OR = 1.2.

1 Several other nonoccupational studies have evaluated the association between
2 formaldehyde exposure and the prevalence of asthma among children (Garrett et al., 1999;
3 Tavernier et al., 2006; Gee et al., 2005; Krzyzanowski et al., 1990; Palczynski et al., 1999).
4 Three studies (Tavernier et al., 2006; Gee et al., 2005; Garrett et al., 1999) were performed by
5 matching children with and without asthma and comparing the levels of formaldehyde in their
6 homes. Gee et al. (2005) selected 100 cases with current asthma and 100 controls from
7 2 primary care facilities in an area of England with low socioeconomic status. Cases were
8 identified through a screening questionnaire that had been validated with diagnoses by
9 physicians. Cases and controls (aged 4–16 years) were matched by age and sex. Median
10 formaldehyde levels were 0.03 ppm in living rooms and 0.04 ppm in bedrooms. Univariate
11 comparisons found no differences in formaldehyde levels between cases of current asthma and
12 controls without asthma. Notably, no association was observed for pollutant indicators of
13 environmental tobacco smoke and current asthma, a recognized risk factor. A subsequent study
14 of the same children in the same homes conducted a more thorough evaluation of risk factors
15 (Tavernier et al. (2006). Again, a one-week average formaldehyde concentration in the living
16 room or bedroom was not found to be associated with current asthma in multivariate analyses
17 adjusted for several indoor variables. Respirable particulates, tobacco specific particles, volatile
18 organic compounds, and nitrogen dioxide also were not associated with current asthma.
19 Tavernier et al. (2006) did not report the measured levels of formaldehyde, but gave the OR for
20 the highest tertile of exposure in the bedroom compared with the lowest tertile of exposure as
21 0.99 (95% CI: 0.39–2.50). The odds ratio for the second tertile compared to the lowest tertile
22 was 1.22 (95% CI: 0.49-3.07). The width of the confidence intervals indicates that study did not
23 have adequate statistical power to detect low level risks and suggests that these findings would
24 still be consistent with a two-fold increase in risk.

25 Garrett et al. (1999) reported on the risk of allergy and asthma-like respiratory symptoms
26 due to formaldehyde exposure in a cross-sectional survey of 80 households in rural Victoria,
27 Australia with children, aged 7–14 years, with ($n = 53$) or without ($n = 88$) doctor-diagnosed
28 asthma. Households were recruited via schools, medical centers, and advertisements in the local
29 press. The study was designed to include asthmatic children in half of the households and the
30 study recruited 43 households with at least one child with asthma diagnosed by a doctor and
31 37 households with no asthmatic children. Formaldehyde exposure was characterized by
32 4 seasonal in-home sampling events in 1994 and 1995 (4-day passive samples) in bedrooms of
33 all participating children and in living rooms, kitchens, and outdoors. Median indoor
34 formaldehyde concentrations were $15.8 \mu\text{g}/\text{m}^3$ (12.6 ppb) with a maximum of $139 \mu\text{g}/\text{m}^3$
35 (111 ppb). The median outdoor concentration was $0.7 \mu\text{g}/\text{m}^3$ with a range of < 0.3 – $15.3 \mu\text{g}/\text{m}^3$.

1 Information on asthma respiratory symptoms during the previous year was obtained through an
2 interview with a parent after sampling was completed. An erratum to the original paper reported
3 that the column headers in two tables were switched but that the summary statistical and
4 conclusions in the 1999 report were correct as published. The proportion of asthmatic children
5 by the highest formaldehyde level measured over four seasons was 0.16, 0.39, and 0.44 for
6 $<20 \mu\text{g}/\text{m}^3$, $20\text{-}50 \mu\text{g}/\text{m}^3$, and $>50 \mu\text{g}/\text{m}^3$, respectively (test for trend, $p < 0.02$). However, in
7 logistic regression models, the ORs for the association did not remain statistically significant
8 after controlling for parental allergy and asthma (ORs and 95% CIs were not provided).

9 A large, representative study of 202 households (mean formaldehyde level of 26 ppb)
10 found that among children aged 6–15 years old and exposed to environmental tobacco smoke,
11 the prevalence of physician-diagnosed asthma was 45.5% for those with measured levels of
12 formaldehyde in the kitchen >60 ppb ($N = 11$). The prevalence of asthma dropped to 0% for
13 levels 41–60 ppb ($N = 12$) and 15.1% for levels ≤ 40 ppb ($N = 106$) (chi-squared trend test
14 $p < 0.05$). No trend in asthma prevalence was seen for children who were not exposed to
15 environmental tobacco smoke (Krzyzanowski et al., 1990).

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

22 A study in Poland randomly selected 120 households with children 5–15 years of age in
23 10 year old apartment houses (Palczynski et al., 1999). Using self-reported asthma prevalence as
24 an outcome, study investigators found no association with levels of formaldehyde (mean
25 $25.9 \mu\text{g}/\text{m}^3$, range $2.0\text{--}66.8 \mu\text{g}/\text{m}^3$) measured using 24-hour samples in the children. Among
26 adults, the authors reported a higher prevalence of allergic diseases in the highest formaldehyde
27 exposure group but that the group was too small for statistical evaluation. However, the
28 prevalence of allergic asthma was higher among adults exposed to $25.1\text{--}50 \mu\text{g}/\text{m}^3$ compared to
29 $<25 \mu\text{g}/\text{m}^3$ and exposed to environmental tobacco smoke ($p = 0.03$).

30 Delfino et al. (2003) conducted a panel study of 22 Hispanic children with a minimum
31 one year history of doctor diagnosed asthma, aged 10–16 years, and living in Los Angeles. The
32 participants were nonsmokers from nonsmoking households, and lived and went to school within
33 3 miles of a central site monitor. The children recorded the severity of asthma symptoms in daily
34 diaries for 3 months. The mean outdoor 24-hour levels of formaldehyde were 7.21 ppb (range
35 4.27–14.02 ppb). A positive association between asthma symptom scores (comparing children

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

7 Other studies of residential exposure to formaldehyde have investigated asthma
8 prevalence and symptoms in adults (Norback et al., 1995; Matsunaga et al., 2008). A cross-
9 sectional study by Norback et al. (1995) reported mean levels of formaldehyde, measured on one
10 day for 2 hours, were $29 \mu\text{g}/\text{m}^3$ (range $<5\text{--}110 \mu\text{g}/\text{m}^3$) in the bedrooms of individuals
11 experiencing nocturnal breathlessness compared with formaldehyde levels of $17 \mu\text{g}/\text{m}^3$
12 ($<5\text{--}60 \mu\text{g}/\text{m}^3$) among those without nocturnal breathlessness. The study sample was
13 88 individuals from a community in Sweden who responded to a screening questionnaire, had
14 lived in the same home during the study period, and agreed to a medical interview and home
15 sampling (58% of recruited). The eligible population was residents of Uppsala, Sweden who had
16 answered yes ($N = 74$) to one of 3 questions regarding attacks of asthma in the previous
17 12 months, nocturnal breathlessness in the past 12 months, or current use of asthma medications
18 or a random sample of those who had answered no ($N = 80$) to all three questions. The OR for
19 nocturnal breathlessness was 12.5 (95% CI: 2.0–77.9) per a 10-fold increase in the indoor
20 concentration in logistic regression models adjusted for age, sex, current smoking, wall to wall
21 carpets and presence of house dust mites. The effect was substantially stronger in magnitude
22 than the associations observed for toluene, terpenes, and volatile organic compounds. Therefore,
23 the association with formaldehyde is likely not entirely explained by confounding by the volatile
24 organic compounds

25 A recent cross sectional study (Matsunaga et al., 2008) found no association between a
26 24-hour personal sample for formaldehyde and prevalence of asthma when pregnant women with
27 an exposure ≥ 47 ppb were compared to those with exposure to < 18 ppb. The adjusted odds ratio
28 from the logistic regression model was 2.65 (95% CI: 0.63–11.11). The small number of women
29 identified with asthma resulted in wide confidence intervals and low statistical power. The
30 authors analyzed baseline data collected in 2001 and 2003 from 998 participants in the Osaka
31 Maternal and Child Health Study in Japan. The prevalence of current asthma, defined as a self
32 report of medical treatment for asthma in the last 12 months, was 2.1% ($N = 21$). A prior
33 diagnosis or treatment prior to the last 12 months was not assessed. However, they did report an
34 increased risk of atopic eczema treated in the last 12 months. Median formaldehyde levels were
35 24 ppb with a maximum of 131 ppb. The odds ratio from atopic eczema for women exposed to

1 ≥ 47 ppb formaldehyde was 2.25 (95% CI 1.01-5.00) in multiple logistic regression models
2 adjusted for age, gestation, parity, family history of asthma, family income, education, mite
3 antigen level in house dust, and season. The association was significantly higher among those
4 with no family history of allergy.

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

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

31 Studies of exposure to formaldehyde at schools have been performed in China (Zhao et
32 al., 2008) and in Sweden (Smedje and Norback, 2001). In a cross-sectional study from China
33 (Zhao et al., 2008), the 7 day mean level of formaldehyde in 31 classrooms at 10 junior high
34 schools was reported to be $2.3 \text{ }\mu\text{g/m}^3$ (range $1.0\text{--}5.0 \text{ }\mu\text{g/m}^3$) indoors and $5.8 \text{ }\mu\text{g/m}^3$ (range
35 $5.0\text{--}7.0 \text{ }\mu\text{g/m}^3$) outdoors. The prevalence of cumulative and doctor diagnosed asthma reported

1 by the 1993 children (90% of eligible) was 1.8% and 1.2%, respectively. In models controlling
2 for outdoor concentrations of formaldehyde, nocturnal attacks of breathlessness were
3 significantly associated with indoor formaldehyde (OR 2.72, 95% CI: 1.03-7.18). In models
4 controlling for indoor concentrations of formaldehyde, cumulative asthma was associated with
5 outdoor formaldehyde levels (OR 4.61, 95% CI 1.09-19.5). An increased risk for daytime
6 attacks of breathlessness also was observed (OR 1.29, 95% CI 0.99-1.68). The 3 level
7 hierarchical logistic models also adjusted for age, sex, parental asthma or allergy and home
8 environmental factors (environmental tobacco smoke etc), and indoor and outdoor
9 concentrations of sulfur dioxide, nitrogen dioxide, and ozone. Moreover, new furniture in the
10 home (a potential source of formaldehyde exposure) was associated with wheeze or whistling in
11 the chest (OR 1.76, 95% CI 1.10-2.81) and daytime attacks of breathlessness (OR 1.31, 95% CI
12 1.0-1.72). In Sweden (Smedje and Norback, 2001), the mean level of formaldehyde measured
13 indoors in school classrooms in 1993 and 1995 were higher than those measured by Zhao et al.
14 (2008) (mean 4, range <5.0–72 $\mu\text{g}/\text{m}^3$).

15 The Swedish investigators (Smedje and Norback, 2001) conducted a 4 year follow-up
16 study among 1732 students from 39 schools aged 7-13, who completed a mailed questionnaire in
17 1993. Both questionnaires were completed by 1347 students, 66% of those invited in 1993. In
18 1993, 6.6% reported having physician diagnosed asthma ($N = 89$) and 34% ($N = 589$) reported a
19 history of atopy defined by an affirmative answer regarding either childhood eczema, allergy to
20 pollens or pet dander. The 4 year incidence of physician diagnosed asthma was 4.5% ($N = 56$).
21 This study did not report an association between formaldehyde exposure and the incidence of
22 asthma (OR 1.2 [95% CI: 0.8–1.7]) among the whole study population. However, when the
23 investigators stratified on history of atopy, they reported that among 22 children without a
24 history of atopy, a new diagnosis of asthma was significantly more likely at higher
25 concentrations of formaldehyde (OR 1.7 per 10 $\mu\text{g}/\text{m}^3$ [95% CI: 1.1–2.6]) and at higher total
26 concentrations of mold (OR = 4.7 per 10-fold increased in total molds [95% CI: 1.2-18.4] in the
27 classroom air. The finding for adverse effects of formaldehyde and mold did not appear to
28 control for the other exposure and no information on the potential correlation between the two
29 exposures was provided.

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

1 $OR^2 = 1.13$ per GSD and the result for mold is $OR^3 = 1.02$ for a comparison of risks at the GSM
2 to $10 \times \text{GSM}$ and $OR^4 = 1.06$ for a comparison of risks at the minimum value of total molds
3 ($5 \times 10^3/\text{m}^3$) to $10 \times \text{minimum}$. As it appears that the magnitude of the formaldehyde effect is
4 substantially stronger than that of the mold effect (following standardization of exposure
5 increment) it can be concluded that the reported formaldehyde effect could not have been the
6 spurious result of uncontrolled confounding by mold. Unfortunately the logistic regression
7 models did not account for the correlated formaldehyde concentrations for children by
8 classroom.

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

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

² $OR \text{ per GSD} = \exp[\ln(OR \text{ per } \mu\text{g}/\text{m}^3)/10 \mu\text{g}/\text{m}^3 * 2.3 \mu\text{g}/\text{m}^3] = \exp[\ln(1.7)/10 * 2.3] = 1.13.$

³ $OR \text{ per GSD} = \exp[\ln(OR \text{ per } 10\text{-fold increase})/(9 * \text{GSM}) * 1.6 \mu\text{g}/\text{m}^3] = \exp[\ln(4.7)/162 * 1.6] = 1.02.$

⁴ $OR \text{ per GSD} = \exp[\ln(OR \text{ per } 10\text{-fold increase})/(9 * \text{Minimum}) * 1.6 \mu\text{g}/\text{m}^3] = \exp[\ln(4.7)/45 * 1.6] = 1.06.$

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1 Rumchev et al. (2002), the OR = 1.24 (95% CI: 1.07-1.45) was somewhat higher than the
 2 OR = 1.17 for all the studies.
 3

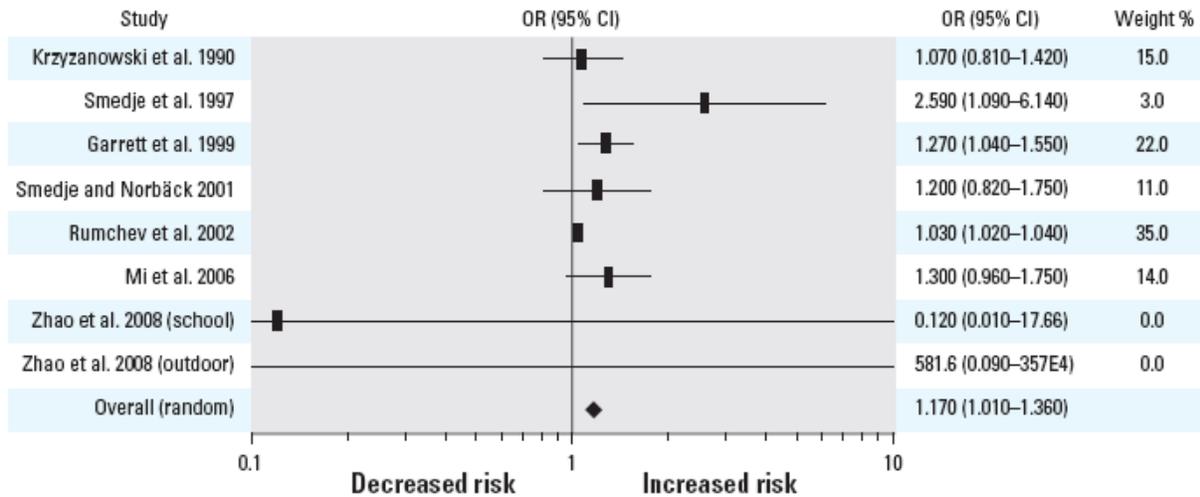


Figure 2. Forest plot of the relative risk estimates and their 95% CIs from the studies included in the meta-analysis of the association between formaldehyde exposure and asthma in children based on a random-effects model.

4
 5
 6 **Figure 4-2. McGwin Forest plot of relative risk estimates and 95% CIs from**
 7 **studies included in a meta-analysis of formaldehyde exposure and asthma in**
 8 **children based on the random effects models.**

9
 10 Source: McGwin et al. (2010).

11
 12
 13 Separate random-effects were fit for the six studies in which the ORs were for self-
 14 reported asthma yielding an OR = 1.26 (95% CI: 0.97–1.64) and for the two studies that used
 15 diagnosed asthma OR = 1.12 (0.88–1.44). Meta-analytic results stratified by study design
 16 yielded an OR = 1.25 (95% CI: 1.08–1.44) for the cross-sectional studies. This systematic
 17 review of the literature on asthma and formaldehyde provide evidence of a concentration-
 18 dependent increased risk of asthma (prevalence and incidence) associated with increased
 19 concentrations of formaldehyde.

20 Garrett et al. (1999) also evaluated the prevalence and severity of allergic sensitization to
 21 12 common allergens and reported increased prevalence with increasing formaldehyde
 22 concentration in the home. A respiratory symptom score, developed using responses by parents
 23 to a validated respiratory questionnaire during an interview, also was increased. The frequency
 24 of each respiratory symptom reported during the past year was categorized into four groups

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1 (none, 1–3 times, 4–12 times, and >12 times) with a score of 0–3, and scores for the eight
2 symptoms (cough, cough in the morning, shortness of breath, waking due to shortness of breath,
3 wheeze/whistling, asthma attacks, chest tightness, and chest tightness in the morning) were
4 combined to construct a total symptom score for each child. Mean respiratory symptom scores
5 increased with categories of the highest of four seasonal 4-day measurements of formaldehyde in
6 a multiple regression model after adjusting for parental asthma and allergy and interactions. For
7 the atopy endpoints, severity/prevalence of allergic sensitization to 12 common allergens was
8 increased in the medium (20–50 $\mu\text{g}/\text{m}^3$) and high (>50 $\mu\text{g}/\text{m}^3$) exposure groups relative to the
9 low (<20 $\mu\text{g}/\text{m}^3$) exposure group, based on the highest of four seasonal 4-day formaldehyde
10 measurements in the home ($p < 0.001$). The proportion of atopic children by three categories of
11 average formaldehyde levels in bedrooms was 0.50, 0.59 and 0.74 for <10 $\mu\text{g}/\text{m}^3$, 10–30 $\mu\text{g}/\text{m}^3$,
12 and >30 $\mu\text{g}/\text{m}^3$, respectively (test for trend, $p = 0.06$). The proportion by highest formaldehyde
13 level was 0.33, 0.64, and 0.75 for <20 $\mu\text{g}/\text{m}^3$, 20–50 $\mu\text{g}/\text{m}^3$, and >50 $\mu\text{g}/\text{m}^3$, respectively (test for
14 trend, $p < 0.001$). In logistic regressions, the crude association for atopy with an increase in
15 bedroom formaldehyde concentration per 10 $\mu\text{g}/\text{m}^3$ was OR = 1.34 which increased when
16 adjusted for parental asthma and gender to an odds ratio of 1.40 per 10 $\mu\text{g}/\text{m}^3$ (95% CI:
17 0.98–2.00). Thus, parental asthma was not a confounder of the association between
18 formaldehyde and prevalence of atopy. The adjusted odds ratio for atopy with an increase in
19 highest recorded formaldehyde per 20 $\mu\text{g}/\text{m}^3$ was 1.42 (95% CI 0.99–2.04). Passive smoking,
20 the presence of pets, indoor nitrogen dioxide concentrations, airborne fungal spores and house-
21 dust-mite allergens also did not influence the effect estimates and were unlikely to be
22 confounders. The association between formaldehyde concentrations and severity of allergic
23 sensitization was analyzed using two measures, number of positive skin prick tests and the ratio
24 of wheal diameters after skin pricks with allergens compared to the histamine wheal size.
25 Average levels of both measures of severity were higher in the two higher formaldehyde groups
26 compared to the lowest group ($p < 0.05$). Further, both measures were linearly related to
27 increasing formaldehyde categories in regression models controlling for parental asthma and
28 allergy and sex. The authors reported that 74% of the children had lived in the residences for at
29 least 5 years and 36% had lived there since birth. While the statistical analysis did not
30 specifically account for correlations in children living in same home (siblings), this
31 methodological limitation would not have induced a bias in the estimate of the effects, rather, the
32 likely impact would have been to underestimate the true variability in the effect estimate due to
33 the unlikely assumption that siblings can be treated as completely independent individuals.
34 Two controlled exposure studies in humans investigated whether exposure to a low level
35 of formaldehyde would enhance inhaled allergen responses among already atopic individuals

1 (Casset et al., 2006; Ezratty et al., 2007). These studies are also described in Section 4.1.1.2.2 on
2 pulmonary function. Casset et al. (2006) evaluated the effect of formaldehyde exposure on the
3 bronchial response to dust mite allergen in sensitized asthma patients. The study included
4 19 nonsmoking subjects (12 women and 7 men) ages 19-35 years with mild asthma. Subjects
5 had positive skin prick tests and specific IgE to *Dermatophagoides pteronyssinus* (dust mites).
6 Subjects had not had a respiratory tract infection in the two weeks priors to the testing.
7 Individuals sensitized to pollen were studied outside the relevant pollen season and people
8 sensitive to pet allergens did not have pets at home. The 30 minute crossover exposures to
9 $100 \mu\text{g}/\text{m}^3$ formaldehyde and air were randomly assigned with a three-week washout period in
10 between. Subjects underwent mite allergen challenge immediately after exposure to
11 formaldehyde or air to determine the dose of allergen that resulted in a 20% reduction in FEV_1
12 (PD_{20}). The dose which induced early-phase bronchial response was significantly lower in the
13 subjects exposed to formaldehyde (34.3 ng vs. 45.4 ng; $p < 0.05$). Late-phase bronchial response
14 was measured over the 6 hours following the dust mite challenge by comparing FEV_1 . The
15 maximum percentage FEV_1 reduction observed was significantly higher after exposure to
16 formaldehyde 15% vs. 11% ($p < 0.05$) (Casset et al., 2006).

17 While the study by Casset et al. (2006) clearly showed that acute formaldehyde exposure
18 ($100 \mu\text{g}/\text{m}^3$) enhanced both early-phase and late-phase bronchial responsiveness to mite allergen
19 in mite-allergen sensitized people with asthma, a subsequent study with a similar protocol did
20 not duplicate this finding. Ezratty et al. (2007) evaluated the response of asthmatics to inhaled
21 allergen after a 60 minute exposure to $500 \mu\text{g}/\text{m}^3$ (0.4 ppm) formaldehyde. The 12 subjects
22 (7 men and 5 women) were 18–44 years old, were nonsmokers, and were diagnosed with
23 intermittent asthma and allergy to pollen. No subjects had contracted an upper respiratory
24 infection for at least 4 weeks before the study. The crossover exposures (60 minutes) to filtered
25 air and $500 \mu\text{g}/\text{m}^3$ formaldehyde occurred 2 weeks apart in random order. Allergen inhalation
26 challenge, using an extract of 5 grass pollen allergens, was conducted immediately after each
27 exposure and the dose producing a 15% decrease in FEV_1 was determined ($\text{PD}_{15}\text{FEV}_1$).
28 Responsiveness to methacholine was determined 8 hours after the allergen inhalation challenge
29 ended. Lung function measurements were taken using a spirometer before, during and 8 hours
30 after the end of the allergen challenge. In addition, PEF and FEV_1 were measured with a
31 portable spirometer every 15 minutes during exposures to filtered air or formaldehyde and every
32 hour until the methacholine challenge. The authors reported that pulmonary function, expressed
33 as percent predicted, was not affected by the formaldehyde exposure, although the data were not
34 presented. The median $\text{PD}_{15}\text{FEV}_1$ for the allergen challenge was 0.80 (0.15–2.0) index of
35 reactivity (IR) after formaldehyde exposure and 0.25 (0.10–2.0) IR after the filtered air exposure

1 ($p = 0.06$). The ratio of response after formaldehyde exposure compared to filtered air exposure
2 was 1 in 7 subjects and higher in 5 subjects. The PD₂₀ for methacholine challenge was 0.23
3 (0.01–3.6) and 0.17 (0.03–4) ($p = 0.42$). The PD₂₀ for methacholine for formaldehyde compared
4 to filtered air was lower in 3 subjects, higher in 4 subjects, and not changed in 5 subjects.

5 There are multiple potential explanation for these seemingly conflicting findings in
6 Casset et al. (2006 and Ezratty et al. (2007) which include the small sample size of these studies,
7 the differencing in the particular allergen tested and difference in the protocols. It is possible
8 that the 12 subjects studied by Ezratty et al. (2007) may not have included individuals who were
9 especially susceptible to formaldehyde. In the Casset et al. (2006) study, the investigators mad
10 specific mention of the size of the particles used for the allergen challenge. They commented in
11 the discussion that dosimetric models of inhaled formaldehyde show that the flux is very large in
12 the first bronchial generations and then decreased rapidly. Casset et al. (2006) used an aerosol
13 with large particles (Mass Median Aerodynamic Diameter of 11.1 μm) specifically because these
14 large particles are deposited in the large airways where formaldehyde flux is higher. Ezratty et
15 al. (2007) did not report the size of the particle used in their challenge but both studies did report
16 the types of dosimeters jet nebulizers that were used and these were different. In a study
17 unrelated to formaldehyde, Praml et al. (2005) compared the physical and biologic doses of
18 methacholine for different nebulizers including the type used by Ezratty et al. (2007). Praml et
19 al. (2005) compared the airway responsiveness of 34 subjects using two types of nebulizers and
20 found that in 17 subjects, neither system caused a 20% decrease in FEV₁, while among
21 8 subjects, both systems were able to provoke a 20% decrease. The remaining 9 subjects
22 responded to only one type of nebulizer. Using the same protocol and the same methacholine
23 agent produced different results based on the type of nebulizer. It may be that the results of
24 Casset et al. (2006) and Ezratty et al. (2007) which did have other differences can be explained
25 thusly. It may also be a difference in particle size plays a role if the pollen allergens used in
26 Ezratty et al. (2007) were smaller and penetrate the lung beyond the upper lung where
27 formaldehyde exposure are greater.

28 Multiple occupational cases reports have documented that formaldehyde can cause the
29 onset of asthma and an epidemiologic study of residential exposures has shown that the risk of
30 incident physician-diagnosed asthma is associated with formaldehyde in a concentration-
31 response relationship after controlling for multiple personal characteristics and other
32 coexposures. The regression slope was statistically significant and increased odds ratios were
33 reported at formaldehyde concentrations of 50–59 $\mu\text{g}/\text{m}^3$ becoming statistically significant at
34 60 $\mu\text{g}/\text{m}^3$ (OR = 1.39). The findings of Rumchev et al. (2002; 2004) are consistent with a causal
35 association of formaldehyde on the incidence of asthma.

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

15 Many of these studies were evaluated quantitatively in a meta-analysis by McGwin et al.
16 (2010). McGwin et al indicated a significant positive association between formaldehyde
17 exposure and childhood asthma both with the Rumchev study (2002) and without it. However,
18 the magnitude of the effect reported by Rumchev et al. (2002) may be smaller than that of other
19 studies in a linear model. As peak expiratory flow rate is a diagnostic criterion of physician-
20 diagnosed asthma incidence, the results of the residential epidemiology study by Krzyzanowski
21 et al. (1990) showing statistically significant decrements in PEFr associated with increased
22 formaldehyde concentration are strongly supportive. Formaldehyde exposure has also been
23 associated with an increased severity of asthma symptoms in children (Garrett et al. 1999;
24 Delfino et al., 2003).

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

30 Garrett et al. observed an increase in the prevalence and severity of allergic sensitization
31 to common allergens associated with formaldehyde at concentrations of $20 \mu\text{g}/\text{m}^3$ and above.
32 The role of formaldehyde in exacerbating allergic responses to common allergens among atopic
33 individuals was demonstrated by Cassett et al. (2006) using formaldehyde concentrations of
34 $100 \mu\text{g}/\text{m}^3$. Another study using different allergens, dosimeters, and study protocol did not
35 report an effect of formaldehyde on allergic responses (Ezratty et al., 2007). Finally, the report

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

4 5 **4.1.1.4. Respiratory Tract Pathology**

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

24 25 **4.1.1.4.1. Nasal lesions.**

26 Ballarin et al. (1992) did a case-control study of 15 workers from a plywood factory
27 where urea-formaldehyde glue is used. Mean levels of formaldehyde exposure (8-hour average)
28 were estimated to be 0.09, 0.1, and 0.39 mg/m³ in three regions of the facility (sawmill, shearing
29 press, and warehouse, respectively). Nasal respiratory samples were obtained. Stained cells
30 were scored for histopathology. Cytology examination revealed increased squamous metaplasia
31 cells in 10 of 15 (67%) factory workers (with an average severity score of 2.3) compared with
32 one of 15 (6%) controls (with an average histology severity score of 1.6). In addition, one
33 formaldehyde exposed worker ($n = 15$) exhibited mild dysplasia and had the highest severity
34 score (3.0). Authors suggest that these results may be due to chronic irritation of the nasal
35 respiratory mucosa. This small study reported only incidence of lesions and did not score based

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

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

21 Edling et al. (1988) collected nasal biopsy samples from workers ($n = 75$) exposed to
22 formaldehyde at three plants (workers in two of these plants were also exposed to wood dust)
23 compared with a referent group ($n = 25$). Concentrations ranged from 0.1 to 1.1 mg/m³ (TWA)
24 with peaks of 5 mg/m³. Nasal histology was scored from 0 to 8 by increasing severity, from
25 normal respiratory epithelium (0) to carcinoma (8). A normal respiratory epithelium was noted
26 in 3 of 75 workers. A loss of cilia and goblet cell hyperplasia (scores of 2) was reported in eight
27 workers. Mixed cuboid/squamous epithelium (metaplasia), stratified squamous epithelium, and
28 keratosis were reported in 58 of 75 workers (those with scores of 3, 4, and 5 were combined).
29 Dysplasia (score of 6) was reported in 6 of 75 formaldehyde-exposed workers. None of the
30 workers had lesions that warranted a histologic score higher than 6. Histologic scores did not
31 correlate with duration of exposure but could not be confirmed due to poor reporting. Data from
32 the referent group were not included. A POD could not be determined from this study.

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

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

14 Pazdrak et al. (1993) exposed human subjects (six men, three women) to 0.4 ppm
15 formaldehyde in a chamber for 2 hours. Approximately half of the subjects suffered from skin
16 hypersensitivity to formaldehyde, while the other subjects were healthy. An evaluation of nasal
17 lavage pretest and following formaldehyde exposure revealed that the hypersensitive and healthy
18 groups had similarly elevated eosinophil counts at 0 hours after exposure (from
19 42×10^3 cells/mL to 72×10^3 cells/mL for healthy subjects [$p < 0.05$] and from
20 39×10^3 cells/mL to 69×10^3 cells/mL for hypersensitive subjects [$p < 0.05$]). Similar
21 eosinophil levels were also seen in both groups at 3 and 18 hours. Both groups had equivalent
22 increases in lavage albumin and total protein levels following exposure, but basophil counts were
23 unchanged. Based on evidence of formaldehyde-induced inflammation, these data provide a
24 LOAEL of 0.4 ppm for nasal histopathology.

25

26 **4.1.1.4.2. Mucociliary clearance.**

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

33 Andersen and Molhave (1983) reviewed five controlled human studies, one of which
34 (Andersen and Lundqvist, 1974) examined mucus flow rate in 16 individuals acutely exposed to
35 0, 0.3, 0.5, 1, or 2 ppm formaldehyde for 4–5 hours in a chamber. Mucus flow rate was

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

5 Holmström and Wilhelmsson (1988) demonstrated reduced mucociliary clearance and
6 nasal mucosal swelling in 70 workers exposed to a mean formaldehyde concentration of
7 0.21 ppm, compared with a referent group of store clerks ($n = 36$) and was further averaged over
8 years of exposure. Mucosal swelling and mucociliary activity was measured in the nasal
9 turbinates. The authors also reported symptoms not only during the weekdays, but also over
10 weekends and vacation periods. Formaldehyde-exposed subjects self-reported significantly more
11 nasal discomfort, eye discomfort, deeper airway discomfort, and frequent headache than the
12 referent group. Groups exposed to formaldehyde had more pronounced mucosal swelling
13 (10.7 nasal resistance score) compared with the reference group (6.5 nasal resistance score).
14 This difference persisted when data were normalized for differential nasal congestion in the
15 subjects. Decreased mucociliary activity was seen in 3% of controls and 20% of formaldehyde-
16 exposed subjects and reached statistical significance ($p < 0.05$). It is not clear whether impaired
17 mucociliary clearance was a consequence of altered cell morphology or increased mucus
18 viscosity. These data provide a LOAEL of 0.21 ppm based on impaired mucociliary clearance.

19 Thus, mild nasal epithelial lesions observed in formaldehyde-exposed workers have been
20 observed consistently at levels of about 0.20 ppm to about 2 ppm (Boysen et al., 1990;
21 Holmström et al., 1989; Edling et al., 1988). Of these, Holmström et al. (1989) and Edling et al.
22 (1988) do not appear to be confounded by exposure to wood dust. Nasal biopsy pathology from
23 formaldehyde-exposed workers is consistent with irritant and reactive properties of
24 formaldehyde (Ballarin et al., 1992; Boysen et al., 1990; Holmström et al., 1989; Edling et al.,
25 1988; Berke, 1987). Moreover, these findings are supported by results from animal toxicity and
26 pharmacokinetic and anatomical airflow studies, indicating that, at concentrations less than
27 1 ppm, inhaled formaldehyde gas does not reach lower regions of the respiratory tract. Of the
28 available human studies that evaluated histopathology, Holmström and Wilhelmsson (1988)
29 appears to be the most robust and sensitive. The study was carefully designed and included a
30 large sample of formaldehyde-exposed subjects who were considered separately from workers
31 exposed to combinations of exposures (formaldehyde and wood dust). Study subjects had been
32 exposed to formaldehyde regularly for many years. The authors reported not only weekday
33 exposures but effects reported on weekends and on vacation. Total exposure was carefully
34 calculated and averaged. The data were controlled for potential confounders, such as smoking.
35 The endpoint of reduced mucociliary clearance has been substantiated by Andersen and Molhave

1 (1983) and Holmström et al. (1989). Animal studies have also reported formaldehyde-induced
2 changes on the nasal mucosa and are highlighted in Section 4.2.1.2.

4 **4.1.1.5. Immunologic Effects**

5 Numerous studies have examined the immunologic responses of individuals exposed to
6 formaldehyde. This section will discuss four specific areas related to immunotoxicity after
7 exposure to formaldehyde: increased upper respiratory tract (URT) infections, systemic immune
8 dysfunction, sensitization and atopy, and production of formaldehyde-protein complexes. Some
9 studies report increased incidence of URT infections after exposure to formaldehyde (Lyapina et
10 al., 2004; Krzyzanowski et al., 1990; Holness and Nethercott, 1989). This effect appears to
11 occur independently of systemic immune changes and may be due to damage to the mucosal
12 barrier, thus facilitating pathogen access. A number of studies have investigated the hypothesis
13 that formaldehyde may induce systemic immunomodulation (Ohtani et al., 2004a, b; Erdei et al.,
14 2003; Thrasher et al., 1990, 1987; Pross et al., 1987). Some studies have also evaluated immune
15 system effects by investigating the role of reactive oxygen species (ROS) from respiratory burst
16 associated with immune cells (Lyapina et al., 2004; Gorski et al., 1992) and by assessing
17 chromosomal damage in immune cells (Orsière et al., 2006; Yu et al., 2005). In addition to the
18 effects of formaldehyde on asthmatics and the potential for formaldehyde exposure to exacerbate
19 asthmatic responses (Pourmahabadian et al., 2006; Herbert et al., 1994; Malaka and Kodama,
20 1990; Alexandersson and Hedenstierna, 1989; Alexandersson et al., 1982), reviewed in
21 Section 4.1.1.2, numerous studies have investigated whether formaldehyde may directly induce
22 sensitization and atopic responses by measuring immunoglobulin E (IgE) levels associated with
23 formaldehyde exposure (Ohmichi et al., 2006; Vandenplas et al., 2004; Doi et al., 2003; Baba et
24 al., 2000; Palczynski et al., 1999; Krakowiak et al., 1998; Wantke et al., 1996a, b; Liden et al.,
25 1993; Salkie, 1991; Grammer et al., 1990; Kramps et al., 1989). Findings are largely negative
26 and suggest that formaldehyde-induced IgE production is not likely. Lastly, studies have
27 investigated the production of formaldehyde-specific antibodies, formaldehyde-albumin
28 complexes, and formaldehyde-heme complexes (Kim et al., 2001; Carraro et al., 1997; Grammer
29 et al., 1993, 1990; Dykewicz et al., 1991; Thrasher et al., 1990). Heme complex formation is not
30 a strict immunologic endpoint but may trigger antibody formation and thus it will be discussed in
31 this section. This section will thus summarize the human studies that have specifically addressed
32 the increased incidence of URT infections, immunotoxic endpoints, atopy and sensitization, and
33 formation of formaldehyde-heme and formaldehyde-albumin complexes.

1 **4.1.1.5.1. Increased URT infections.**

2 Three studies have investigated the possibility that formaldehyde exposure leads to
3 increased URT infections (Lyapina et al., 2004; Krzyzanowski et al., 1990; Holness and
4 Nethercott, 1989). Lyapina et al. (2004) studied 29 workers who were occupationally exposed
5 occupationally to formaldehyde for an average of 12.7 years through contact with carbamide-
6 formaldehyde glue. The mean values of the average shift concentrations of formaldehyde in the
7 application of carbamide-formaldehyde glue was found to be 0.71 ppm TWA with a range of
8 0.32 to 1.57 ppm. The workers were divided into two subgroups, one ($n = 12$) that suffered from
9 either a long history (with clinical findings) of chronic mucous inflammation of the URT with
10 multiple relapses and a second group ($n = 17$) whose URT inflammations were short, acute, and
11 predominantly viral. Twenty-one healthy subjects served as controls. A statistically significant
12 association of self-reported chronic bronchitis and decreased resistance to URT infection was
13 reported in all the exposed workers compared with controls ($p = 0.02$). Of the workers, 41% had
14 a history of chronic respiratory infection and frequent long-lasting infectious inflammatory
15 relapses (group 1a). Another group (group 1b) consisted of 17 exposed workers, 12 of whom
16 had no history of recurrent viral infections of the URT. There was a statistically significant
17 association of frequency and duration of inflammatory relapses between groups 1a and 1b. No
18 dates were provided regarding when these measurements were made or over what period of time
19 they were calculated.

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

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

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

4 **4.1.1.5.2. Immune function.**

5 A number of studies have evaluated the ability of formaldehyde to induce systemic
6 immunotoxic effects (Erdei et al., 2003; Thrasher et al., 1990, 1987; Pross et al., 1987). Some
7 studies have reported altered innate immune responses associated with formaldehyde exposure in
8 immunologically compromised children (Erdei et al., 2003), while others have noted adaptive
9 immune response suppression associated with formaldehyde exposure (Thrasher et al., 1990,
10 1987) and changes associated with alterations to a predominant T—lymphocyte helper 2 (Th2)
11 pattern (Ohtani et al., 2004a, b). In contrast, Pross et al. (1987) did not observe formaldehyde-
12 associated changes in systemic immune function.

13 Erdei et al. (2003) found that *Haemophilus influenzae* humoral biomarker (H.in.IgG),
14 *Klebsiella pneumoniae* biomarker (K.pn.IgG), and elevated monocyte concentrations were
15 significantly associated with high formaldehyde concentrations in asthmatic children, compared
16 with nonsensitive children. Briefly, Erdei et al. (2003) compared the immune system responses
17 in 9- to 11-year-old Hungarian school children whose respiratory systems were immunologically
18 compromised (chronic respiratory disease, asthma) and normal children who were exposed to
19 indoor air pollutants, including formaldehyde. In the homes of the children with the highest
20 levels of pollutants, 49.3% of formaldehyde measurements exceeded the Hungarian indoor
21 standard of 0.01 ppm, while 20% exceeded the World Health Organization’s (WHO’s) suggested
22 indoor level of 0.09 ppm. The authors excluded from consideration all measurements that
23 exceeded WHO’s air quality guidelines in one unidentified city to prevent a “city-related bias,”
24 since these measurements occurred entirely in that city. The average formaldehyde
25 concentration in the 123 homes tested was 14 ppm with a range of 0.5 to 46 ppm. H.in.IgG and
26 K.pn.IgG were significantly associated with high formaldehyde concentrations ($p < 0.013$ and
27 $p < 0.049$, respectively) in sensitive children compared with nonsensitive children. These
28 markers were also correlated with high levels of nitrogen dioxide, the number of cigarettes
29 smoked, and exposure to paint, volatile organic compounds, and solvents. Additionally, indoor
30 formaldehyde exposure was significantly associated with increased monocyte concentrations
31 ($p < 0.017$) that are important to the innate immune response (inflammation) in diseased tissue.
32 The authors concluded that the elevation of immune biomarkers in sensitive children with
33 respiratory disease is likely the result of high concentrations of toxic indoor air pollutants,
34 including formaldehyde.

1 Thrasher et al. (1987) assessed the effects of formaldehyde exposure on cellular
2 immunity and antibody formation in eight exposed and eight unexposed individuals. The
3 exposed group consisted of three males and five females. Seven of the exposed individuals had
4 resided in mobile homes for periods ranging from 2 to 7 years; the eighth was a laboratory
5 worker who resided in a newly decorated, energy-efficient apartment. Air monitoring in four of
6 the homes revealed formaldehyde vapor concentrations ranging from 0.07 to 0.55 ppm. Venous
7 blood samples were collected from all subjects and T- and B-cells were counted and monitored
8 for blastogenesis. When IgG and IgE antibodies to formaldehyde were monitored in serum, no
9 IgE antibodies to formaldehyde were detected in exposed or control subjects. IgG antibody titers
10 in exposed subjects ranged from 1:8 to 1:256 but essentially were undetected (1:4) in seven of
11 the controls. T- and B-cell numbers were significantly lower ($p < 0.05$) in mobile home residents
12 (48 and 12.6%, respectively) compared with those of control subjects (65.9 and 14.75%,
13 respectively). As determined by incorporation of ^3H -thymidine into 48-hour unaltered
14 lymphocytes, phytohemagglutinin-stimulated T- and B-cell blastogenesis was significantly
15 depressed ($p < 0.01$) in cells of mobile home residents compared with those of control subjects
16 (17,882 and 28,576 cpm, respectively). Thrasher et al. (1987) concluded that exposure to
17 formaldehyde decreases the proportion of peripheral T cells.

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

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

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

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

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

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

3 Lyapina et al. (2004) also reported effects of formaldehyde exposure on neutrophil
4 respiratory burst activity (NRBA), the capacity of polymorphonuclear leukocytes to produce
5 reactive oxygen radicals in response to chemical or microbial stimuli using flow cytometry.
6 Briefly, Lyapina et al. (2004) studied 29 workers who were occupationally exposed to
7 formaldehyde for an average of 12.7 years through contact with carbamide-formaldehyde glue
8 with a mean value of the average shift concentration of formaldehyde reported as 0.71 ppm
9 TWA with a range of 0.32 to 1.57 ppm. The workers were divided into two subgroups, one
10 ($n = 12$) that suffered from either a long history (with clinical findings) of chronic mucous
11 inflammation of the URT with multiple relapses, and a second group ($n = 17$) whose URT
12 inflammations were short, acute, and predominantly viral. Twenty-one healthy subjects served
13 as controls. A suite of hematological tests and flow cytometric analysis for respiratory burst
14 activity were performed. Although no significant difference was observed in the spontaneous
15 and stimulated NRBA (median percentage of oxidizing cells) between the 29 exposed workers
16 with URT inflammation and the healthy controls (0.83 versus 1.35, respectively), a separate
17 comparison of the NRBA of 12 workers with chronic, repeating URT infections and 17 workers
18 with short, infrequent episodes of URT inflammations was significant (0.45 versus 1.00,
19 $p = 0.037$). When the NRBA of the group with chronic URT infections ($n = 12$) was separately
20 compared with that of the healthy controls ($n = 21$), the results were also significant (0.45 versus
21 1.35, $p = 0.012$). Individuals with chronic URT infections have reduced NRBA that could be
22 due to formaldehyde exposure. Neutrophils respond to tissue damage or local invasion of
23 microorganisms and act to phagocytize foreign cells. If neutrophilic activity is hampered or
24 altered by formaldehyde exposure, then the ability to fight infection will be diminished, leading
25 to prolonged infection. However, no dose-response pattern of formaldehyde exposure could be
26 determined from this study.

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

1 genotoxic agents. Study participations were excluded if workers had a history of radio- or
2 chemotherapy or had used therapeutic medications that were known to be mutagenic.
3 Occupational exposure was determined through 15-minute and 8-hour personal air sampling
4 during a typical workday. Mean formaldehyde concentrations were 2 ppm (range:
5 <0.1–20.4 ppm) for 15-minute sampling and 0.1 ppm (range: <0.1–0.7 ppm) for 8-hour
6 sampling. No change in DNA damage was found between the beginning and end of the workday
7 among exposed workers (3.9 ± 0.6 versus 3.6 ± 0.5 relative light units/ng DNA). However,
8 exposed workers had significant elevations in the binucleated micronucleated cell rate (BMCR)
9 per 1,000 cells compared with controls (16.9 ± 9.3 versus $11.1 \pm 6.0\%$; $p < 0.001$) suggesting a
10 clastogenic response, but BMCR did not appear to be correlated with formaldehyde
11 concentration. Linear regression analysis showed that the effect for exposure remained after
12 adjusting for gender, age, smoking, and drinking habits. For 18 exposed and 18 control workers
13 who underwent cytokinesis-blocked micronucleus assay (CBMA) combined with fluorescent in
14 situ hybridization (FISH) with pan-centromeric DNA probe, results showed that the frequency of
15 micronuclei (MN) containing only one centromere (C1+MN) was elevated among the exposed
16 compared with unexposed workers ($11.0 \pm 6.2\%$ versus $3.1 \pm 2.4\%$; $p < 0.001$). The effect of
17 exposure remained significant after controlling for gender, age, smoking, and drinking habits.
18 Results from Yu et al. (2005) and Orsière et al. (2006) suggest that formaldehyde exposure may
19 promote chromosomal damage leading to micronucleated lymphocytes.

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

22

23 **4.1.1.5.3. Sensitization and atopy.**

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

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

7 Palczynski et al. (1999) evaluated whether exposure to formaldehyde might facilitate
8 specific sensitization to common allergens. The study population was comprised of residents of
9 apartments built in 1989–1990. Only households with children from 5–15 years were eligible
10 for the study. A random sample of 120 apartments was selected in which lived a total of
11 465 persons aged 5–65 years. Individual demographic characteristics and medical histories were
12 determined by questionnaire. Residents were tested, using the skin-prick method, for allergen
13 response to a variety of materials, such as household dust, pollens, and feathers. Total serum IgE
14 levels were measured, and the presence of formaldehyde-specific IgE antibodies was determined.
15 Measured mean levels of formaldehyde were 21.05 ± 8.94 ppb. No significant relationship
16 between respiratory allergy prevalence and indoor exposure to formaldehyde was detected.
17 Significant increases in serum IgE levels were found in children exposed to both environmental
18 tobacco smoke and formaldehyde.

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

25 Several studies have examined serum for formaldehyde-specific IgE antibodies in groups
26 of formaldehyde-exposed humans (Ohmichi et al., 2006; Krakowiak et al., 1998; Wantke et al.,
27 1996a, b; Salkie, 1991; Grammer et al., 1990; Kramps et al., 1989). While formaldehyde-
28 specific IgE was reported in one study (Wantke et al., 1996a), results from most other studies
29 failed to find a consistently strong association between formaldehyde-specific IgE or IgG
30 antibodies in groups of formaldehyde-exposed humans.

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

1 (23/62), cough (15/62), and nosebleeds (14/62). The number of children with symptoms in each
2 classroom decreased with decreasing formaldehyde concentration (49, 47, and 24, respectively,
3 for the 0.075, 0.069, and 0.043 ppm classrooms). However, the investigators reported that
4 elevated levels of specific IgE did not correlate with the number and severity of symptoms.
5 When the children were evaluated after 3 months in a new school that did not have particleboard
6 paneling and had lower ambient formaldehyde concentrations (0.029, 0.023, and 0.026 ppm), the
7 number of children reporting symptoms decreased significantly from earlier figures, and, when
8 measured in 20 of the children, the mean serum levels of formaldehyde-specific IgE declined
9 significantly compared with premoving mean levels.

10 In contrast, a study by Krakowiak et al. (1998) measured serum IgE levels in asthmatic
11 and healthy subjects as part of a larger study to characterize the mechanism of
12 formaldehyde-induced nasal and bronchial response in asthmatic subjects with suspected
13 formaldehyde allergy. Ten subjects reported to have formaldehyde rhinitis and asthma and
14 10 healthy subjects underwent a 2-hour inhalation challenge in an exposure chamber with
15 formaldehyde at a concentration of 0.5 mg/m³ (0.41 ppm). Formaldehyde-specific serum IgE
16 antibodies were measured, and cellular, biochemical, and mediator changes were assessed in
17 nasal lavage before, immediately after, and at 4 and 24 hours after challenge. Challenges with
18 formaldehyde caused only transient symptoms of rhinitis in both groups. Furthermore, none of
19 the subjects thought to have occupational asthma developed clinical symptoms of bronchial
20 irritation. No specific IgE antibodies to formaldehyde were detected in persons with
21 occupational exposure to formaldehyde. No differences in the nasal response to formaldehyde
22 were found between subjects reported to have occupational allergic respiratory diseases and
23 healthy subjects ($p > 0.05$). The study showed that inhaled formaldehyde at a level as low as
24 0.5 mg/m³ did not induce a specific allergic response either in the upper or in the lower part of
25 the respiratory tract. In addition, it demonstrated that there was no difference in nasal response
26 to formaldehyde between asthmatic subjects occupationally exposed to formaldehyde and
27 healthy subjects.

28 Similarly, formaldehyde-specific IgE antibodies were detected in only 1 serum sample
29 (out of 86) from four groups of formaldehyde-exposed subjects (Kramps et al., 1989). The
30 subject with detected formaldehyde-specific IgE displayed allergic symptoms. The groups
31 included (1) 28 subjects living or working in places with formaldehyde-containing construction
32 materials (e.g., chipboard) and estimated formaldehyde concentrations ranging from 0.08 to
33 0.37 ppm, (2) 18 occupationally exposed subjects from an anatomy laboratory and in other
34 unspecified industries where air concentrations were not measured, (3) 12 hospital attendants
35 who worked with formaldehyde-sterilized hemodialysis equipment, and (4) 28 hemodialysis

1 patients coming into contact with equipment sterilized with formaldehyde. Other subjective
2 symptoms, such as headache, eye irritation, and respiratory complaints, were reported by
3 24/28 subjects in the construction material group and confirm that formaldehyde is an irritant
4 (reviewed in Section 4.1.1.1). Durations of exposure or length of employment were not reported
5 for the subjects in this study.

6 Grammer et al. (1990) studied the immunologic nature of formaldehyde sensitivity in
7 37 workers who were examined by a group of physicians in response to complaints of
8 formaldehyde-related illness. Air sampling of formaldehyde ranged from 0.003 to 0.078 ppm,
9 but specific levels were not tied to specific workplace areas. Blood samples were collected and
10 assayed for IgE and IgG activity against formaldehyde-human serum albumin (formaldehyde-
11 HSA) and HSA alone. None of the workers had IgG activity against formaldehyde-HSA. Five
12 workers had comparable IgE activity against both formaldehyde-HSA and HAS that was more
13 than twice the normal control serum levels. No IgE antibodies were detected in the other
14 32 workers. The authors concluded that there was no evidence of an immunologically mediated
15 response to formaldehyde in this group of workers.

16 Formaldehyde-specific IgE was not detected in any of a group of 45 medical students
17 before or after the students attended a 4-week anatomy dissecting course (Wantke et al., 1996b).
18 Estimates of ambient air concentrations of formaldehyde ranged from 0.059 to 0.219 ppm
19 (0.124 ± 0.05 ppm; mean \pm SD). However, the survey revealed frequencies of irritation
20 symptoms that were consistent with other studies (e.g., itching of the skin in 33/45 students,
21 headache in 15/45, and burning eyes in 13/45).

22 Similarly, Ohmichi et al. (2006) were unable to correlate formaldehyde exposure with
23 specific IgE production among eight students attending a gross anatomy laboratory.
24 Formaldehyde exposure was estimated to range from 0.33 to 1.47 ppm during the laboratory
25 sessions. The sample size was small, and IgE levels varied substantially (ranging from <19 to
26 >5,000 international units/mL). Compared with IgE levels taken 90 minutes prior to the start of
27 the first session, IgE levels measured shortly after the last session and up to 23 days following
28 the last session showed no association with exposure.

29 Salkie (1991) investigated the prevalence of formaldehyde-specific IgE in practicing
30 pathologists who complained of formaldehyde sensitivity. Exposure levels were not reported.
31 Serum samples were assayed for total IgE and formaldehyde-specific IgE. Of the 46 subjects,
32 29 self-reported atopy that was confirmed in 12 subjects by positive IgE. Moreover, 29 subjects
33 complained of formaldehyde-specific sensitivity. However, zero subjects had formaldehyde-
34 specific IgE, and there was no evidence that atopic individuals were more sensitive to

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

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

9 A clinical study by Liden et al. (1993) evaluated IgE-specific antibodies against
10 formaldehyde in 23 subjects who had previously tested positive for skin sensitization by skin
11 prick test. Subjects were exposed to formaldehyde by skin patch (1% formaldehyde in water).
12 Ten of the subjects were classified as atopic. Though 15 of 23 of the sensitized subjects were
13 also sensitive to formaldehyde applied by skin patch, formaldehyde-IgE was positive in 2 of
14 15 individuals who were not classified as atopic. No dose-response relationship could be
15 determined from the study design of this study.

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

24 **4.1.1.5.4. Formaldehyde-albumin and formaldehyde-hemoglobin complexes.**

25 Numerous studies have shown that formaldehyde can bind to blood proteins such as
26 hemoglobin (Hb) and human serum albumin (HSA) forming formaldehyde-Hb (Bono et al.
27 2006) and formaldehyde-HSA complexes (Carraro et al., 1997; Grammer et al., 1993, 1990;
28 Dykewicz et al., 1991; Thrasher et al., 1990). Kim et al. (2001) failed to identify IgE against
29 formaldehyde-HSA complexes in one case-control subject following industrial occupational
30 exposure to formaldehyde. These complexes may serve to traffic formaldehyde throughout the
31 bloodstream and throughout the body. While formaldehyde may be too small to engender an
32 immune response, these complexes may be able to trigger formaldehyde-protein-specific
33 antibodies, leading to an immune response, including sensitization.

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

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

8 Grammer et al. (1990) studied the immunologic nature of formaldehyde sensitivity in
9 37 workers who were examined by a group of physicians in response to complaints of
10 formaldehyde-related illness. Air sampling of formaldehyde ranged from 0.003 to 0.078 ppm,
11 but specific levels were not tied to specific workplace areas. Blood samples were collected and
12 assayed for IgE and IgG reactivity against HSA alone and formaldehyde-HSA. None of the
13 workers had IgG activity against formaldehyde. Five workers had IgE against both HSA alone
14 and against formaldehyde-HSA complexes. No IgE antibodies were detected in the other
15 32 workers. The authors concluded that there was no evidence of an immunologically mediated
16 response to formaldehyde in this group of workers.

17 Grammer et al. (1993) described the evaluation of a worker with bronchospasm
18 symptoms caused by formaldehyde exposure. The worker was evaluated by means of ELISA,
19 cutaneous tests, and methacholine and formaldehyde inhalation challenges. The ELISA showed
20 that the worker had positive IgE and IgG titers to formaldehyde-HSA. The worker also had a
21 positive cutaneous test for formaldehyde-HSA but a negative methacholine challenge at
22 25 mg/mL and negative formaldehyde inhalation challenges at exposure concentrations of 0.3, 1,
23 3, and 5 ppm for 20 minutes. The worker might have developed a positive response if a higher
24 concentration of formaldehyde had been used for the challenge, but it is more probable that the
25 worker's symptoms were not caused by immunologically mediated asthma.

26 Dykewicz et al. (1991) evaluated whether IgE or IgG antibodies to formaldehyde were
27 related to formaldehyde exposure or to respiratory symptoms arising from such an exposure.
28 The authors studied 55 potentially exposed subjects (hospital histology technicians, internal
29 medicine residents, pathology residents, current smokers, and persons with known workplace
30 exposure to formaldehyde) and compared them to controls with no history of formaldehyde
31 exposure. Reported workplace formaldehyde concentrations were 0.2–0.64 ppm for pathology
32 residents, 0.64 ppm for histology technicians, and 0.6–11 ppm for miscellaneous formaldehyde
33 exposure scenarios. Workplace air concentrations were not measured for the other occupations.
34 Occupational exposure to formaldehyde averaged 12.45 years for histology technicians,
35 0.38 years for medical residents, 3.21 years for pathology residents, and 18.34 years for five

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

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

21 Given that formaldehyde is a sensory irritant that is particularly bothersome to
22 individuals with compromised lung function or asthma, numerous studies have assessed the
23 ability of formaldehyde to induce immunotoxic effects (Wieslander et al . 1997; Norback et al .
24 1995; Grammer et al . 1993; Pross et al . 1987). Some studies have documented increased rates
25 of chronic bronchitis and upper respiratory tract infections associated with exposure to
26 formaldehyde, which suggests a possible immunomodulatory effect (Gorski and Krakowiak
27 1991; Krzyzanowski et al . 1990; Malaka and Kodama 1990; Holness and Nethercott 1989;
28 Tuthill 1984). However, of the numerous articles that have investigated systemic
29 immunomodulatory effects due to formaldehyde (Lyapina et al., 2004; Gorski et al., 1992;
30 Thrasher et al., 1990, 1988, 1987; Pross et al., 1987), few have reported significant immune
31 modulation related to formaldehyde exposure (Thrasher et al., 1990, 1988, 1987; Pross et al.,
32 1987). Significant decreases in specific adaptive immune cell populations do not appear
33 correlated to formaldehyde exposure (Erdei et al., 2003; Gorski et al., 1992; Thrasher et al.,
34 1990, 1987; Pross et al., 1987). Thus, the tendency for increased infection rates associated with
35 formaldehyde may not be related to altered immune function. Perhaps altered mucociliary

1 clearance and disturbed mucosal barrier may provide greater access for pathogens and result in
2 greater infection rates. Moreover, formaldehyde has been associated with exacerbation of
3 asthmatic or atopic responses, particularly in sensitized individuals. However, this effect does
4 not appear to occur by increased IgE or formaldehyde-specific IgE levels (Ohmichi et al., 2006;
5 Palczynski et al., 1999; Krakowiak et al., 1998; Wantke et al., 1996b; Salkie, 1991; Grammer et
6 al., 1990; Kramps et al., 1989). Thus, formaldehyde-associated enhanced allergic responses does
7 not appear to be due to direct induction of sensitization and may not occur via an immunologic
8 mechanism. Lastly, the formation of formaldehyde-heme and formaldehyde-HSA complexes
9 has been well documented (Grammer et al., 1993, 1990; Dykewicz et al., 1991; Thrasher et al.,
10 1990) and may serve as a biomarker of exposure (Carraro et al., 1997). Moreover, these
11 complexes may provide a means by which formaldehyde travels throughout the bloodstream and
12 may drive antibody formation that may lead to immune activation.

13

14 **4.1.1.6. *Neurological/Behavioral***

15 There is some suggestion of neurological impairment in humans following occupational
16 exposure to formaldehyde; the data are limited and the results from several studies are potentially
17 confounded by exposure to other solvents. Two studies of histology technicians with
18 occupational exposure to formaldehyde and other solvents found neurological deficits and poorer
19 performance on neurocognitive tests associated with formaldehyde exposure (Kilburn et al.,
20 1987, 1985). In another study, Kilburn and Warshaw (1992) found no change from initial
21 performance, for as long as 4 years, in follow-up evaluations of histology technicians with
22 continuing exposure to formaldehyde. In a preliminary report from a prospective study,
23 Weiskopf et al. (2009) found a strong association between duration of formaldehyde exposure
24 and death from amyotrophic lateral sclerosis (ALS). In a controlled exposure study, Bach et al.
25 (1990) found that, when workers with chronic formaldehyde exposure were challenged with an
26 acute formaldehyde exposure, they exhibited poorer performance on some neurocognitive tests
27 compared with workers without chronic exposure undergoing the same acute challenge
28 conditions. In another controlled exposure study, Lang et al. (2008) found equivocal changes in
29 reaction time following an acute exposure.

30

31 **4.1.1.6.1. *Epidemiological studies.***

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

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

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

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

4 **4.1.1.6.2. *Controlled exposure studies.***

5 Bach et al. (1990) examined whether cognitive and motor performance of humans
6 responded acutely to formaldehyde exposure and whether previous chronic exposure to
7 formaldehyde affected the responses observed following acute exposure. Thirty-two men with at
8 least 5 years of occupational exposure to formaldehyde and 29 matched controls were exposed to
9 formaldehyde at concentrations of 0.04, 0.21, 0.48, or 1.10 mg/m³ (32, 170, 390, or 890 ppb) for
10 5.5 hours. During the exposure period, symptoms were assessed by using a standardized
11 questionnaire, and subjects were evaluated in four tests designed to estimate several aspects of
12 cognitive function. Testing was performed once prior to exposure and twice during the exposure
13 period. The authors noted that the typical dose-related symptoms of respiratory irritation were
14 not seen in this study. Previously unexposed subjects reported more headaches, “heavy head,”
15 and physical tiredness than the exposed workers ($p < 0.025$). In both occupationally exposed and
16 unexposed subjects, decreased performance in an addition test was significantly correlated with
17 increasing concentration of formaldehyde (decreased number of additions, $p < 0.025$; increased
18 number of errors, $p < 0.05$). Compared with previously unexposed subjects, occupationally
19 exposed subjects showed significantly decreased performance in three other tests (digit symbol
20 test [pooled exposure groups, $p < 0.025$]; digit span [total digit sum], $p < 0.025$; graphic
21 continuous line test, $p < 0.05$), although the effect was not dose related. The study did not adjust
22 for several potential confounders, including prior exposure to other chemical agents, and the age
23 and health status of the cases and controls. Authors concluded that their data indicated that acute
24 exposure to formaldehyde might cause acute effects on CNS functions at exposures of 0.40 and
25 1.2 mg/m³ (equivalent measured doses of 390 or 890 ppb, with a NOAEL of 170 ppb), but that
26 more investigation was needed to verify their results.

27 In a study evaluating chemosensory irritation, Lang et al. (2008) assessed possible
28 changes in reaction time during an acute (4-hour) exposure to formaldehyde concentrations
29 between 0–0.5 ppm (some exposure sessions also included short concentration peaks of up to
30 1 ppm) with or without a masking agent (ethyl acetate). Twenty-one healthy volunteers were
31 exposed once per day to each of 10 different exposure combinations in random order (for a total
32 of 10 sessions per subject). Reaction time was tested before and after each exposure session.
33 Significant increases in reaction time were seen at 0.3 ppm formaldehyde, with or without
34 masking agent, but not at 0.5 ppm. The significance of these findings is unclear.

1 Performance of 16 healthy volunteers on addition, multiplication, and card punching
2 tasks was measured by Andersen and Molhave (1983) before and during a 5-hour exposure to
3 formaldehyde at concentrations up to 2 mg/m³. The authors reported that formaldehyde
4 exposure had no effect on performance, but results were not presented.

6 **4.1.1.6.3. Summary.**

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

12 **4.1.1.7. Developmental and Reproductive Toxicity**

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

25 **4.1.1.7.1. Spontaneous abortion.**

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

33 The earliest report of an association between spontaneous abortion and formaldehyde
34 exposure comes from a Swedish cohort study of female laboratory workers (Axelsson et al.,
35 1984). Subjects were women born in 1935 or later and worked in a university laboratory during
36 1968–1979. There were 745 women who responded to a mailed questionnaire (response

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1 rate = 95%), 556 of whom reported on 1,180 pregnancies that resulted in 997 births. Exposure to
2 formaldehyde was estimated based on answers to the questionnaires. Formaldehyde exposure
3 was reported only in connection with 10 pregnancies, of which 5 went to term, 3 were reported
4 as miscarriages, and 2 were terminated by induced abortion. Excluding the latter, the
5 spontaneous abortion rate among women exposed to formaldehyde in the first trimester was 3/8
6 (37.5%) compared with 14/148 (9.5%) in the population of laboratory workers not exposed to
7 any solvent in the first trimester.

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

16 In a 1988 Health Hazard Evaluation, the National Institute for Occupational Safety and
17 Health (NIOSH) investigated complaints of adverse reproductive outcomes at a plant where
18 work pants were cut and sewn with a fabric that was treated with a resin that releases
19 formaldehyde (Seitz and Baron, 1990). In a NIOSH laboratory, the fabric released 163 to
20 1,430 µg of formaldehyde/gram of cloth. TWA personal breathing space formaldehyde levels
21 ranged from trace to 0.46 ppm, while workstation values ranged from 0.32 to 0.70 ppm. The
22 investigators studied the outcomes by using a mailed questionnaire. The response rate for
23 current employees was 98%. There were 296 pregnancies among a cohort of 188 women. The
24 investigators found increased rates of spontaneous abortion, premature birth, and congenital
25 malformations. The crude rate of spontaneous abortion was 21% among women working at the
26 plant while pregnant (4 of 19 pregnancies), 15% among women employed elsewhere while
27 pregnant (11 of 71 pregnancies), and 5% among women at home while pregnant (10 of 206
28 pregnancies). The investigators did not explain how workers employed elsewhere or at home
29 during pregnancy were categorized compared with current workers, nor did they calculate RRs.
30 As calculated from data presented in Table 5 of the monograph, the crude OR (not corrected for
31 multiple observations per woman) for those pregnant while currently working at the plant
32 compared with all others was 3.2 (95% CI: 0.8–12). There were also excess congenital
33 malformations (13 versus 2%) and premature births (13 versus 4%) among the live births (both
34 based on two births each in the exposed group) from the women who were pregnant while

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

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

8 A cohort study of effects of paternal occupational exposures in Finland by Lindbohm et
9 al. (1991) found that exposure to formaldehyde had little effect on the rate of spontaneous
10 abortions among 99,186 pregnancies listed in the national hospital discharge register. The
11 analysis was limited to births/spontaneous abortions in 1976 and from May 1980 to April 1982.
12 Spontaneous abortion incidence came from the hospital discharge register and data from
13 outpatient clinics. There were 808 pregnant wives among potentially formaldehyde-exposed
14 fathers. Exposure to formaldehyde was based on employment information listed in the Finnish
15 1980 census. Compared with pregnancies among wives of unexposed spouses, the age and
16 socioeconomic level-adjusted ORs were 1.1 for low paternal exposure to formaldehyde and 1.0
17 for moderate to high paternal exposure. Paternal occupational exposures to ethylene oxide,
18 gasoline/benzene, and rubber industry chemicals were associated with spontaneous abortion.
19 The authors hypothesized that the mode of action (MOA) for spontaneous abortion following
20 male exposure to chemicals is genetic damage to germ cells.

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

26 A case-control study by Taskinen et al. (1994) of effects of maternal occupational
27 exposure to chemicals used in laboratories in Finland indicated that exposure to formalin, which
28 is a 37% aqueous solution of formaldehyde, was related to an increased risk of spontaneous
29 abortion. The investigators studied subjects from payrolls of Finnish state-employed laboratory
30 workers, the laboratory workers’ union, and a register of workers occupationally exposed to
31 carcinogens. These records were cross-referenced with the hospital discharge register. The
32 investigators selected women who had a single spontaneous abortion during the period
33 1973–1986 and two controls who had delivered a baby without malformations. The final sample
34 size was 208 cases and 329 controls after refusals and other exclusions. The response rate was
35 82.4%.

1 Information on occupational exposure, health status, medication, contraception, and
2 pregnancy history came from mailed questionnaires. Industrial hygienists' construction of an
3 exposure index was based on the subjects' descriptions of their work assignments, use of
4 solvents including estimates of quantity used, and use of a fume hood. ORs were adjusted for
5 employment, smoking, alcohol consumption, parity, previous miscarriage, birth control failure,
6 febrile disease during pregnancy, and other organic solvents found in laboratory work.
7 Spontaneous abortion was associated with 3–5 days per week of formalin exposure (OR 3.5
8 [95% CI: 1.1–11.2]). A contemporaneous study of formaldehyde concentrations in similar
9 Finnish workplaces (pathology and/or histology laboratories) reported workroom air to range
10 from 0.01 to 7 ppm with a mean of 0.45 ppm formaldehyde (Heikkilä et al., 1991 [as cited in
11 Taskinen et al., 1994]) and that the highest exposures occurred during emptying of sample
12 containers, dish washing, and preparation of formaldehyde solution.

13 Although the results of this study indicate an increased risk between spontaneous
14 abortion and exposure to formaldehyde/formalin, the women were also exposed to several
15 chemicals concurrently, of which toluene (OR 4.7 [95% CI: 1.4–15.9]) and xylene (OR 3.1
16 [95% CI: 1.3–7.5]) were also significantly associated with the incidence of spontaneous
17 abortion. However, the investigators reported that the women were more likely to be coexposed
18 to formalin and xylene, which would make confounding by toluene less likely, and, since xylene
19 was not as strongly associated with the outcome as was formaldehyde, it too is unlikely to fully
20 explain the reported relationship between formaldehyde and increased risk of spontaneous
21 abortion. While it is possible that exposure misclassification may have occurred because of the
22 indirect assessment of workplace chemical exposure, an overall conclusion is that, since the
23 exposure assessment was conducted by industrial hygienists, it is unlikely that this form of bias
24 will have impacted the results of the study to any great extent.

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

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

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

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

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

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

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

6 Time-to-pregnancy data were analyzed by a discrete proportional hazards regression
7 procedure with, as the outcome, a fecundability density ratio (FDR), in which a ratio of average
8 incidence densities of pregnancies for exposed women was compared with that of the employed,
9 unexposed women. As explained by Taskinen et al. (1999), an FDR significantly below unity
10 suggests that conception was delayed. The age-, employment-, smoking-, alcohol consumption-,
11 parity-, and menstrual irregularity-adjusted FDR was 0.64 (95% CI: 0.43–0.92) for women
12 exposed to high formaldehyde levels compared with the unexposed controls, indicating that there
13 was a substantial delay in time to conception in this group of women. Among a subset of women
14 with high exposure who did not use gloves, the FDR was even lower (0.51 [95% CI:
15 0.28–0.92]), suggesting that these results might be explained in part through dermal contact with
16 formaldehyde or might indicate an individual’s failure to follow appropriate precautions, which
17 might have increased inhalation exposures in other ways. Exposure to solvents, wood dust and
18 other dusts, and phenols was not associated with decreased fecundability.

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

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

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

7 Data on pregnancy history, including spontaneous abortions, were collected by
8 questionnaire. Spontaneous abortion is the most common adverse outcome of pregnancy (Klein
9 et al., 1989), and retrospective self-report of spontaneous abortion has been found to match well
10 with prospectively collected reproductive histories (Wilcox and Horney, 1984). Many
11 spontaneous abortions, however, are missed with self-reporting with the magnitude likely
12 exceeding 25%, but only rarely do women self-report false positive events (Wilcox and Horney,
13 1984). The effect of such an undercount is to cause a bias towards the null when the likelihood
14 of undercounting is unrelated to formaldehyde exposure. The implication is that the observed
15 association of increased risk of spontaneous abortion associated with occupational exposure to
16 formaldehyde may be an underestimation of the true risk.

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

1 The range of formaldehyde concentrations measured in sterilizing units has been reported as
2 0.03–3.5 ppm.

3 It is not clear that the unexposed women who served as controls were an appropriate
4 comparison group to the sterilizing staff. The investigators reported that, among the sterilizing
5 staff, those women who were unexposed during pregnancy experienced a rate of spontaneous
6 abortion of 4.6% but that, among the comparison population of nursing auxiliaries who were
7 presumed to be unexposed, the rate of spontaneous abortion was 10.5%. Had the nursing
8 auxiliaries been an appropriate comparison group, it would be expected that their rate of
9 spontaneous abortion would be similar to the unexposed sterilizing staff. Given this anomaly in
10 study design and the unknown concentrations of formaldehyde exposure that were assessed as
11 positive or negative by supervisory nurses regarding occupational exposures in the previous
12 30 years, it is concluded that this report of no association between formaldehyde exposure and
13 the risk of spontaneous abortion does not temper the conclusion that formaldehyde exposure has
14 been shown to increase the risk of spontaneous abortion.

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

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

1 may have attenuated any true adverse effect. Nevertheless, the overall reported meta-analytic
2 RR for parental formaldehyde exposure based on eight maternal and paternal exposure studies
3 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
4 cohort studies the RR was 1.7 (95% CI: 1.2–2.3). Collins et al. (2001b) argued that the method
5 of exposure evaluation may have influenced the observed results; they stated that several of the
6 studies whose exposures were based on the investigator’s judgment were likely misclassified,
7 which may have obscured the true relationship, while other studies that assessed exposure based
8 on self-reporting could have suffered from recall bias. They report that RRs were higher for
9 studies based on self-reported exposures (RR = 1.9 [95% CI: 1.3–2.6]) than those based on
10 objective exposure assessments (RR = 1.5 [95% CI: 0.6–3.7]) and suggested that this difference
11 might reflect recall bias in the exposure assessment. However, for recall bias to have been
12 operable in these studies, the women who provided self-reported data on pregnancy history and
13 occupational exposure would have had to appreciate that the hypothesis of interest was the
14 specific effect of formaldehyde on the risk of spontaneous abortion. In the specific case of the
15 study by Taskinen and colleagues (1999), the investigator also looked at the effects of other
16 exposures, such as organic solvents, dust, and phenols, and did not report adverse effects. It is
17 therefore unlikely that the women providing exposure data were doing so in a manner indicative
18 of recall bias. If the supposition of nondifferential misclassification error in exposure is indeed
19 correct, the observed results of the meta-analysis would likely have been biased towards the null.
20 Therefore, the true RR for maternal formaldehyde could be higher than Collins et al. (2001b)
21 reported and would likely be statistically significant. Had the study of paternal exposure been set
22 aside, the meta-analysis almost surely would have shown a statistically significant increase in the
23 risk of spontaneous abortion associated with maternal formaldehyde exposure. This single study
24 reported a null finding based on exposure assessment from census records of employment, and,
25 as the largest of the studies in the meta-analysis, it contributed the greatest weight.

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

1 exposures and their potentially confounding effects, the investigators reported an OR of 2.1
2 (95% CI: 1.0–4.3) for the use of formaldehyde-based disinfectants. The laboratory workers
3 studied by Axelsson et al. (1984) were potentially coexposed to a wide range of solvents, but the
4 miscarriage rate was highest among those exposed to formaldehyde, and, for a potential
5 confounder to entirely explain an observed effect of another exposure, it must be more strongly
6 associated with the adverse outcome.

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

10 The findings by Taskinen et al. (1999) of reduced fertility and increased risk of
11 spontaneous abortion are internally consistent and coherent with other reports of increased risk
12 of pregnancy loss associated with exposure to formaldehyde (John et al., 1994; Taskinen et al.,
13 1994; Seitz and Baron, 1990; Axelsson et al., 1984). Absent evidence of alternative explanation
14 for these findings, it is concluded that exposure to formaldehyde is associated with pregnancy
15 loss and diminished fertility.

16 17 **4.1.1.7.2. Congenital malformations.**

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

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

30 A Lithuanian study (Dulskiene and Gražulevičiene, 2005) for which only a brief
31 summary is available in English investigated the risk of congenital heart malformations as a
32 result of exposure to 43 different agents. The number of births included in the study was not
33 given in the English abstract. Exposure to residential ambient formaldehyde concentrations of
34 $>2.42 \mu\text{g}/\text{m}^3$ (0.002 ppm) was associated with a 24% increase in the risk of congenital heart
35 malformations (OR = 1.24 [95% CI: 0.81–2.07]). The details of this study are unavailable in

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

4 **4.1.1.7.3. Low birth weight.**

5 A case-control study by Gražulevičiene et al. (1998) examined the association of low
6 birth weight (<2,500 grams) and air pollutants, including formaldehyde, particulates, sulfur
7 dioxide, lead, ozone, and nitrogen dioxide, measured in 12 areas in the city of Kaunas, Lithuania.
8 This city has conducted environmental pollutant measurements since 1993, and the investigators
9 classified formaldehyde exposure based on the area of residence of the study subjects.
10 Formaldehyde levels in the 12 districts of Kaunas in 1994 ranged from 1.36 to 5.28 $\mu\text{g}/\text{m}^3$
11 (0.0011–0.0043 ppm), with a citywide average of 3.14 $\mu\text{g}/\text{m}^3$ (0.0026 ppm). Information on
12 infants came from a birth registry. There were 244 cases of low birth weight and 4,089 normal
13 controls born in 1994. Personal data came from record-based prenatal interviews, and pregnancy
14 data came from hospital records.

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

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

33 **4.1.1.7.4. Summary.**

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

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

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

23 24 **4.1.1.8. *Oral Exposure Effects on the Gastrointestinal Tract***

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

27 28 **4.1.1.9. *Summary: Noncarcinogenic Hazard in Humans***

29 Formaldehyde has clearly and consistently been shown to be a potent sensory irritant
30 with a variety of adverse health effects. Eye, nose, and throat irritation as a result of
31 formaldehyde exposure has been documented in a wide range of epidemiologic studies (Ritchie
32 and Lehnen, 1987; Hanrahan et al., 1984; Liu et al., 1991; Kriebel et al., 1993; Horvath et al.,
33 1988; Holmström and Wilhelmsson, 1988; Akbar-Khanzadeh et al., 1994). Workers chronically
34 exposed to formaldehyde have exhibited signs of reduced lung function consistent with BC,
35 inflammation, or chronic obstructive lung disease (Malaka and Kodama, 1990; Herbert et al.,

1 1994; Alexandersson and Hedenstierna, 1989). A well-conducted residential epidemiology study
2 has convincingly shown a concentration response for decreased pulmonary function among
3 children with increased formaldehyde exposures (Krzyzanowski et al., 1990). Several cross-
4 sectional studies have described associations between increased concentrations of formaldehyde
5 and increased prevalence of asthma (Garrett et al., 1999; Krzyzanowski et al., 1990; Norback et
6 al., 1995; Zhao et al., 2008). In addition, a case-control study that focused on risk factors for the
7 initial physician diagnosis of asthma, which is indicative of atopic switching, has shown
8 concentration-dependent adverse effects associated with formaldehyde exposure (Rumchev et
9 al., 2002).

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

18 Finally, there is epidemiologic evidence that formaldehyde is associated with adverse
19 reproductive outcomes. Four of six occupational studies found an increased risk of spontaneous
20 abortion among formaldehyde-exposed women (Taskinen et al., 1999, 1994; John et al., 1994;
21 Seitz and Baron, 1990; Axelsson et al., 1984). Results of other studies suggested associations
22 among formaldehyde and congenital malformations, low birth weight, and endometriosis
23 (Hemminki et al., 1985; Gražulevičiene et al., 1998; Taskinen et al., 1999). The strongest
24 evidence of an association between formaldehyde and an adverse reproductive outcome came
25 from a well-conducted study of infertility in women employed in the wood processing industry
26 (Taskinen et al., 1999). This study found a greater than threefold increased risk of spontaneous
27 abortion, a nearly 50% decrease in a measure of delayed conception indicating reduced fertility,
28 and increased time to pregnancy associated with average daily formaldehyde exposures of
29 0.15–1 ppm.

30 31 4.1.2. Cancer Health Effects

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

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

5 6 **4.1.2.1. *Respiratory Tract Cancer***

7 **4.1.2.1.1. *Nasopharyngeal Cancer (NPC)***

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

23 24 **4.1.2.1.1.1. *Cohort studies of nasopharyngeal cancer.***

25 Several studies examined exposure to formaldehyde in relation to solid tumor-related
26 mortality risk at 10 production facilities included in the cohort study conducted by the National
27 Cancer Institute (NCI) (Hauptmann et al. 2004; Blair et al., 1987, 1986). This cohort consisted
28 of 25,619 workers from 10 manufacturers of formaldehyde, formaldehyde resins, molding
29 compounds, plastic products, film or plywood who were first employed prior to 1966.
30 Occupational histories from company records and exposure to formaldehyde was estimated for
31 each individual job category from work histories, with calendar-time and plant-specific estimates
32 based on assessments of job titles and tasks associated with those jobs using plant visits by
33 industrial hygienists and monitoring data (Blair et al. 1986; Stewart et al. 1986; Blair and Stewart
34 1990). Exposures were categorized by highest peak exposure, average intensity of exposure,
35 cumulative exposure and duration of exposure. The highest peak exposure categories were
36 defined as nonexposed, low (>0– <2.0 ppm), medium (2.0– <4.0 ppm), and high (> 4.0 ppm).

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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)	
			<i>Trend p < 0.001 (Trend on categorical data)</i>					
			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)	
			<i>Trend p = 0.066 (Trend on continuous data among exposed only)</i>					
			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)	
<i>Trend p = 0.025 (Trend on continuous data among exposed only)</i>								
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)				
<i>Trend p = 0.147 (Trend on continuous data among exposed only)</i>								

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)

^aExposure estimates by Hauptmann et al. (2004) were 10 times higher than those of Marsh et al. (2002, p. 259).

^bAdjusted 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, <i>n</i> = 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, <i>n</i> = 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, <i>n</i> = 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, <i>n</i> = 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 <table border="1"> <tr> <td><25</td> <td>RR</td> <td>1.3</td> <td>(95% CI: 0.6–3.2)</td> </tr> <tr> <td>25 or more</td> <td></td> <td>2.9</td> <td>(95% CI: 1.1–7.6)</td> </tr> </table> Age at first exposure (years) <table border="1"> <tr> <td><25</td> <td>RR</td> <td>2.7</td> <td>(95% CI: 1.1–6.6)</td> </tr> <tr> <td>25 or older</td> <td></td> <td>1.2</td> <td>(95% CI: 0.5–3.3)</td> </tr> </table>	<25	RR	1.3	(95% CI: 0.6–3.2)	25 or more		2.9	(95% CI: 1.1–7.6)	<25	RR	2.7	(95% CI: 1.1–6.6)	25 or older		1.2	(95% CI: 0.5–3.3)								
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<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, <i>n</i> = 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, <i>n</i> = 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) <table border="1"> <tr> <td>1–5</td> <td>OR</td> <td>0.9</td> <td>(95% CI: 0.4–2.1)</td> </tr> <tr> <td>6–17</td> <td></td> <td>1.9</td> <td>(95% CI: 0.9–4.4)</td> </tr> <tr> <td>18 or more</td> <td></td> <td>2.7</td> <td>(95% CI: 1.2–6.0)</td> </tr> </table> Trend <i>p</i> = 0.014 Cumulative exposure (ppm-years) <table border="1"> <tr> <td>0.05–0.40</td> <td>OR</td> <td>0.9</td> <td>(95% CI: 0.4–2.0)</td> </tr> <tr> <td>0.41–1.10</td> <td></td> <td>1.8</td> <td>(95% CI: 0.8–4.1)</td> </tr> <tr> <td>≥1.10</td> <td></td> <td>3.0</td> <td>(95% CI: 1.3–6.6)</td> </tr> </table> Trend <i>p</i> = 0.033	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)	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)
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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)																								

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)			<p>Probable or definite exposure (27 cases, 30 controls)</p> <table border="1"> <tr> <td>Ever</td> <td>OR</td> <td>2.1</td> <td>(95% CI: 1.1–4.2)</td> </tr> <tr> <td colspan="4">Duration (years)</td> </tr> <tr> <td>1–5</td> <td>OR</td> <td>2.0</td> <td>(95% CI: 0.8–5.0)</td> </tr> <tr> <td>6–17</td> <td></td> <td>3.3</td> <td>(95% CI: 0.9–12)</td> </tr> <tr> <td>18 or more</td> <td></td> <td>1.6</td> <td>(95% CI: 0.5–5.6)</td> </tr> <tr> <td colspan="4" style="text-align: right;"><i>Trend p = 0.069</i></td> </tr> <tr> <td colspan="4">Cumulative exposure (ppm-years)</td> </tr> <tr> <td>0.05–0.40</td> <td>OR</td> <td>1.9</td> <td>(95% CI: 0.7–4.9)</td> </tr> <tr> <td>0.41–1.10</td> <td></td> <td>2.6</td> <td>(95% CI: 0.7–9.5)</td> </tr> <tr> <td>≥1.10</td> <td></td> <td>2.2</td> <td>(95% CI: 0.7–7.0)</td> </tr> <tr> <td colspan="4">Definite exposure (10 cases, 2 controls)</td> </tr> <tr> <td>Ever</td> <td>OR</td> <td>13.3</td> <td>(95% CI: 2.5–70)</td> </tr> </table>	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)	<i>Trend p = 0.069</i>				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)
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Definite exposure (10 cases, 2 controls)																																																			
Ever	OR	13.3	(95% CI: 2.5–70)																																																
Hildesheim et al. (2001)	Population-based, <i>n</i> = 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	<p>All cases and controls</p> <table border="1"> <tr> <td>Ever</td> <td>OR</td> <td>1.4</td> <td>(95% CI: 0.93–2.2)</td> </tr> <tr> <td colspan="4">Duration</td> </tr> <tr> <td>>0–≤ 10</td> <td>OR</td> <td>1.3</td> <td>(95% CI: 0.69–2.3)</td> </tr> <tr> <td>> 10</td> <td></td> <td>1.6</td> <td>(95% CI: 0.91–2.9)</td> </tr> <tr> <td colspan="4">Cumulative exposure (ppm-yrs)</td> </tr> <tr> <td>> 0– <25</td> <td>OR</td> <td>1.3</td> <td>(95% CI: 0.70–5.8)</td> </tr> <tr> <td>≥ 25</td> <td></td> <td>1.5</td> <td>(95% CI: 0.88–2.7)</td> </tr> </table>	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)																				
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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 1 st exposure, and age at 1 st exposure. Analysis of nonkeratinizing or undifferentiated tumors yielded similar results as overall analysis	<p>EBV positive cases and controls</p> <p>Ever OR 2.7 (95% CI: 0.12–6.2)</p> <p>Duration</p> <p>>0–≤ 10 OR 2.8 (95% CI: 0.83–9.7)</p> <p>> 10 2.6 (95% CI: 0.87–7.7)</p> <p>Cumulative exposure (ppm-yrs)</p> <p>> 0– <25 OR 4.0 (95% CI: 0.92–17)</p> <p>≥ 25 2.2 (95% CI: 0.80–5.8)</p>

^aEBV 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.

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

12 Subjects were initially followed to January 1, 1980, accruing approximately 600,000
13 person-years of follow-up (Blair et al., 1987). Information on potential formaldehyde exposures
14 after 1980 was unavailable. Hauptmann et al. (2004) updated the cohort through December 31,
15 1994 reporting a total accrual of person-time of 865,708 person-years with a median duration of
16 follow-up of 35 years. Cause of deaths was taken from death certificates. A total of 10 deaths
17 from nasopharyngeal cancer were identified. Among them 8 were classified as ever exposed and
18 2 never exposed.

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

25 In addition to the SMR analysis, Hauptman et al. (2004) provided both RRs based on
26 internal referent groups (unexposed employees, and the low exposed workers) and regression
27 analysis of all exposed workers. Use of an internal referent group reduces potential selection
28 bias due to the healthy worker effect and potential confounding from area- and employment-
29 related factors. Both of these analyses were conducted for four different exposure metrics
30 Exposure measures used in the Poisson regression were based on a standardized exposure
31 assessment protocol (summarized in Table 4-2), and included measures of highest peak
32 exposure, average exposure intensity, cumulative exposure, and duration of exposure to
33 formaldehyde (Hauptmann et al., 2004). RRs for nasopharyngeal cancer increased with various
34 exposure metrics with trend *p*-values (based on continuous exposure measures) < 0.001 and
35 0.025 for the peak exposure and cumulative exposure metrics, respectively (see Table 4-2).

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

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

25 Following these reports of increased risk of nasopharyngeal cancer associated with
26 formaldehyde exposure, a series of post hoc analyses of a subset of the NCI cohort were
27 undertaken by Marsh and coworkers (Marsh et al., 2007a, b, 2002, 1996; Marsh and Youk,
28 2005). Briefly, these studies focused on the specific findings from a single plant in the NCI
29 cohort (Wallingford, Connecticut) that generated the majority of the nasopharyngeal cancer
30 cases. The Marsh et al. (2002) study had both a cohort analysis and a nested case-control
31 analysis. The cohort study followed 7328 workers employed in the plant from 1941 to 1984,
32 extending the follow-up period to 1998 and independently evaluated the exposure assessment
33 (Marsh et al., 1996 present the earlier analysis with follow-up through 1984). Seven deaths from
34 nasopharyngeal cancer were seen in this cohort, for SMRs of 4.94 (95% CI 1.99-10.19) using the
35 United States population as the referent group and 5.00 (95% CI 2.01-10.30) using the local

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

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

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

1 controls, with family member interviews used for most of the cases, and self-interviews used for
2 most of the controls. Details of the content and structure of the interview were not provided, but
3 the authors say that little useful information was obtained from these interviews (Marsh et al.
4 2007a, 2002). Additional sources of work history included data from the plant job application,
5 city directories and genealogy-based search strategies. An association was seen between
6 nasopharyngeal cancer risk and work in silversmithing occupations (including brass plating or
7 other jobs relating to silver or brass, OR 14.4, 95% CI 1.3, 758; 4 exposed cases); the odds ratio
8 for other types of metal work (steel work and welding) was 3.61 (95% CI 0.50-22.7; 3 exposed
9 cases) and for the combined category of silversmithing and other metal work the OR was 7.31
10 (9% CI 1.08-82.1; 5 exposed cases). There are no prior citations of an association between
11 silversmithing exposures and nasopharyngeal cancer in the medical literature, but Marsh et al.
12 review the literature pertaining to related exposures (sulfuric acid mists, metal dusts) and
13 respiratory and laryngeal cancer to support this association. However, the results for these
14 exposures and laryngeal cancer are inconsistent, and data pertaining to these exposures and
15 nasopharyngeal cancer are quite limited. Despite these limitations, Marsh et al. (2007) suggest
16 that the observed associations between nasopharyngeal cancer and formaldehyde exposure in the
17 Wallingford plant are due to these other occupational exposures. Marsh et al. (2007) do note that
18 history of silversmithing and other metal work was not associated with formaldehyde exposure,
19 and so was not a confounder of the formaldehyde results as reported for the Wallingford Plant.
20 In addition, there was no evidence of confounding due to smoking history. Regardless, Marsh et
21 al. (2007a) suggest that these other occupational exposures, rather than formaldehyde, could
22 explain the risk of nasopharyngeal cancer seen in the Wallingford cohort.

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

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

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

13 Other studies also examined nasopharyngeal cancer risk in occupational cohorts, but are
14 limited by the small number of expected cases. Pinkerton et al. (2004) updated of a cohort study
15 of 11,030 workers (82% female) followed from 1955 or the beginning date of exposure through
16 1982 in three garment factories conducted by Stayner et al. (1988). Formaldehyde resins were
17 used to treat fabrics in these factories beginning in 1955 and 1959. Although formaldehyde
18 levels were available on a subset of the employees from monitoring data available from surveys
19 completed in 1981 and 1984, they were not used in this analysis. The geometric mean 8-hour
20 time weighted average exposure across all departments and plants was 0.09-0.20 ppm. The
21 geometric mean formaldehyde concentration across all departments was estimated at 0.15 ppm.
22 No cases of nasopharyngeal cancer were observed compared to an expected number of one case.

23 Gardner et al. (1993) studied a cohort of 14,017 workers exposed to formaldehyde in the
24 British chemical industry and followed until the end of 1989. Exposure to formaldehyde was
25 classified according to job title and assigned to categories including nil/background (<0.1 ppm),
26 low (0.1-0.5 ppm), moderate (0.6-2.0 ppm) and high (over 2.0 ppm). Results were stratified by
27 date of first employment and among men first employed before 1965, the SMR for cancer of the
28 pharynx was 1.47 (95% CI: 0.59-3.03). No cases were reported among the men first employed
29 after 1965. Although the cause-specific cancer mortality was for cancer of pharynx (ICD-9
30 146–9) and would include nasopharyngeal cancer, the authors stated separately that there were
31 1.3 cases of nasopharyngeal cancer that were expected but no deaths recorded for
32 nasopharyngeal cancer. Coggon et al. (2003) updated this cohort study with additional follow-up
33 through the end of 2000 and reported the effects of formaldehyde exposure on mortality from
34 cancer among 14,014 workers. Coggon et al. (2003) reported only one case compared to an

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

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

8 9 **4.1.2.1.1.2. Case-control studies of nasopharyngeal cancer.**

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

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

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

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

18 Roush et al. identified 173 cases of nasopharyngeal cancer in men from the Connecticut
19 Tumor Registry who dies of any cause between 1935 and 1975. Male controls ($n = 605$) were
20 selected from state death certificates during the same time period. Four exposure categories
21 were created based on the probability and duration of formaldehyde exposure based on industrial
22 hygienist evaluations of subject's job titles, industry, specific employment and duration of
23 employment. Logistic regression adjusted for potential confounders including age at death, year
24 of death. For the lowest exposure category defined as 'probably exposed most of working life,'
25 the OR = 1.0 (95% CI: 0.6-1.7); for the second exposure category defined as 'probably exposed
26 most of working life and probably exposed 20+ years before death,' the OR = 1.3 (95% CI: 0.7-
27 2.4); for the third category of exposure defined as 'probably exposed most of working life and
28 probably to high level in some year,' the OR = 1.4 (95% CI: 0.6-3.1); and for the fourth exposure
29 category defined as 'probably exposed most of working life and probably exposed to high level
30 20+ years before death,' the OR = 2.3 (95% CI: 0.9-6.0).

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

1 hygienist who was blinded to case-control status evaluated subject's occupational histories to
2 estimate likely exposures to formaldehyde, solvents, dusts, exhausts, and pesticides. Conditional
3 logistic regression was used to control for coexposures. Controlling for education, dust,
4 processed meat and fresh fish consumption, mosquito coil usage, and use of herbal medicine, the
5 RR of occupational formaldehyde exposure more than 25 years earlier was 4.0 (95% CI:L 1.3-
6 12.3). The independent effect of dust was also statistically significant for exposure more than 35
7 years prior (OR = 4.4; 95% CI: 1.1-17.5) as was the independent effect of daily use of mosquito
8 coils (OR = 5.9; 95% CI: 1.7-20.1). While the authors mentioned only that the compounds in the
9 anti-mosquito coil smoke might be of further interest, they did identify those compounds.
10 However, independent testing of 6 brands of East Asian mosquito coils evaluated the emission
11 rates of carbonyl compounds in the mosquito smoke and reported that formaldehyde and
12 acetaldehyde had the highest emission rates (Liu et al., 2003). Among the three experiments on
13 each of the six brands, the range of formaldehyde concentrations was from 0.87 $\mu\text{g}/\text{m}^3$ (1 ppb) to
14 25 $\mu\text{g}/\text{m}^3$ (31 ppb).

15 Armstrong et al. (2000) studied 282 ethnically Chinese residents of Malaysia with
16 recently diagnosed nasopharyngeal cancer (diagnosis from 1987–1992, with case identification
17 occurring between 1990 and 1992). Cases were individually matched to 282 controls by age and
18 sex; structured interviews collected residential and work history data. No association was seen
19 between formaldehyde exposure and nasopharyngeal cancer (adjusted OR = 0.71 [95% CI:
20 0.34–1.43]), controlling for wood dust and industrial heat.

21 In a population-based case-control study of incident nasopharyngeal cancer cases
22 identified through the U.S. SEER cancer registry, Vaughn et al. (2000) used work history data
23 collected from interviews (see Table 4-3) to estimate each individual worker's formaldehyde
24 exposure. Workers with more than 1.10 ppm-years of cumulative exposure were found to be at
25 significantly higher risk of nasopharyngeal cancer, with an odds ratio (OR) of 3.0 (95% CI
26 1.3–6.6). Both duration of exposure and cumulative exposure were positively associated with
27 increased risk of nasopharyngeal cancer (trend $p = 0.014$ and 0.033 , respectively). The OR
28 increased in magnitude as the probability of "Ever" having occupational exposure increased,
29 from OR = 1.6 (95% CI 1.0–2.8) among the 61 cases whose exposure was judged to be
30 "Possible, probable or definite", to 2.1 (5% CI 1.1, 4.2) among the 27 cases with "probable or
31 definite" exposure, to OR = 13.3 (95% CI 2.5–70) among the 10 cases with "definite" exposure
32 (p -trend < 0.001) (Vaughn et al., 2000).

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

1 produced modest risk elevations: for duration of exposure, OR = 1.6 for 10 years or less and 1.2
2 for over 10 years of exposure; for cumulative exposure, OR = 1.3 for <25 years of exposure and
3 1.5 for 25+ years of exposure. Among those with EBV, the OR was 2.7 (95% CI: 1.2–6.2) for
4 ever-exposed persons. The risk was higher among exposed persons whose work history was
5 within the last 10 years (OR = 4.7 [95% CI: 1.1–20.0]) and for those followed 20+ years after
6 exposure (OR = 2.8 [95% CI: 1.1–7.6]). The analysis of these data, however, was conducted
7 using unconditional logistic regression. The authors stated that conditional logistic regression
8 was not used "to avoid the loss of information from cases and controls without a matched pair."
9 This loss of information was particularly acute among the EBV+ strata in which 360/375 cases
10 were EBV+ while only 94/325 controls were EBV+. While the investigators did include age, sex
11 and other potential confounders in the analytic models, this analytic methodology may not be
12 sufficient to effectively control for these factors given the individual matching used in the study.
13 Failure to condition on the matching factors can create bias that is unpredictable in direction or
14 magnitude (Cox and Hinkley, 1974, p. 292 and Breslow and Day, 1980, p. 249). Had the study
15 design specified frequency matching rather than individual matching, then the logistic regression
16 analysis with the inclusion of the matching factors would have been appropriate; however, this
17 apparent weakness in all the analyses by Hildesheim et al. (2001) raises considerable uncertainty
18 regarding the results and less weight should be assigned to this study in the overall evaluation.
19

20 **4.1.2.1.1.3. Summary of nasopharyngeal cancers.**

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

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

5 Hauptmann et al. (2004) reported that for mortality from all types of cancer combined,
6 the workers experienced fewer deaths than would be expected in the general population
7 (SMR = 0.90, 95% CI: 0.86-0.95). This typically reflects that fact that workers are healthier than
8 the general population which includes nonworkers. In spite of this ‘healthy worker effect,’ the
9 Hauptmann et al. (2004) analysis reported a doubling of nasopharyngeal cancer mortality risk in
10 workers from 10 formaldehyde producing or using factories (SMR = 2.1, 95% CI: 1.05–4.21).
11 Additional analyses demonstrated that relative risks increased with average exposure intensity
12 (p -trend = 0.066), cumulative exposure (p -trend = 0.025), highest peak exposure (p -trend <
13 0.001) and duration of exposure to formaldehyde (p -trend = 0.147).

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

19 The case-control studies also provide a robust collection of data supporting a similar
20 overall magnitude of association between formaldehyde exposure and nasopharyngeal cancer
21 (i.e., relative risk estimates in the range of 1.5–3.0) (see Table 4-3). These studies were
22 conducted in several different countries and work settings. Only one study (Armstrong et al.,
23 2000) did not observe a risk consistent with these estimates.

24 Of particular note were three sets of effect estimates of unusual magnitude, especially
25 when the definitions of exposure to formaldehyde were more specifically defined. Vaughan et
26 al. (1986b) reported that the odds ratio for living in a mobile home for 1-9 years was 2.1 (95%
27 CI: 0.7-6.6) compared to not living in a mobile home, while living in a mobile home for more
28 than 10 years had an OR = 5.5 (95% CI: 1.6-19.4). Vaughan et al. (1986b) also reported results
29 that could be considered as consistent with an exposure-response relationship. Compared to
30 having no exposure to mobile homes, they reported OR = 1.7 (95% CI: 0.5-5.7) for working in a
31 mobile home, OR = 2.8 (95% CI: 1.0-7.9) for living in a mobile home and OR = 6.7 (95% CI:
32 1.2-38.9) for both living in and working in a mobile home. While exposure to mobile homes is a
33 crude proxy for formaldehyde exposure, the gradient in response and the magnitude of effect at
34 the highest exposure level is striking.

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

7 The study by West et al. (1993) was also remarkable for the exposure-response shown for
8 formaldehyde and for anti-mosquito coils which have been shown to release high concentrations
9 of formaldehyde. In multivariate analyses, compared to never having had occupational exposure
10 to formaldehyde, having occupation exposure within the last 25 years had an OR = 1.2 (95% CI:
11 0.41-3.6) while having occupational exposure more than 25 years earlier had an OR = 4.0 (95%
12 CI: 1.1-17.5). Compared to never using anti-mosquito coils, the OR for using them less than
13 daily was 1.4 (95% CI: 0.64-2.8) while daily use of anti-mosquito coils had an OR = 5.9 (95%
14 CI: 1.7-20.1).

15 Establishment of a concentration-response relationship is important evidence of causality.
16 An attenuation of risk is often seen at the highest dose level in epidemiologic studies, however, it
17 may possibly be due to exposure misclassification and of the use of inappropriate exposure
18 models (Stayner 1985, 1988). Identification of concentration-response relationships in spite of
19 potentially substantial effect measure attenuation provides even stronger evidence of causality.
20 Findings from the large NCI cohort studies of nasopharyngeal cancer risk (Hauptmann et al.,
21 2004) show a pattern of increased risk with increased formaldehyde exposures across multiple
22 exposure metrics.

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

1 increased risk of nasopharyngeal cancer (Hauptmann et al., 2004). The described association of
2 a potential occupational relationship with silversmithing (brass plating, silver and other brass
3 work) and nasopharyngeal cancer has little support in the medical literature. In addition, if
4 silversmithing exposures are indeed independent risk factors for nasopharyngeal cancer, it would
5 be expected that the rates of nasopharyngeal cancer in the surrounding counties with historical
6 silver-and other metal-related exposures would be elevated. However they are not increased, as
7 evidenced by the comparability of the increased rates of nasopharyngeal cancer among the plant
8 workers based on the national and based on local county rates (Marsh et al., 2007a). The
9 comparable rates indicate the counties' rates of nasopharyngeal cancer were very similar to the
10 national rates. Given the limitations and the lack of comparability in the source of the work
11 history data in the nested case-control study of Marsh et al. (2007a) (next of kin interviews for
12 cases and self-interviews for controls) and the many post hoc re-examinations of alternative
13 hypotheses to explain the original NCI findings, EPA believes the association seen with
14 silversmithing does not represent an unmeasured confounder of the results of Hauptman et al.
15 (2004). A more likely explanation for the increased risk seen in short-term workers at the
16 Wallingford, CT plant is the specific work conditions of those jobs at that plant. It is also
17 plausible that the observed association at the Wallingford plant reflects higher formaldehyde
18 exposures than at other plants. Marsh and Youk (2005) reported that the exposure levels at Plant
19 2 were even higher than at the Wallingford plant; the fivefold increase in risk of nasopharyngeal
20 cancer seen at this plant, although based on only a single observed case, supports the results seen
21 in Wallingford, CT.

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

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

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

11 **4.1.2.1.2. Nasal and paranasal (sinonasal) cancer.**

12 **4.1.2.1.2.1. Cohort studies of nasal and paranasal cancers.**

13 IARC (1995) reported the results of several cohort studies of professional and industrial
14 workers exposed to formaldehyde for the induction of nasal and paranasal cancers (Andjelkovich
15 et al., 1995; Gardner et al., 1993; Hall et al., 1991; Hayes et al., 1990; Bertazzi et al., 1989;
16 Edling et al., 1987; Blair et al., 1986; Stroup et al., 1986; Harrington and Oakes, 1984; Levine et
17 al., 1984; Walrath and Fraumeni, 1984, 1983; Friedman and Ury, 1983). The likelihood of
18 finding this rare tumor type in even long-term cohort study is low, and only a few studies
19 reported any cases of nasal and paranasal cancer. No cases of this type of cancer were reported
20 in any of the studies of professional workers examined by the IARC. Only two cases (2.2
21 expected) were reported in the NCI cohort of more than 25,000 workers by Blair et al. (1986)
22 while the update by Hauptmann et al. (2004) identified three cases (2.5 expected). Marsh et al.
23 examined a subset of the NCI cohort from the Wallingford, Connecticut plant and identified two
24 cases compared to 0.5 in the 1984 follow-up (Marsh et al., 1996) and three cases against one
25 expected in the 1998 follow-up (Marsh et al., 2002). The cohort study of 14,017 British
26 chemical industry workers exposed to formaldehyde by Gardner et al. (1993) identified only one
27 case (1.7 expected). Coggon et al. (2003) updated this cohort study with additional follow-up
28 and reported only two cases compared to an expected number of 2.3 cases. The other large
29 cohort studies of more than 11,000 garment workers by Stayner et al. (1988) and Pinkerton et al.
30 (2004) did not identify any cases of sinonasal cancer. In contrast, a large population-based study
31 using nationwide Danish Product Register data found an increased risk of sinonasal cancer
32 (standardized proportional incidence ratio [SPIR]= 2.3 [95% CI: 1.3–4.0] based on 13 observed
33 cases; 2,041 of 91,182 cancer patients had at least 10 years of continuous formaldehyde-related
34 work experience before diagnosis (Hansen and Olsen, 1995).

1 **4.1.2.1.2.2. Case-control studies of nasal and paranasal cancers.**

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

10 Three other studies conducted analyses limited to a specific cell type (Luce et al., 1993;
11 Hayes et al., 1986; Olsen and Asnaes, 1986) (see Table 4-4). Hayes et al (1986) conducted a
12 case-control study of occupational exposure to formaldehyde and the risk of nasal and paranasal
13 cancer in the Netherlands based on 116 cases and 259 controls. Analyses were done by
14 histological type of tumor and controlled for history of tobacco use and occupational exposure to
15 wood dust. Controlling for potential confounding by wood dust was important as wood dust was
16 reported to be strongly associated with sinonasal cancer (Hayes et al., 1986). Two independent
17 assessments of formaldehyde exposure were conducted with RRs of 3.0 (90% CI 1.3-6.4) and 1.9
18 (90% CI 1.0-3.6) seen for squamous cell carcinoma based on assessments A and B, respectively.

19 Olsen and Asnaes (1986) conducted a case-control of 287 histologically verified cancers
20 of the nasal cavity and 179 cases of paranasal cancer in Denmark. Controlling for wood dust
21 exposures, the investigators reported adjusted RR for formaldehyde exposure of 2.2 (95% CI:
22 0.9-5.8) for squamous cell carcinoma and RR = 2.3 (95% CI: 0.7-7.2) for adenocarcinoma. The
23 results for squamous cell carcinoma did not appear to be influenced by coexposure to wood dust;
24 however, for adenocarcinoma, among those who were never exposed to wood dust, the RR of
25 formaldehyde exposure was 7.0 (95% CI: 1.1-43.9) and among those who were ever exposed to wood
26 dust the RR of formaldehyde exposure was 39.5 (95% CI: 22.0-70.8). Given the differences
27 between the stratum-specific results, which indicate effect modification, and between the
28 stratum-specific results and the adjusted results, it is the stratum-specific results that provide the
29 most valid measures of effect. The analyses by Olsen and Asnaes (1986) did not control for
30 smoking as that data was unavailable but tobacco use was not shown to be a confounder in the
31 study by Hayes et al (1986) or the study by Luce et al. (2002).

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

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

7 More recently, Luce et al. (2002) pooled data from 12 case-control studies. Pooled
8 studies are different from meta-analyses in that they compile the original raw data at the level of
9 the individual into a single pooled dataset while meta-analyses examine the summary results
10 across studies. Luce et al. (2002) assessed the associations between sinonasal cancer and
11 occupational exposures to formaldehyde after developing a common job exposure matrix
12 specifically for this study which standardized the different classifications of exposures and
13 potential confounders. Disease coding was also standardized to a single system to increase
14 compatibility. Combined, these studies contributed 195 adenocarcinomas and 432 squamous cell
15 carcinomas of the sinonasal passages compared with 3,136 controls. Analyses controlled for age
16 and study and were conducted separately by gender. Potential confounders were evaluated and
17 controlled for. Among men, the investigators were able to control for wood dust and leather dust
18 when evaluating the effects of formaldehyde on adenocarcinoma. The authors reported a
19 statistically significant increase in the risk of sinonasal adenocarcinoma in men (adjusted
20 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
21 probability of exposure to formaldehyde. Luce et al. (2002) checked for residual confounding by
22 wood dust by regrouping wood dust exposure into 5 categories, using the cumulative level of
23 wood dust as a continuous variable and using the highest lifetime level of exposure and reported
24 that the results were ‘not markedly changed’ indicating no evidence of residual confounding.
25 For squamous cell carcinomas, the ORs were more modest: OR = 1.2 in men and OR = 1.5 in
26 women for a high probability of exposure to formaldehyde. In an analysis of 11 formaldehyde-
27 exposed cases of sinonasal adenocarcinomas who were not exposed to wood dust, there was an
28 elevated risk in men (OR = 1.9; 3 cases) and a significantly increased risk in women (OR = 11.1
29 [95% CI: 3.2–38.0]; 5 cases) with a high probability of exposure to formaldehyde. Limitations
30 of these studies were the lack of information about the actual levels or intensity of exposure to
31 formaldehyde, exposure to multiple occupational carcinogens, and the small number of cases in
32 some subgroups. In spite of those limitations, which generally obfuscate the observation of a
33 true underlying effect, the studies evaluated by Luce et al. (2002) identified effects of
34 formaldehyde that were statistically significant predictors of sinonasal cancers and this
35 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: 0.9–3.8)	
			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)	
			1 st exposure >10 years or more before diagnosis	RR	4.1	(95% CI: 0.2.3–7.3)	
Vaughan et al. (1986a), Washington	Population-based, <i>n</i> = 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.	<p>Squamous cell carcinoma</p> <p>Industrial hygienist A</p> <table border="1"> <tr> <td>Any exposure</td> <td>RR</td> <td>3.0</td> <td>(90% CI: 1.3–6.4)</td> </tr> <tr> <td>Moderate exposure</td> <td></td> <td>2.7</td> <td>(90% CI: 1.0–7.2)</td> </tr> <tr> <td>High exposure</td> <td></td> <td>3.1</td> <td>(90% CI: 0.9–10.0)</td> </tr> </table> <p>Industrial hygienist B</p> <table border="1"> <tr> <td>Any exposure</td> <td>RR</td> <td>1.9</td> <td>(90% CI: 1.0–3.6)</td> </tr> <tr> <td>Moderate exposure</td> <td></td> <td>1.4</td> <td>(90% CI: 0.5–3.4)</td> </tr> <tr> <td>High exposure</td> <td></td> <td>2.4</td> <td>(90% CI: 1.1–5.1)</td> </tr> </table>	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)	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)
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)																								
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.	<p>Squamous cell carcinoma/lymphoepithelioma</p> <p>Ever vs. never</p> <table border="1"> <tr> <td>Formaldehyde only</td> <td>RR</td> <td>2.0</td> <td>(95% CI: 0.7–5.9)</td> </tr> <tr> <td>Formaldehyde + wood dust</td> <td>RR</td> <td>1.6</td> <td>(95% CI: 0.8–3.3)</td> </tr> </table> <p>10 or more years since first exposure</p> <table border="1"> <tr> <td>Formaldehyde only</td> <td>RR</td> <td>1.4</td> <td>(95% CI: 0.3–6.4)</td> </tr> <tr> <td>Formaldehyde + wood dust</td> <td>RR</td> <td>1.8</td> <td>(95% CI: 0.7–4.4)</td> </tr> </table> <p>Adenocarcinoma</p> <p>Ever vs. Never</p> <table border="1"> <tr> <td>Formaldehyde only</td> <td>RR</td> <td>7.0</td> <td>(95% CI: 1.1–44)</td> </tr> <tr> <td>Formaldehyde + wood dust</td> <td>RR</td> <td>40.0</td> <td>(95% CI: 22–71)</td> </tr> </table>	Formaldehyde only	RR	2.0	(95% CI: 0.7–5.9)	Formaldehyde + wood dust	RR	1.6	(95% CI: 0.8–3.3)	Formaldehyde only	RR	1.4	(95% CI: 0.3–6.4)	Formaldehyde + wood dust	RR	1.8	(95% CI: 0.7–4.4)	Formaldehyde only	RR	7.0	(95% CI: 1.1–44)	Formaldehyde + wood dust	RR	40.0	(95% CI: 22–71)
Formaldehyde only	RR	2.0	(95% CI: 0.7–5.9)																								
Formaldehyde + wood dust	RR	1.6	(95% CI: 0.8–3.3)																								
Formaldehyde only	RR	1.4	(95% CI: 0.3–6.4)																								
Formaldehyde + wood dust	RR	1.8	(95% CI: 0.7–4.4)																								
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 1 st 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 1 st 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 1 st exposed (years)	
			≤20	OR 2.03 (95% CI: 0.63–6.54)
>20	2.36 (95% CI: 0.76–7.33)			
Date 1 st 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.	<p>Adenocarcinoma</p> <table border="1"> <tr> <td>High probability of exposure</td> <td></td> <td></td> <td></td> </tr> <tr> <td>Men</td> <td>OR^d</td> <td>3.0</td> <td>(95% CI: 1.5–5.7)</td> </tr> <tr> <td>Women</td> <td>OR^e</td> <td>6.2</td> <td>(95% CI: 2.0–20)</td> </tr> </table> <p>Squamous cell carcinoma</p> <table border="1"> <tr> <td>High probability of exposure</td> <td></td> <td></td> <td></td> </tr> <tr> <td>Men</td> <td>OR^e</td> <td>1.2</td> <td>(95% CI: 0.8–1.8)</td> </tr> <tr> <td>Women</td> <td>OR^e</td> <td>1.5</td> <td>(95% CI: 0.6–3.8)</td> </tr> </table>	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)	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)
High probability of exposure																											
Men	OR ^d	3.0	(95% CI: 1.5–5.7)																								
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Men	OR ^e	1.2	(95% CI: 0.8–1.8)																								
Women	OR ^e	1.5	(95% CI: 0.6–3.8)																								

1 **4.1.2.1.2.3. Summary of nasal and paranasal cancers.**

2 Rare outcomes are difficult to study due to a lack of statistical power to identify risks.
3 Most cohort studies observed no more than one case of sinonasal cancer and may not have had
4 sufficient statistical power to show an association. The exception is the national population-
5 based study linking the Danish cancer registry and industry data in which 13 cases were
6 identified for a SPIR of 2.3 (95% CI 1.3–4.0) (Hansen and Olsen, 1995). The case-control
7 studies of sinonasal cancer in which no excess risk was seen did not distinguish cancer type and
8 thus may have aggregated a truly causal relationship with adenocarcinoma and a noncausal
9 relationship with squamous cell carcinoma. Among all the studies of sinonasal cancer, the
10 pooled case-control study of Luce et al. (2002) provides the strongest causal evidence of an
11 association between formaldehyde exposure and increased risk of sinonasal adenocarcinoma. In
12 summary, there appears to be increased risk of sinonasal cancer associated with formaldehyde
13 exposure with or without exposure to wood dust (Luce et al., 2002). The effect appears to be
14 stronger when the risk is stratified by cancer type with higher risks of adenocarcinoma compared
15 with squamous cell carcinoma. Taken together with the nasopharyngeal cancer findings in the
16 neighboring tissue, EPA concludes that there is evidence of a causal association of sinonasal
17 cancer associated with exposure to formaldehyde.
18

19 **4.1.2.1.3. *Cohort studies of lung cancer.***

20 Several industrial cohort studies (Andjelkovich et al., 1995; Stayner et al., 1988; Edling
21 et al., 1987) reported no significant excess risks of lung cancer from exposure to formaldehyde.
22 Bertazzi et al. (1986) studies a cohort of 1322 workers producing formaldehyde resins in Italy
23 and reported an increased risk of lung cancer in the initial analysis with an SMR of 1.86 (95%
24 CI:1.10-2.93) based on 18 cases; however, the reanalysis of this cohort with six additional years
25 of follow-up (Bertazzi et al., 1989) showed no excess risk with 24 cases of lung cancer compared
26 to 23.9 expected. No consistent association between formaldehyde exposure and lung cancer
27 was found in several reports of the NCI 10-plant cohort study originally investigated by Blair et
28 al. (1987, 1986). Hauptmann et al. (2004) give the most recent report on this cohort, which has
29 been studied in part or in its entirety by several others (Marsh et al., 1994, 1992a, b; Sterling and
30 Weinkam, 1994, 1989a, b, 1988; Robins et al., 1988; Liebling et al., 1984; Fayerweather et al.,
31 1983; Wong, 1983; Marsh, 1982). Hauptmann et al. (2004) reported 641 cases of lung cancer
32 against 660.8 expected for an SMR of 0.97 (95% CI: 0.90-1.05).

33 Gardner et al. (1993) found a modest association between lung cancer and formaldehyde
34 exposure in the British chemical industry cohort study ($n = 14,016$) among men hired before
35 1965 with a SMR of 1.23 (95% CI: 1.10–1.36) based on national rates of expected deaths and a

1 SMR of 1.12 (95% CI: 1.00-1.24) based on local deaths rates. In workers hired after 1964, the
2 corresponding SMRs were 1.14 (95% CI: 0.85-1.48) and 1.13 (95% CI: 0.85-1.47). No trends by
3 level or duration of exposure were found (Gardner et al., 1993). Coggon et al. (2003) updated
4 the Gardner et al. (1993) cohort study with additional follow-up through the end of 2000 and
5 reported the effects of formaldehyde exposure on mortality from lung cancer among
6 14,014 workers. Coggon et al. (2003) reported an SMR of 1.22 (95% CI: 1.12-1.32) for lung
7 cancer in the entire cohort. In the analysis of the combined data from the 6 factories, an excess
8 risk of lung cancer was seen in the high-exposure category when compared with British national
9 mortality rates (SMR = 1.58 [95% CI: 1.40–1.78]) and with local mortality rates (SMR = 1.28
10 [95% CI: 1.13–1.44]). Pinkerton et al. (2004) and Stayner et al. (1988) studied a cohort of
11 11,030 workers in three garment plants and found an SMR of 1.14 for lung cancer (95% CI:
12 0.81-1.56). Callas et al. (1996) reanalyzed the cumulative exposure of 279 lung cancer cases
13 among white male workers from the NCI study, which comprised 80% of the NCI cohort (Blair
14 et al., 1986). The analysis revealed RRs of 1.46, 1.27, and 1.38 for lung cancer in the cumulative
15 exposure categories of 0.05 to 0.5 ppm-years, 0.51 to 5.5 ppm-years, and > 5.5 ppm-years,
16 respectively. None of these RRs were statistically significant.

17 None of the cohort studies of workers in specific professions indicated excess risks of
18 lung cancer. Of the professional studies reviewed, the RRs range from an extremely low SMR
19 value of 0.2 in Hall et al. (1991), based on nine deaths, to an RR (proportional mortality ratio) of
20 1.1, based on 70 lung cancer deaths in Walrath and Fraumeni (1983). Matanoski (1991) reported
21 a significant deficit in the risk of respiratory cancer (SMR = 0.56 [95% CI: 0.44–0.70]; 77
22 observed) in pathologists presumably exposed to formaldehyde based on U.S. mortality rates.

23

24 **4.1.2.1.4. Case-control studies of lung cancer.**

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

1 OR = 3.0 (95% CI: 1.6–5.8), with an exposure-response trend seen across exposure levels (trend
2 $p = 0.004$).

3
4 **Summary of lung cancer.** Evidence of a relationship between formaldehyde exposure and lung
5 cancer is relatively weak and conflicting, with some studies showing modest increases (e.g.,
6 relative risks of 1.2, as seen in Coggon et al., 2003) while others show inverse associations
7 between exposure and risk. In all studies of formaldehyde and lung cancer, smoking remains an
8 important confounder and possibly an effect modifier. Residual confounding of smoking or
9 other respiratory exposures (e.g., wood dust or chemical or particular exposures) must always be
10 considered. A meta-analysis of workers exposed to formaldehyde by Bosetti et al. (2008)
11 reported a summary effect estimate of RR = 1.06 (95% CI: 0.92-1.23) for industry workers and
12 RR = 0.63 (95% CI: 0.47-0.84) for professionals. The meta-analysis by Blair et al. (1990)
13 reported a RR = 0.9 for professional and nonoccupational exposures and RR = 1.1 among
14 industrial workers. The meta-analysis by Partanen (1993) largely confirmed the results of Blair
15 et al. (1990). Except for the findings of De Stefani et al. (2005), other studies of lung cancer and
16 exposure to formaldehyde have not supported the finding by Coggon et al. (2003), including
17 several well-done cohort studies that were specifically designed to evaluate lung cancer.

18 19 **4.1.2.1.5. Other respiratory tract cancers.**

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

22 23 **4.1.2.1.5.1. Cohort studies of other respiratory tract cancers.**

24 In studies of industrial worker cohorts where buccal/pharyngeal cancer was examined
25 (Andjelkovich et al., 1995; Stayner et al., 1988; Blair et al., 1986), only one (Stayner et al., 1988)
26 reported an excess risk of death from this tumor (SMR = 3.4, based on four deaths in a cohort of
27 6,741 white women). Of six cohort studies of buccal/pharyngeal cancer in studies of
28 professionals reviewed by IARC (Hayes et al., 1990; Logue et al., 1986; Stroup et al., 1986;
29 Levine et al., 1984; Walrath and Fraumeni, 1984, 1983), no evidence of a risk associated with
30 exposure to formaldehyde was reported.

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

1 hypopharyngeal cancer was 1.41 (based on one case), and the SMR for laryngeal cancer was
2 1.47 (based on six cases). The latter risks were elevated even when SMRs were derived from
3 Connecticut mortality rates.

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

18 Hauptmann et al. (2004) combined upper respiratory tract cancers (cancers of the salivary
19 gland, mouth, nasopharynx, nasal cavity, and larynx; $n = 11$ observed deaths). For average
20 intensity of exposure, the RR was 1.47, 1.69, and 2.21 for the low ($> 0-0.5$ ppm), medium (0.5
21 to <1.0 ppm) and high (≥ 1.0 ppm) exposure categories, respectively, (trend $p = 0.122$).
22 Confidence intervals were not provided for these RR; however, a footnote states that for the
23 RR = 2.21 for the highest exposure category (≥ 1.0 ppm), the 95% confidence interval does not
24 include 1.00. For peak exposure, the RR was 1.32, 1.24, and 1.65 for the low ($> 0- <2.0$ ppm),
25 medium (2.0- <4.0 ppm) and high (≥ 4.0 ppm) exposure categories, respectively (trend
26 $p = 0.142$). For cumulative exposure, the RR was 1.24, 1.92 and 0.86 for the low ($> 0-1.5$ ppm-
27 yrs), medium (1.5- <5.5 ppm-yrs) and high (≥ 5.5 ppm-yrs) exposure categories (trend
28 $p = 0.0.765$). Increased relative risks of cancer of the buccal cavity (which included salivary
29 glands, the floor of the mouth, other mouth and the nasopharynx) were reported for the medium
30 and high exposure categories with 95% confidence intervals that did not include 1.00 for the
31 medium exposure category. Hauptmann et al. (2004) concluded that in spite of the small number
32 of deaths from these rare cancers of the upper respiratory tract, the positive associations of
33 increased cancer risk with increased formaldehyde exposure were consistent with the
34 carcinogenicity of formaldehyde at these sites of first contact.

1 **4.1.2.1.5.2. Case-control studies of other respiratory tract cancers.**

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

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

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

3 4 **4.1.2.1.5.3. Summary of other respiratory tract cancers.**

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

17 18 **4.1.2.1.5.4. Summary of respiratory tract cancers.**

19 The epidemiologic studies of nasopharyngeal cancer provide strong support for a causal
20 role of formaldehyde exposure to the etiology of this rare cancer. The Hauptmann et al. (2004)
21 analysis of the large NCI cohort study of 10 worksites in the United States reported a doubling of
22 nasopharyngeal cancer mortality risk in workers (SMR = 2.1, 95% CI: 1.05–4.21). Hauptmann
23 et al. demonstrated that relative risks increased with average exposure intensity (p -
24 trend = 0.066), cumulative exposure (p -trend = 0.025), highest peak exposure (p -trend < 0.001)
25 and duration of exposure to formaldehyde (p -trend = 0.147). The case-control studies also
26 provide a robust collection of data supporting a similar association between formaldehyde
27 exposure and nasopharyngeal cancer (i.e., relative risk estimates in the range of 1.5–3.0). The
28 association between formaldehyde and nasopharyngeal cancer persisted when adjusted for the
29 effect of potential confounders. Data from several of these studies have identified a
30 concentration-response relationship (Hauptmann et al., 2004; Marsh et al., 2002; Vaughan et al.,
31 2000; West et al., 1993), adding weight to the causal evidence of an association between
32 formaldehyde exposure and cancer. The studies of the single Wallingford plant by Marsh et al.
33 (2002, 1996, 1994) and Marsh and Youk (2005) also revealed a dose-response trend, although
34 the absolute exposure level estimates were much lower according to Marsh et al. (2002).

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

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

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

30 31 **4.1.2.2. Nonrespiratory Tract Cancer**

32 **4.1.2.2.1. Lymphohematopoietic (LHP) cancers.**

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

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1 studies of professional groups were reviewed in an IARC (1995) monograph and are briefly
2 discussed in the next section regarding their findings on cancer of the LHP system. One case-
3 control study of non-Hodgkin’s lymphoma is discussed at the end of this section.
4

5 **4.1.2.2.1.1. Professional cohort studies.**

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

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

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

30 Hall et al. (1991) expanded the above study by including the newest members of the
31 Royal College of Pathologists. The cohort totaled 4,512 individuals, although only 3,069 males
32 and 803 females were included in the analysis. The reasons for this discrepancy were not
33 specified, although the authors mentioned that an unknown number of expected deaths for
34 Northern Irish and female Scottish pathologists were not calculated, 32 pathologists were lost in
35 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.	<u>Cohort study</u>				
			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)			
<u>Nested case-control analysis</u>							
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	<i>p</i> = 0.06			
			Cumulative exposure	<i>p</i> = 0.07			

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

16 Walrath and Fraumeni (1983) conducted a proportionate mortality study of all embalmers
17 and funeral directors licensed in the state of New York between 1902 and 1980 who were known
18 to have died between 1925 and 1980. While the investigators called their study a proportionate
19 mortality ratio (PMR) study, the methods described the comparison population with which
20 cancer deaths as the general population and not other deaths in the study population. The authors
21 computed their expected number of cause-specific deaths based on the US male population
22 stratified by 5-year age and calendar time periods. As such, their PMR meets the modern
23 definition of the more methodologically appropriate standardized mortality ratio (SMR). The
24 authors did also compute what they called the proportionate cancer mortality ratio (PCMR)
25 which is not standardized and may overestimate effects. The investigators requested death
26 certificates for 1,678 persons but received only 1,263 (75%). The investigators restricted their
27 analysis to 1,132 males. The distribution of the causes of death was compared with the age-,
28 race-, and calendar-year-specific proportions of deaths for each cause among the male U.S.
29 population. Duration of exposure was approximated by time since first license. While the
30 methodology could not be applied in all calculations because of data gaps, excess risks were
31 found for lymphatic and hematopoietic cancers, with a SMR of 1.2 (observed 25), and for
32 leukemia, with a SMR of 1.32 and proportionate cancer mortality ratio (PCMR) of 1.19
33 (12 observed). The SMRs were not affected when the estimates were stratified by latency
34 (<35 years or 35 years since first license) or by age at first license. Because the cause of death
35 could not be determined for nearly 25% of the study group, the risk estimates could be

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

5 Using what was actually the standardized mortality ratio methods (although referring to it
6 as the proportionate mortality method), Walrath and Fraumeni (1984) studied 1,007 deceased
7 white male embalmers, members of the California Bureau of Funeral Directing and Embalming,
8 whose deaths occurred between 1925 and 1980. The decedents had to have been licensed to
9 practice between 1916 and 1978. For lymphatic and hematopoietic cancer, the PMR (actually an
10 SMR) was 1.22 (19 deaths observed). For leukemia alone, the PMR was 1.75 and significant
11 (12 deaths observed, $p < 0.05$). Among embalmers licensed for 20 years or longer, the risk of
12 leukemia increased and was also significant (PMR (actually an SMR) 2.21; 8 observed;
13 $p < 0.05$).

14 Levine et al. (1984) conducted a cohort mortality study of 1,477 male Ontario
15 undertakers first licensed between 1928 and 1957 and followed until the end of 1977. Out of
16 359 subjects who had died, there were 8 deaths from lymphatic and hematopoietic cancers
17 compared with 6.5 expected. Additionally, there were 4 deaths from leukemia versus
18 2.5 expected. Because death rates were not available for Ontario before 1950, person-years and
19 deaths before 1950 could not be counted. No actual exposure estimates are available for these
20 undertakers.

21 Stroup et al. (1986) conducted an historic cohort mortality study of 2,317 men who were
22 members of the American Association of Anatomists between 1888 and 1969. The investigators
23 derived SMRs from the U.S. white male population and used members of the American
24 Psychiatric Association (APA) as a comparison group. Vital status was ascertained between
25 1925 and 1979. Women were excluded from analysis because of the small numbers. Only
26 738 deaths were observed versus 1,133.9 expected, based on U.S. death rates (SMR 0.65),
27 possibly indicating a sizable HWE. However, a slight increase in the risk of lymphatic and
28 hematopoietic cancers (SMR 1.2; 18 observed) and the risk of leukemia (SMR 1.5; 10 observed)
29 was evident. A significant increase in the risk of brain cancer (SMR 2.7; 10 observed; $p < 0.05$)
30 was also reported. When the leukemia analysis was restricted to the myeloid type, the SMR
31 increased to 8.8, based on five deaths ($p < 0.05$). The analysis using the APA group was
32 restricted to deaths that occurred between 1900 and 1969. This restriction removed five
33 leukemia deaths and person-years from the analysis because they likely died after 1969. Because
34 of this, there were only 3 leukemia deaths versus 3.6 expected, based on APA death rates. The
35 investigators concluded that the etiological agent had not been definitively identified, mentioning

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

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

17 Hayes et al. (1990) conducted a cohort study of 3,649 deceased white and 397 deceased
18 nonwhite U.S. male embalmers and funeral directors who had died between 1975 and 1985,
19 using records from local licensing boards, state funeral directors' associations in 32 states and
20 the District of Columbia, the National Funeral Directors' Association, and state offices of vital
21 statistics ($n = 894$). Expected deaths by cause were derived from 5-year age- and calendar-year-
22 specific proportions of deaths among appropriate race groups from the U.S. population. No
23 measured exposure data were available. A PCMR would be derived by excluding noncancer
24 causes of death. Statistically significant excesses in hematopoietic and lymphatic cancers were
25 found in white (PMR (actually SMR) 1.31 [95% CI: 1.06–1.59]; 100 observed) and nonwhite
26 (PMR (actually SMR) 2.41 [95% CI: 1.35–3.97]; 15 observed) embalmers and funeral directors.
27 The combined PMR (actually SMR) was 1.39 (95% CI: 1.15–1.63). The excess risk for all men
28 was higher for myeloid leukemia (ML) (PMR (actually SMR) 1.57 [95% CI: 1.01–2.34]; 24
29 observed) and for other unspecified leukemias (PMR (actually SMR) 2.28 [95% CI: 1.39–3.52];
30 20 observed) in white males.

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

1 age- and calendar-time-specific death rates from 1925 were used to develop SMRs. Separate
2 SMRs were based on psychiatrists' death rates. The risk of hematopoietic cancer (excluding
3 Hodgkin's disease) was elevated (SMR 1.25; 57 observed) based on U.S. white males. For
4 leukemia, the SMR was 1.35 (31 observed). The SMR for leukemia among psychiatrists was
5 0.83 (35 observed). Compared with leukemia in psychiatrists, the SMR for pathologists was
6 1.68 (95% CI: 1.14–2.38). The SMR for other lymphatic cancers was 1.53 (16 observed) and for
7 LHP cancer 1.22 (64 observed). Comparing the pathologists' death rates to those of psychiatrists
8 could be thought to have greater validity than if death rates for the U.S. population as a whole
9 had been used, because of shared socioeconomic circumstances and access to medical care
10 between the two professional groups. Differences in access to health care might have been
11 greater for subjects in the earlier part of the study, because improved diagnosis and medical care
12 for LHP cancers became more broadly available later in the study period. By using SMRs based
13 on U.S. death rates, which include those who do not have adequate access to medical care, the
14 difference between expected and observed deaths would be reduced. This is less likely to occur
15 when one professional group is compared with another professional group, assuming
16 psychiatrists and pathologists have equal access to care.

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

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

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

7 Odds ratios were estimated for all lymphohematopoietic cancers combined, cancers of
8 lymphoid as well as nonlymphoid origin, and specifically for myeloid leukemia using the
9 following exposure metrics: ever embalming, number of embalmings, number of years of
10 working in embalming, and four quantitative estimates of exposure to formaldehyde (see
11 Table 4-5). Exposure to formaldehyde as estimated as "ever embalming" was not associated
12 with an increased risk for all lymphohematopoietic cancers combined (OR = 1.4, 95% CI:
13 0.8–2.6). The ORs for lymphohematopoietic cancers of nonlymphoid origin which includes
14 Hodgkin disease as well as other specific subtype were generally elevated but there did not
15 appear to be any clear patterns of association. However there was an increased risk for
16 lymphohematopoietic cancers of nonlymphoid origin (OR = 3.0, 95% CI: 1.0-9.5). An
17 exposure-response trend showed that more years of working in embalming was associated with
18 an increase in risk of lymphohematopoietic cancers of nonlymphoid origin ($p < 0.05$); the other
19 exposure metrics did not demonstrate significant exposure-response relationships. Statistically
20 significant increases were observed for lymphohematopoietic cancers of nonlymphoid origin for
21 the highest exposure category for durations of working in embalming jobs (OR = 3.7, 95%
22 CI:1.1-12.2), for the number of embalmings (OR = 3.9, 95% CI: 1.2–12.8), for cumulative
23 exposure (OR = 4.0, 95% CI: 1.2–13.2), as well as for the cumulative mid-level exposures
24 (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
25 TWA intensity of exposure, and for the highest exposure category for peak exposure (OR = 3.8,
26 95% CI: 1.1–12.7).

27 The highest reported increases in risk for ever embalming were for myeloid leukemia
28 (OR = 11.2, 95% CI: 1.3-95.6). Duration of employment in jobs with embalming demonstrated
29 an exposure-response relationship with increased risk of myeloid leukemia ($p = 0.02$). The
30 number of embalming was also significantly associated with increased risk of myeloid leukemia
31 in the middle (OR = 12.7, 95%: 1.4-116.7) and highest exposure levels (OR = 12.7, 95% CI: 1.6-
32 119.7). Cumulative exposure was associated with increased risk of myeloid leukemia, with the
33 highest category of exposure showing OR = 13.2 (95% CI: 1.5-115.4).

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

1 14.8, and 9.5), and the test for an exposure-response trend was of borderline statistical
2 significance ($p = 0.058$). The risk of myeloid leukemia mortality was also very strongly and
3 significantly associated with 8-hour TWA exposure for mid-level exposures (OR = 13.6, 95%
4 CI: 1.5-125.8) and high-level exposures (OR = 12.0, 95% CI: 1.3-107.4), as well as for peak
5 exposure in two of three categories (0-7 ppm OR = 15.2, 95% CI: 1.6-141.6; 7-9.3 ppm
6 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,
7 there was also a statistically significant exposure-response trend ($p = 0.036$).

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

22 23 **4.1.2.2.1.2. Industry worker cohort studies.**

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

29 Blair et al. (1986) reported on 4,396 deaths from all causes in the 10 formaldehyde-
30 associated factories that made up the NCI cohort of 26,561 workers employed before January 1,
31 1966. There was little evidence of an association with LHP system cancer (SMR 0.91; 56
32 observed) in exposed white men, who dominated the cohort. Marsh et al. (1994), in an early
33 study of the Wallingford plant, which is also part of the Hauptmann et al. (2004, 2003) and Blair
34 et al. (1986) studies, found SMRs of 0.89 and 0.91, based on U.S. and county death rates,

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

3 Hauptmann et al. (2003) updated the cohort mortality study of Blair et al. (1986) that
4 consisted of predominantly the same (25,619) workers from 10 plants. The primary focus of this
5 analysis was cancer of the LHP system, including leukemia. The description and demographics
6 of the current study are the same as those reported by Blair et al. (1986) and Stewart et al.
7 (1986). In this update, follow-up was extended through December 31, 1994. The additional 15
8 years of follow-up increased the number of deaths from 4,349 to 8,486. However, in the course
9 of updating this cohort with follow-up through 2004, Beane Freeman et al. (2009) (including
10 Hauptmann) discovered that the matching of death certificates in Hauptmann et al. (2003) had
11 inexplicatively missed 1,006 deaths. Beane Freeman et al. (2009) reanalyzed the cohort with
12 follow-up through 1994 in order to replicate the analyses in Hauptmann et al. (2003) and
13 provided those results in supplementary tables. Nonetheless, the focus of the most recent re-
14 analysis of the NCI cohort was on follow-up completed through 2004.

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

26 In the latest update to the large NCI cohort, Beane Freeman et al. (2009) extended the
27 follow-up through 2004. This cohort is based on workers from 10 manufacturers of
28 formaldehyde, formaldehyde resins, molding compounds, plastic products, film or plywood who
29 were first employed prior to 1966. The median follow-up time for workers was 42 years,
30 representing 998,106 person-years of follow-up among 25,619 workers (Beane Freeman et al.,
31 2009). Exposure to formaldehyde was estimated for each individual job category from work
32 histories, with calendar-time and plant-specific estimates based on assessments of job titles and
33 tasks associated with those jobs using plant visits by industrial hygienists and monitoring data
34 (Blair et al. 1986; Stewart et al. 1986; Blair and Stewart 1990). Exposures were categorized by
35 peak exposure, average intensity of exposure and cumulative exposure. Peak exposure

1 categories were defined as nonexposed, low (0.1–1.9 ppm), medium (2.0–3.9 ppm), and high
2 (4.0 ppm or greater). Average intensity categories of exposure were defined as nonexposed, low
3 (0.1–0.4 ppm), medium (0.5 to <0.9 ppm), and high (≥ 1.0 ppm). Cumulative exposure was
4 defined as nonexposed, low (0.1–1.4 ppm-years), medium (1.5–5.4 ppm-years), and high (≥ 5.5
5 ppm-years). Duration of exposure was defined as 0, 0.1–4.9 years, 5.0–14.9 years, and ≥ 15
6 years. The presence of formaldehyde-containing particulates and other potential chemical
7 coexposures in the plants were indentified. Information on potential formaldehyde exposures
8 after 1980 was unavailable.

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

15 A total of 319 deaths from all lymphohematopoietic cancers were identified. Among
16 them, there were 286 ever-exposed and 33 never-exposed workers. SMR analyses based on
17 external comparison of populations showed that mortality from lymphohematopoietic cancers
18 was not elevated in either the ever-exposed (SMR = 0.94, 95% CI: 0.84-1.06) or never-exposed
19 workers (SMR = 0.86, 95% CI: 0.61-1.21). The ratio of the exposed to unexposed SMR was
20 1.09 (95% CI: 0.76-1.57)⁵. Table 4-6 shows the ratios of the SMRs in the exposed workers to
21 the SMRs in the unexposed workers. An elevated risk for Hodgkin lymphoma among exposed
22 workers was observed (SMR = 1.42, 95% CI: 0.96-2.10) which was twice the SMR for the
23 unexposed workers but not significantly different from the null. The SMR for lymphatic
24 leukemia was only 1.15 among exposed workers but this was more than four times higher than
25 the SMR for lymphatic leukemia among unexposed worker although not significantly so.
26 Mortality from other subtypes of lymphohematopoietic cancers among the exposed workers did
27 not show increased mortality rates compared with the U.S. population. It is important to note
28 that workers are generally healthier than the general population and, as such, are expected to
29 have lower baseline risk for cancer and mortality. This healthy worker effect effectively biases
30 many external rate and risk comparisons to yield SMR values below unity. Internal analyses can
31 provide a more appropriate comparison, as exposed and unexposed workers are likely to have
32 background risks of cancer and mortality that are more similar.

33
34

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

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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

^aVariance(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,

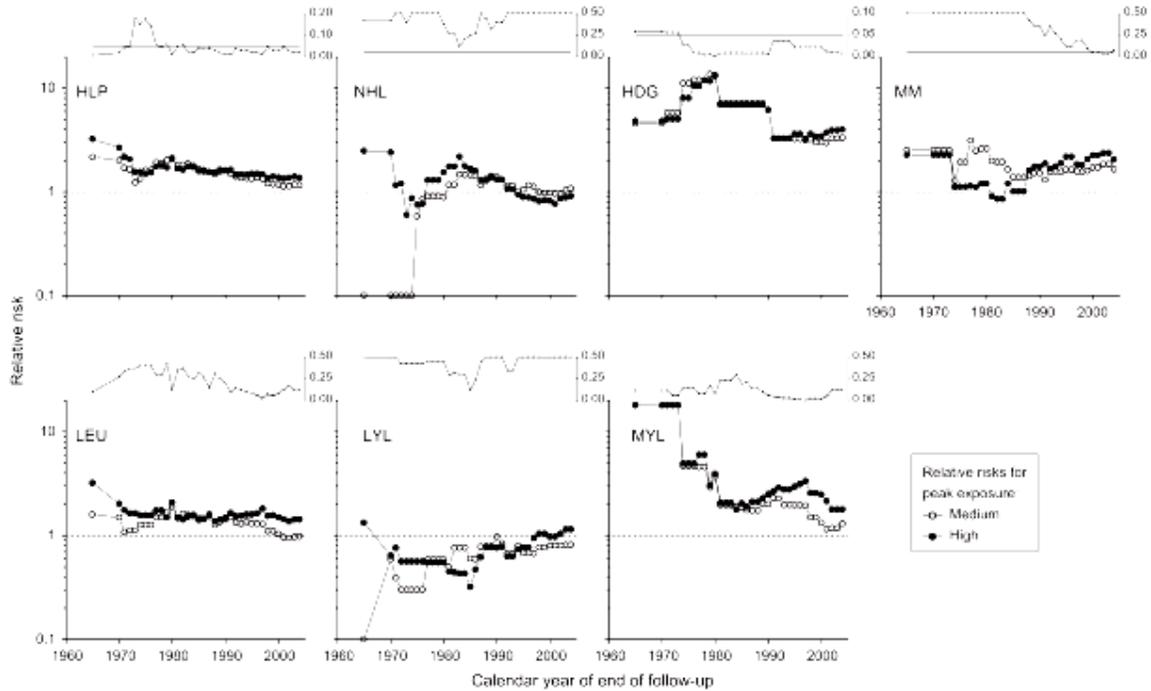
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1 there was no evidence of an exposure-response trend including the never-exposed workers. The
2 association of multiple myeloma with formaldehyde exposure was also shown throughout the
3 cohort experience (see Figure 4-3). Regarding leukemia mortality,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

8 Beane Freeman et al. (2009) further showed plots presenting the RR from the internal
9 analyses for each endpoint and for each year of follow-up. The association of Hodgkin
10 lymphoma with formaldehyde exposure is not only seen for the complete 2004 follow-up, but
11 throughout the cohort experience (see Figure 4-3). These plots show that during the 1970's, the
12 relative risk of Hodgkin lymphoma with peak exposure was greater than 10 and diminished to
13 approximately RR = 8 in the 1980's and remained at about RR = 4 through the end of follow-up
14 in 2004. Similarly, the relative risks for myeloid leukemia were greater than 10 in the early
15 1970's and diminished with extended follow-up. Beane Freeman et al. (2009) stated that “this
16 pattern could reflect the increased precision of the relative risk estimates with accrual of
17 additional person-years and myeloid leukemia or could reflect a relatively short induction-
18 incubation time for myeloid leukemia because by time since first exposure and first high peak
19 both indicate highest risks within the first 25 years.”

20 The exposure metric based on the average intensity of formaldehyde exposure was
21 associated with increased risks of Hodgkin lymphoma mortality but not with other specific
22 subtypes of lymphohematopoietic cancer mortality. Average exposures in the highest exposure
23 category were associated with an increase in Hodgkin lymphoma deaths (RR = 2.48, 95% CI:
24 0.84-7.32), comparing death rates among workers with peaks of ≥ 4 ppm to those with > 0 to 2.0
25 ppm. Across the three categories of average intensity, there was a statistically significant
26 exposure-response trend ($p = 0.05$). The exposure-response trend including the never exposed
27 workers was also statistically significant ($p = 0.03$). As with peak exposure, there is a consistent
28 trend across the years of follow-up for RR for myeloid leukemia and multiple myeloma of the
29 mid and high exposed individuals be elevated, although only nearing significance at a few points
30 (see Figure 4-4).

31



1
2
3 **Figure 4-3. Association between peak formaldehyde exposure and the risk of**
4 **lymphohematopoietic malignancy.**
5

6 Relative risks for medium-peak (2.0 to <4.0 ppm) and high-peak (≥ 4.0 ppm)
7 formaldehyde exposure categories compared with the low exposed category (>0
8 to <2.0 ppm) and *P* values for trend tests among the exposed person-years for
9 lymphohematopoietic malignancies are shown by year of end of follow-up, 1965-
10 2004. Values plotted at 0.1 represent RR = 0 due to no cases in the exposure
11 category values plotted at 20 represent RR = infinity due to no cases in the
12 referent category. The **small graphs** above the relative risk plots represent the
13 exposure-response trend *P* values based on two-sided likelihood ratio tests (1 df)
14 of zero slope for continuous formaldehyde exposure among exposed person-years
15 only. The **points** represent the relative risk estimates based on the cumulative
16 number of cases and person-years accrued from the start of the study to that point
17 in time and for 2004 are equivalent to the relative risk estimates presented in
18 Table 2 (Beane Freeman et al., 2009). HLP = lymphohematopoietic
19 malignancies, NHL = non-Hodgkin lymphoma, HDG = Hodgkin lymphoma,
20 MM = multiple myeloma, LEU = leukemia, LYL = lymphatic leukemia,
21 MYL = myeloid leukemia, RR = relative risk.
22

23 Source: Beane Freeman et al. (2009)
24

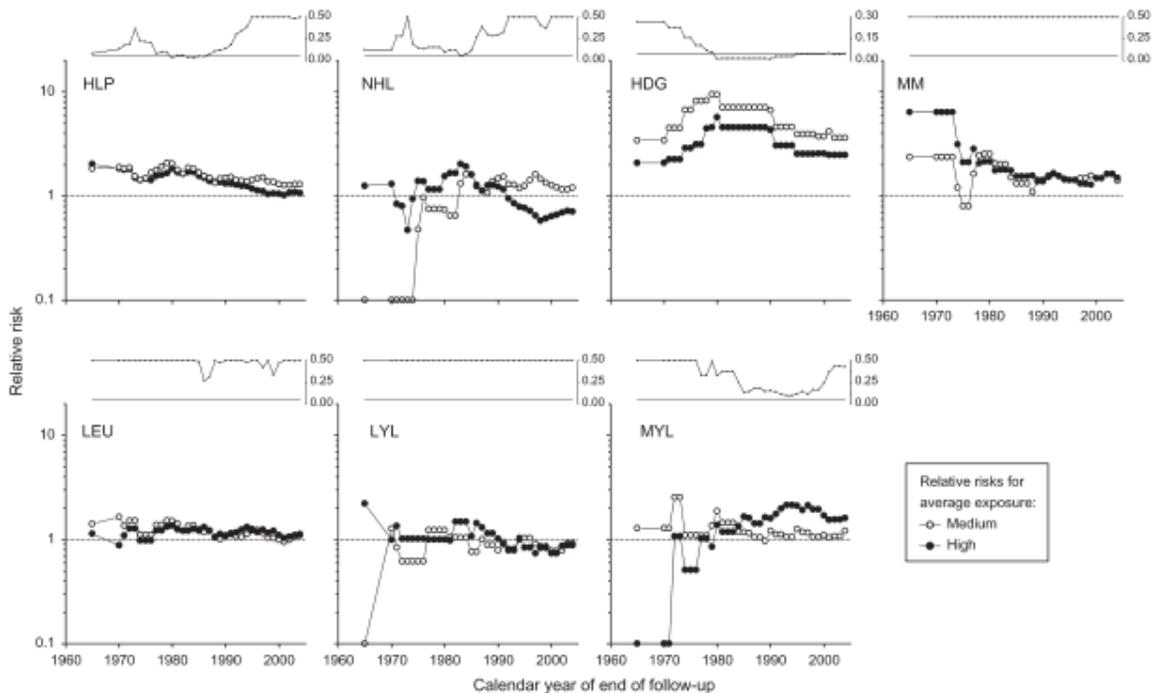


Figure 4-4. Association between average intensity of formaldehyde exposure and the risk of lymphohematopoietic malignancy.

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)

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

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

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

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

7 Beane Freeman et al. (2009) concluded that the pattern of attenuating
8 lymphohematopoietic risk over time was “consistent with a causal association within the
9 relatively short induction-incubation periods, characteristic of leukemogenesis.” To understand
10 the implication of this conclusion, it should be noted that if the etiologically-relevant window of
11 time for formaldehyde-induced lymphohematopoietic cancer mortality is relatively short, then
12 extending a follow-up of the cohort well beyond the upper bound of that window will not allow
13 for the additional inclusion of more formaldehyde-induced lymphohematopoietic cancer deaths.
14 Rather, it allows for the inclusion of unrelated lymphohematopoietic cancer mortality which
15 serves to dilute any true effect of formaldehyde exposure. The median follow-up time in the
16 Beane Freeman et al. (2009) was 42 years and the analyses reported that exposure lags between 2
17 and 25 years were tested for fit, with an 18-year lag having the best fit. Since exposures were
18 not considered to have continued beyond 1980 and the cohort mortality was complete through
19 2004, the mortality in the later years of follow-up was unlikely to be related to formaldehyde as
20 the deaths may have been outside of any causal exposure window. It should be noted that the
21 analyses present in Beane Freeman et al. (2009) were based on a 2-year lag, as there was not
22 strong enough support for assuming either a longer or a shorter lag.

23 Two sensitivity analyses evaluated the assumption of no formaldehyde exposure after
24 1980. If exposure was considered to continue at 1980 levels until age 65 years or death, risk
25 patterns for all lymphohematopoietic cancers were reported to be similar to those observed in the
26 primary analysis. If the cohort follow-up was censored two years after the last job for the
27 2,810 individuals who were still exposed in 1979 and alive two years later, the association for
28 myeloid leukemia with peak and average intensity of exposure was stronger than that observed in
29 the primary analyses. The investigators did not report the results of the sensitivity analyses for
30 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	<i>NR</i>	(156)
			LHP cancers	SMR	2.0	<i>p</i> < 0.01	(8)
			Hodgkin’s disease	SMR	1.4	<i>NR</i>	(1)
			Leukemia	SMR	0.6	<i>NR</i>	(1)
			<u>Technicians</u>				
			All cause mortality	SMR	0.67	<i>NR</i>	(154)
			LHP cancers	SMR	0.5	<i>NR</i>	(3)
			Hodgkin’s disease	SMR	–	<i>NR</i>	(0)
			Leukemia	SMR	0.5	<i>NR</i>	(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	<i>NR</i>	(8)
			Leukemia	SMR	1.60	<i>NR</i>	(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)	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.	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)
Previous reports:			Unexposed	SMR	0.86	(95% CI: 0.61–1.52)	(33)
Hauptmann et al. (2003)	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.	Exposures to 11 other compounds were	<u>Peak exposure (ppm)</u>				
			0	RR	1.07	(95% CI: 0.70–1.62)	(33)
Blair et al. (1986)			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)
			<i>Trend p = 0.02</i>				
			<u>Average exposure (ppm)</u>				

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	<i>Reference value</i>	(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)			
			<i>Trend p > 0.50</i>							
			<u>Cumulative exposure(ppm-years)</u>							
			0	RR	0.89	(95% CI: 0.59–1.34)	(33)			
			0.1–1.4	RR	1.00	<i>Reference value</i>	(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)			
			<i>Trend p = 0.25</i>							
			Leukemia							
			<u>Peak exposure (ppm)</u>							
			0	RR	0.59	(95% CI: 0.25–1.36)	(7)			
			0.1–1.9	RR	1.00	<i>Reference value</i>	(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)						
<i>Trend p = 0.012</i>										
<u>Average exposure (ppm)</u>										
0 ppm	RR	0.54	(95% CI: 0.24–1.22)	(7)						
0.1–0.4	RR	1.00	<i>Reference value</i>	(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)			<i>Trend p > 0.50</i>				
			<u>Cumulative exposure (ppm-years)</u>				
			0	RR	0.53	(95% CI: 0.23–1.21)	(7)
			0.1–1.4	RR	1.00	<i>Reference value</i>	(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)
			<i>Trend p = 0.12</i>				
			Hodgkin Lymphoma				
			<u>Peak exposure (ppm)</u>				
			0	RR	0.67	(95% CI: 0.12–3.60)	(2)
			0.1–1.9	RR	1.00	<i>Reference value</i>	(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)
			<i>Trend p = 0.01</i>				
<u>Average exposure (ppm)</u>							
0	RR	0.46	(95% CI: 0.05–3.93)	(2)			
0.1–0.4	RR	1.00	<i>Reference value</i>	(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)			
<i>Trend p = 0.05</i>							
<u>Cumulative (ppm-years)</u>							
0	RR	0.42	(95% CI: 0.09–2.05)	(2)			
0.1–1.4	RR	1.00	<i>Reference value</i>	(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)			
			<i>Trend p = 0.08</i>							
			Myeloid Leukemia							
			<u>Peak exposure (ppm)</u>							
			0	RR	0.82	(95% CI: 0.25–2.67)	(4)			
			0.1 to 1.9	RR	1.00	<i>Reference value</i>	(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)			
			<i>Trend p = 0.13</i>							
			<u>Average exposure (ppm)</u>							
			0	RR	0.70	(95% CI: 0.23–2.16)	(4)			
			0.1 to 0.4	RR	1.00	<i>Reference value</i>	(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)			
<i>p = 0.43</i>										
<u>Cumulative (ppm-years)</u>										
0	RR	0.61	(95% CI: 0.20–1.91)	(4)						
0.1-1.4	RR	1.00	<i>Reference value</i>	(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)						
<i>Trend p > 0.50</i>										

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)	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.	Occupational history obtained by interviews with next of kin and coworkers using detail questionnaires.	All LHP cancers				
<u>Previous reports:</u>			<u>Embalming</u>				
Hayes et al. (1990)	Odds ratios calculated using unconditional logistic regression	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.	Never	OR	1.0	Reference value	(24)
Walrath and Fraumeni (1983)			Ever	OR	1.4	(95% CI: 0.8–2.6)	(144)
Walrath and Fraumeni (1984)	<u>Related re-analyses:</u>		<u>Duration of working in jobs with embalming (years)</u>				
Marsh et al. (2007a)			0	OR	1.0	Reference value	(24)
Marsh et al. (2007b)	Marsh and Youk (2005)		> 0 to 20	OR	0.8	(95% CI: 0.4–1.8)	(28)
Marsh et al. (1996)			> 20 to 34	OR	1.5	(95% CI: 0.8–2.8)	(50)
			> 34	OR	1.8	(95% CI: 1.0–3.4)	(66)
			<i>Trend p = 0.058</i>				
			<u>Number of embalming</u>				
			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)
			<i>Trend p = 0.477</i>				
			<u>Cumulative exposure (ppm-hours)</u>				
			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)
			<i>Trend p = 0.422</i>				

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)			<u>Average formaldehyde intensity while embalming (ppm)</u>				
			0	OR	1.0	<i>Reference value</i>	(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)
			<i>Trend p = 0.591</i>				
			<u>Time-weighted average exposure over 8 hours (ppm)</u>				
			0	OR	1.0	<i>Reference value</i>	(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)
			<i>Trend p = 0.635</i>				
			<u>Peak formaldehyde exposure (ppm)</u>				
			0	OR	1.0	<i>Reference value</i>	(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)			
<i>Trend p = 0.555</i>							
Myeloid leukemia							
<u>Embalming</u>							
Never	OR	1.0	<i>Reference value</i>	(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)			<u>Duration of working in jobs with embalming (years)</u>				
			0	OR	1.0	<i>Reference value</i>	(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)
			<i>Trend p = 0.02</i>				
			<u>Number of embalmings</u>				
			0	OR	1.0	<i>Reference value</i>	(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)
			<i>Trend p = 0.314</i>				
			<u>Cumulative exposure (ppm-hours)</u>				
			0	OR	1.0	<i>Reference value</i>	(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)
			<i>Trend p = 0.192</i>				
			<u>Average formaldehyde intensity while embalming (ppm)</u>				
			0	OR	1.0	<i>Reference value</i>	(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)			
<i>Trend p = 0.058</i>							

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)			<p><u>Time-weighted average exposure over 8 hours (ppm)</u></p> <table border="1"> <tr> <td>0</td> <td>OR</td> <td>1.0</td> <td><i>Reference value</i></td> <td>(1)</td> </tr> <tr> <td>> 0 to 0.10</td> <td>OR</td> <td>8.4</td> <td>(95% CI: 0.8–79.3)</td> <td>(8)</td> </tr> <tr> <td>> 0.1 to 0.18</td> <td>OR</td> <td>13.6</td> <td>(95% CI: 1.5–125.8)</td> <td>(13)</td> </tr> <tr> <td>> 0.18</td> <td>OR</td> <td>12.0</td> <td>(95% CI: 1.3–107.4)</td> <td>(12)</td> </tr> </table> <p style="text-align: center;"><i>Trend p = 0.396</i></p> <p><u>Peak formaldehyde exposure (ppm)</u></p> <table border="1"> <tr> <td>0</td> <td>OR</td> <td>1.0</td> <td><i>Reference value</i></td> <td>(1)</td> </tr> <tr> <td>> 0 to 7.0</td> <td>OR</td> <td>15.2</td> <td>(95% CI: 1.6–141.6)</td> <td>(12)</td> </tr> <tr> <td>> 7.0 to 9.3</td> <td>OR</td> <td>8.0</td> <td>(95% CI: 0.9–74.0)</td> <td>(9)</td> </tr> <tr> <td>> 9.3</td> <td>OR</td> <td>13.0</td> <td>(95% CI: 1.4–116.9)</td> <td>(12)</td> </tr> </table> <p style="text-align: center;"><i>Trend p = 0.036</i></p>	0	OR	1.0	<i>Reference value</i>	(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)	0	OR	1.0	<i>Reference value</i>	(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)
0	OR	1.0	<i>Reference value</i>	(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)																																							
0	OR	1.0	<i>Reference value</i>	(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)																																							
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.	<p>Non-Hodgkin lymphoma</p> <p><u>Formaldehyde</u></p> <table border="1"> <tr> <td>Never</td> <td>OR</td> <td>1.0</td> <td><i>Reference value</i></td> <td>(398)</td> </tr> <tr> <td>Ever</td> <td>OR</td> <td>1.3</td> <td>(95% CI: 1.0–1.7)</td> <td>(203)</td> </tr> </table> <p><u>Average exposure intensity</u></p> <table border="1"> <tr> <td>Never</td> <td>OR</td> <td>1.0</td> <td><i>Reference value</i></td> <td>(398)</td> </tr> <tr> <td>Low</td> <td>OR</td> <td>1.4</td> <td>(95% CI: 1.0–1.8)</td> <td>(129)</td> </tr> <tr> <td>Medium-High</td> <td>OR</td> <td>1.2</td> <td>(95% CI: 0.8–1.7)</td> <td>(74)</td> </tr> </table> <p style="text-align: center;"><i>Trend p = 0.21</i></p>	Never	OR	1.0	<i>Reference value</i>	(398)	Ever	OR	1.3	(95% CI: 1.0–1.7)	(203)	Never	OR	1.0	<i>Reference value</i>	(398)	Low	OR	1.4	(95% CI: 1.0–1.8)	(129)	Medium-High	OR	1.2	(95% CI: 0.8–1.7)	(74)															
Never	OR	1.0	<i>Reference value</i>	(398)																																							
Ever	OR	1.3	(95% CI: 1.0–1.7)	(203)																																							
Never	OR	1.0	<i>Reference value</i>	(398)																																							
Low	OR	1.4	(95% CI: 1.0–1.8)	(129)																																							
Medium-High	OR	1.2	(95% CI: 0.8–1.7)	(74)																																							

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)			<u>Average exposure probability</u>				
			Never	OR	1.0	<i>Reference value</i>	(398)
			Low	OR	1.3	(95% CI: 1.0–1.7)	(129)
			Medium-High	OR	1.4	(95% CI: 0.9–2.3)	(74)
			<i>Trend p > 0.50</i>				
			<u>Both average exposure intensity and average exposure probability</u>				
			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				
			<u>Peak exposure (ppm)</u>				
			0	RR	0.59	(95% CI: 0.25–1.36)	(7)
			0.1–1.9	RR	1.00	<i>Reference value</i>	(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)			
<i>Trend p = 0.012</i>							
<u>Average exposure (ppm)</u>							
0 ppm	RR	0.54	(95% CI: 0.24–1.22)	(7)			
0.1–0.4	RR	1.00	<i>Reference value</i>	(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)			
			<i>Trend p > 0.50</i>							
			<u>Cumulative exposure (ppm-years)</u>							
			0	RR	0.53	(95% CI: 0.23–1.21)	(7)			
			0.1–1.4	RR	1.00	<i>Reference value</i>	(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)			
			<i>Trend p = 0.12</i>							
			Hodgkin Lymphoma							
			<u>Peak exposure (ppm)</u>							
			0	RR	0.67	(95% CI: 0.12–3.60)	(2)			
			0.1–1.9	RR	1.00	<i>Reference value</i>	(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)			
<i>Trend p = 0.01</i>										
<u>Average exposure (ppm)</u>										
0	RR	0.46	(95% CI: 0.05–3.93)	(2)						
0.1–0.4	RR	1.00	<i>Reference value</i>	(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)						
<i>Trend p = 0.05</i>										

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)			<u>Cumulative (ppm-years)</u>				
			0	RR	0.42	(95% CI: 0.09–2.05)	(2)
			0.1–1.4	RR	1.00	<i>Reference value</i>	(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)
			<i>Trend p = 0.08</i>				
			Myeloid Leukemia				
			<u>Peak exposure (ppm)</u>				
			0	RR	0.82	(95% CI: 0.25–2.67)	(4)
			0.1 to 1.9	RR	1.00	<i>Reference value</i>	(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)
			<i>Trend p = 0.13</i>				
			<u>Average exposure (ppm)</u>				
			0	RR	0.70	(95% CI: 0.23–2.16)	(4)
0.1 to 0.4	RR	1.00	<i>Reference value</i>	(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)			
<i>p = 0.43</i>							
<u>Cumulative (ppm-years)</u>							
0	RR	0.61	(95% CI: 0.20–1.91)	(4)			
0.1-1.4	RR	1.00	<i>Reference value</i>	(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)			
<i>Trend p > 0.50</i>							

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)			
			<u>Mortality since 1960</u>							
			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 1 st exposure	SMR	1.91	<i>p</i> > 0.05	(13)			
			Multiple cause leukemia							
			10+ years of exposure and 20+ years since 1 st exposure	SMR	1.92	(95% CI: 1.08–3.17)	(15)			
			Multiple cause ML							
20+ years since 1 st exposure	SMR	2.02	(95% CI: 1.13–3.34)	(15)						
10+ years of exposure and 20+ years since 1 st 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 High exposure Leukemia Overall High exposure Multiple myeloma Overall High exposure	SMR 0.98 SMR 0.89 SMR 0.91 SMR 0.71 SMR 0.86 SMR 1.18	(95% CI: 0.67–1.39) (95% CI: 0.41–1.70) (95% CI: 0.62–1.29) (95% CI: 0.31–1.39) (95% CI: 0.48–1.41) (95% CI: 0.48–2.44)	(31) (9) (31) (8) (15) (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 Leukemia	SMR 0.59 SMR 0.43	(95% CI: 0.23–1.21) (95% CI: 0.05–1.57)	(7) (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)					
				SMR				
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.	<u>All LHP cancers</u>					
			Hourly workers	SMR	0.93		(28)	
			Salaried workers	SMR	1.69		(23)	
			<u>Leukemia</u>					
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)
			<u>Race</u>				
			White	SMR ^b	1.31	(95% CI: 1.06–1.59)	(100)
			Nonwhite	SMR ^b	2.41	(95% CI: 1.35–3.97)	(15)
			<u>Occupation on death certificate</u>				
			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)
			<u>Age at death</u>				
			<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)

^aAdjusted for age, state, smoking, family history of malignant proliferative disease, agricultural exposure to pesticides, use of dye, and direct/surrogate response to interview.

^bWalrath 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.

1 Stayner et al. (1988) conducted a cohort study of 11,030 workers (82% female) followed
2 from 1955 or the beginning date of exposure through 1982 in three garment factories. Personnel
3 records from three garment manufacturing facilities, one in Pennsylvania and two in Georgia,
4 were used to assemble a cohort of workers who attained a minimum of 3 months of exposure
5 after the introduction of formaldehyde into these facilities. Formaldehyde resins were used to
6 treat fabrics, beginning in 1955 and 1959. Although formaldehyde levels were available on a
7 subset of the employees from monitoring data available from surveys completed in 1981 and
8 1984, they were not used in this analysis. Instead, the results were stratified by duration and
9 latency. SMRs were based on U.S. population mortality rates. Based on six cases, the SMRs for
10 leukemia were 2.43 and 3.81 among workers with 20 or more years since first exposure or at
11 least 10 years of exposure, respectively. In their conclusions, the authors suggested that,
12 although the numbers of deaths from LHP cancers were small, the risks were related to duration
13 and latency.

14 Pinkerton et al. (2004) updated the Stayner et al. (1988) study by adding 16 years of
15 follow-up. No new exposure information was added. The mean TWA exposure in 1981–1984
16 for the three plants was 0.15 ppm. No additional information regarding earlier industrial hygiene
17 data was available, although the authors stated that the levels of exposure to formaldehyde were
18 greater in the years before 1980. Stayner et al. (1988) cited independent studies of exposure
19 levels in similar garment factories in the 1960s that seemed to indicate that the formaldehyde
20 levels during that period ranged from 0.9 to 2.7 ppm (Blejer and Miller, 1966) in one garment
21 manufacturing area. Another report (Shipkovitz, 1966) of 10-minute personal exposure samples
22 indicated a range from 0.3 to 2.7 ppm in eight garment plants. In another study (Ahmad and
23 Whitson, 1973), the levels ranged from 2 to 10 ppm. Goldstein (1973) calculated that
24 concentrations in the cutting rooms of garment plants dropped from 10 ppm in 1968 to less than
25 2 ppm in 1973 because of an improvement in the resin treating process. The authors assumed
26 that exposure ceased in 1981 and 1983. This produced an underestimate of exposure based on
27 duration of employment for about 11% of the cohort who were still actively employed after those
28 dates. Stayner et al. (1988) speculated that the risks of cancer of the buccal cavity, leukemia, and
29 other LHP neoplasia may have been due to exposure to the highest potential formaldehyde levels
30 in the industry between 1955 and 1962, because the resin used to treat permanent press fabrics
31 still contained a relatively large amount of formaldehyde.

32 The SMRs were derived from age-, race-, and calendar-time-adjusted U.S. mortality
33 rates. The analysis was repeated using Georgia or Pennsylvania mortality rates. In addition to
34 the primary analysis of the underlying cause of death, the analysis used all causes listed on the
35 death certificates to evaluate multiple cause mortality. As a referent for this, the analysis relied

1 on multiple cause death rates available since 1960 from the National Death Index maintained by
2 the U.S. Centers for Disease Control and Prevention (CDC).

3 Altogether, 608 cancer deaths were observed (Pinkerton et al. 2004). The SMR for all
4 cancer was 0.89 (95% CI: 0.82–0.97). The overall SMR for leukemia was 1.09 (24 deaths) and
5 1.44 (15 deaths) for ML. After 10 years of exposure, the risk for ML was 2.19. Exposure prior
6 to 1963 was associated with a risk of 1.61. Among garment workers followed for 20 or more
7 years from initial exposure, the SMR was significantly elevated for ML (1.91; $p < 0.05$; 13
8 deaths), as was the SMR for multiple cause leukemia (1.92 [95% CI: 1.08–3.17]; 15 deaths) in
9 the subgroup with 10 or more years of exposure to formaldehyde and who were followed for 20
10 or more years after first exposure. The multiple cause mortality for ML for this subgroup of
11 workers was also significant (SMR 2.55 [95% CI: 1.10–5.03]; 8 deaths).

12 The study by Stayner et al. (1988) has only limited power to detect excess risks of rare
13 cancers, such as NPC and nasal cancer (13 and 16%, respectively). Limitations to the
14 interpretations of the findings include a lack of any monitoring data before 1981, particularly
15 during the critical time period 1955 to 1962, and lack of personal exposure estimates for any
16 members of the cohort. The possibility exists that misclassification may still be present because
17 the intensity of exposure to formaldehyde decreased as improvements were made in the resin
18 systems used to treat fabrics (e.g., a person who worked 5 years beginning in 1955 might have
19 been subject to greater exposure than a person who worked 5 years beginning in 1993).
20 However, workers from the 1950s and 1990s were both placed in the same category of having
21 worked fewer than 10 years. The median duration of exposure was 3.3 years. Work histories
22 were not updated in the follow-up study; however, the low or background exposure levels that
23 probably existed after 1981 were not likely to contribute substantially to the risk of cancer. The
24 use of mortality data to estimate risk, when the case fatality rate was less than 100% for most
25 cancer sites evaluated, could potentially produce an underestimate of the actual risk. Despite
26 these limitations, this study provides additional evidence of an association between leukemia,
27 especially ML, and formaldehyde in comparison with the general population.

28 Gardner et al. (1993) reported that the risk of leukemia was not statistically significant
29 (SMR 0.9) based on 15 deaths among workers employed before 1965. Only four leukemia
30 deaths were observed after 1964 through 1989, producing an SMR of 0.9.

31 When Coggon et al. (2003) updated the above cohort study of 14,014 men first employed
32 before 1965 in six factories by adding 11 additional years of follow-up (ending December 31,
33 2000), no increase in the risk of leukemia or related cancers of the hematopoietic system was
34 reported, either in the entire cohort (SMR 0.91; 31 observed) or in the group with the highest
35 formaldehyde exposure (>2 ppm) (SMR 0.71; 8 observed). Similar results were obtained for

1 Hodgkin’s disease, non-Hodgkin’s lymphoma, and multiple myeloma. No other cancers of the
2 hematopoietic system were evaluated, and no additional analyses were performed to assess a
3 possible leukemia risk. However, the main finding from this study was a marked association of
4 lung cancer with formaldehyde (discussed in the lung cancer section). This study’s main focus
5 was respiratory disease, lung cancer, and stomach cancer, not LHP cancers. For cancers of the
6 LHP system, there was neither latency evaluation nor internal comparisons. The HWE is also
7 potentially a problem.

8 Andjelkovich et al. (1995) studied a cohort of 3,929 male iron foundry workers
9 potentially exposed to formaldehyde between January 1, 1960, and December 31, 1989, in which
10 127 cancer deaths had occurred during the observation period. An industrial hygienist, after
11 reviewing work histories, categorized formaldehyde exposure into four levels corresponding to
12 the approximate midpoint of the ranges: none, low (0.05 ppm), medium (0.55 ppm), and high
13 (1.5 ppm) for exposure to formaldehyde. Boundaries of these exposure categories were not
14 given. The authors warned that the assignment of exposure levels was not perfect because
15 “subjective judgment had to be applied in many instances.” SMRs were based on U.S. male
16 mortality rates, but actual ranges were not specified. The authors also compared the exposed to
17 2,032 nonexposed workers from the same company. The population-based SMR for
18 hematopoietic cancer in the exposed population was 0.59 (based on seven observed deaths). For
19 leukemia the SMR was 0.43, based on two deaths. There were no other analyses for leukemia or
20 LHP cancers in this study. Because of the uncertainty about workers’ true formaldehyde
21 exposure, there was no analysis by level of exposure, duration, or latency. There were also very
22 few LHP cancers in the cohort. Thus, these results neither support nor refute an association of
23 formaldehyde exposure with LHP cancers. The main focus of this paper was on lung cancer risk.

24 Bertazzi et al. (1989, 1986), in a cohort mortality study, followed 1,330 male workers
25 from 1959 through 1986 at a formaldehyde resin plant in Italy. The workers had to have been
26 employed for at least 30 days at the plant sometime between 1959 and 1980 to be included in the
27 study. Their mortality was compared with national and local rates adjusted for age and calendar
28 time period. No individual exposure estimates were available, but mean levels were estimated to
29 be between 0.2 and 3.8 mg/m³ (0.16 and 3.1 ppm) during the period 1974–1979. The authors
30 found an SMR of 2.01 (five deaths observed) for cancer of the lymphatic and hematopoietic
31 system. The study’s limitations included incomplete work histories, small numbers of deaths,
32 and a follow-up period that may not have been sufficient to allow for a latency period for the
33 development of LHP cancers. As before, the results neither support nor refute an association of
34 formaldehyde exposure with LHP cancers.

1 Edling et al. (1987) reported on the incidence of disease in a cohort of 521 blue collar
2 Swedish workers in plants where abrasives bound with formaldehyde resins were manufactured.
3 Formaldehyde levels ranged from 0.1 to 1.0 mg/m³ (0.08–0.8 ppm). The workers in the cohort
4 were employed between 1955 and 1983, and incidence rates were calculated from 1958 through
5 1981. There were only 24 total cancer cases (28.5 expected) of which 2 (1.0 expected) were
6 lymphomas and 2 (0.5 expected) were multiple myelomas. Expected cases were determined
7 through the Swedish National Cancer Register. No other LHP cancers were observed. This
8 study lacked the power to detect any significant associations between LHP cancer and exposure
9 to formaldehyde.

10 Dell and Teta (1995) conducted a cohort mortality study of 5,932 male employees of a
11 New Jersey plastics manufacturing, research, and development facility that produced phenol-
12 formaldehyde resins. The workers, who had been employed during the period 1946–1967, were
13 followed-up for an average of 32 years. SMRs were based on U.S. and New Jersey mortality
14 rates. Hourly workers ($n = 3,853$) were analyzed separately from the 2,079 salaried employees.
15 Although no excess risk was evident for hematopoietic cancer in hourly workers (SMR 0.93;
16 28 observed), there was an SMR of 1.69 (95% CI: 1.07–2.53; 23 observed) among salaried
17 workers. This association was further narrowed to mainly research and development workers
18 (eight leukemia deaths observed with three expected, for an SMR of 2.67). No common
19 exposure was found when work history records were examined. The decedents were mostly
20 associated with process development in two research pilot plants, where chemical engineers, lab
21 technicians, and plant operators executed small-scale product development. Although notebooks
22 referred to benzene and toluene solvents, no definite connection was made with formaldehyde or
23 any of the solvents. No ambient air measurements of formaldehyde were available. The
24 findings cannot be assumed to be due to formaldehyde exposure because of the presence of other
25 potential leukemogens.

26 Blair et al. (1993) conducted a study that evaluated the risk of non-Hodgkin's lymphoma
27 from exposure to formaldehyde. This was a population-based, case-control, interview-based
28 study of 1,867 white males of whom 622 cases had the disease and 1,245 were controls.
29 Subjects had lived in Iowa and Minnesota between 1980 and 1983. This study was exploratory
30 and designed to find associations with any environmental exposures and non-Hodgkin's
31 lymphoma. Subjects or next of kin were interviewed to determine what exposures the cases and
32 controls may have received based on agricultural exposures, work histories, medical conditions,
33 and family history. Extra effort was made to collect information about occupation, industrial
34 exposures, and other selected exposures. The analysis revealed an OR of 1.2 for exposure to
35 formaldehyde. Similar associations were found for metals and other substances in the study.

1 This study, because it did not select cases and controls from a population with possible
2 formaldehyde exposure, could not detect specific relationships between formaldehyde and
3 non-Hodgkin's lymphoma.

4 Wang et al. (2009) assessed the effect of formaldehyde exposure on the risk of
5 non-Hodgkin lymphoma in a population-based case-control study among women in Connecticut.
6 Incident cases ($N = 601$) were frequency matched to 717 controls by age. A standardized
7 questionnaire was used to gather information on lifetime occupational history and other risk
8 factors. Exposure to organic solvents and formaldehyde for each job was assessed by linking
9 study participant's occupational data to a job-exposure matrix created by industrial hygienists at
10 the National Cancer Institute (Dosemeci et al., 1994; Gomez et al., 1994). Semiquantitative
11 exposure metrics included average exposure intensity and average exposure probability which
12 were evaluated individually and together. Analyses use unconditional logistic regression and
13 controlled for age, family history of hematopoietic cancers, alcohol consumption and race. For
14 the low average exposure intensity category the OR = 1.4 (95% CI: 1.0-1.8), while for the
15 Medium-High category the OR = 1.2 (95% CI: 0.8-1.7). For the Low average exposure
16 probability category the OR = 1.3 (95% CI: 1.0-1.7), while for the Medium-High category the
17 OR = 1.4 (95% CI: 0.9-2.3). When both factors were considered jointly, the Low intensity and
18 Low probability exposure category had OR = 1.4 (95% CI: 1.1-1.9); Medium-High intensity and
19 Low probability exposure category had OR = 1.0 (95% CI: 0.7-1.6); Low intensity and Medium-
20 High probability exposure category had OR = 1.1 (95% CI: 0.5-2.4); Medium-High and
21 Medium-High probability exposure category had OR = 1.6 (95% CI: 0.9-3.1). The investigators
22 also examined the risk of non-Hodgkin lymphoma among major subtypes. The risk of follicular
23 lymphoma and chronic lymphocytic leukemia/Small lymphocytic lymphoma was slightly
24 elevated but the risk of diffuse large B-cell lymphoma was OR = 1.9 (95% CI: 1.3-2.6) for ever
25 having been exposed to formaldehyde. For Low average intensity exposure, the risk was
26 OR = 2.1 (95% CI: 1.3-2.6) while for Medium-High average intensity exposure, the risk was
27 OR = 1.5 (95% CI: 0.9-2.4). Even so, an exposure-response relationship was demonstrated using
28 the continuous parameterization of average intensity rather than the categorical ($p = 0.03$).
29 Likewise, an exposure-response relationship was demonstrated using the continuous
30 parameterization of average probability of exposure rather than the categorical ($p = 0.01$).

31 The findings of Wang et al. (2009) provide some support for an association between
32 formaldehyde exposure and non-Hodgkin lymphoma. It should be noted that a population-based
33 case-control study, where incidence rather than mortality defines the case—may be more
34 appropriate for cancers with relatively low mortality (i.e., CCL, large B-cell lymphoma, Small
35 lymphocytic lymphoma).

1 **4.1.2.2.1.3. Meta-analyses of epidemiological studies for lymphohematopoietic malignancies.**

2 Several meta-analyses have reported risks for all lymphohematopoietic cancers and
3 leukemia (Collins and Lineker, 2004; Bosetti et al., 2008; Zhang et al., 2009). The meta-analysis
4 conducted by Collins and Lineker (2004) was based on 18 studies. Fixed-effects models were
5 used to obtain summary relative risk values and 95% confidence intervals, and random effects
6 models were used to evaluate heterogeneity across studies. The summary RR across all studies
7 was 1.1 (95% CI = 1.0 to 1.2) for leukemia. The effect estimates varied by type of study,
8 country of study population, type of industry, year of publication, and study size. The cohort
9 studies had a summary RR = 1.0 (95% CI: 0.9-1.2) while the summary estimate for the case-
10 control studies was RR = 2.4 (95% CI: 0.9-6.5). For industrial type jobs the RR was 0.9 (95%
11 CI: 0.8-1.0) while for embalmers the RR was 1.6 (95% CI: 1.2-2.0) and for pathologists and
12 anatomists the RR was 1.4 95% CI: 1.0-1.9).

13 Bosetti et al. (2008) reviewed cohort studies of industry workers and health professional
14 using fixed-effect and random-effect models depending on the degree of heterogeneity among
15 the individual cohorts. They did not indentify sufficient heterogeneity among the studies and
16 used the fixed effect models to estimate the relative risk of lymphatic and hematopoietic cancers
17 among four studies of industrial workers exposed to formaldehyde with RR = 0.85 (95% CI:
18 0.74-0.96) and among eight studies of health professionals with RR = 1.31 (95% CI: 1.16-1.47).
19 Likewise for leukemia, they used the fixed effect model to the estimate the RR among four
20 studies of industry workers of lymphatic and hematopoietic cancers among workers exposed to
21 formaldehyde with RR = 0.90 (95% CI: 0.75-1.07) and among eight studies of health
22 professional with RR = 1.39 (95% CI: 1.15-1.68).

23 Zhang et al. (2009) reviewed many of the same studies of lymphohematopoietic cancers
24 and all leukemia that were included in the Bosetti et al. (2008) meta-analysis using a different
25 methodology that focused on the highest exposure groups in each study in order to increase the
26 statistical power of the meta-analysis by minimizing bias from type II error. This method of
27 focusing on the highest exposure groups also reduces the likelihood that any individual study
28 results were confounded or otherwise biased (Greenland, 1998). The authors noted that in the
29 presence of a true causal association, “combining workers with very low exposures with workers
30 with high exposures into one overall exposed group can dilute relative risk estimates towards the
31 null.” The authors also preferentially selected the results for myeloid leukemia over all leukemia
32 when these data were reported. This methodology is appropriate if the underlying hypothesis is
33 that formaldehyde causes myeloid leukemia which has the ancillary effect of increasing the risk
34 of all leukemias. By increasing the specificity of the endpoint definition, this meta-analytic
35 method does not create any bias but rather increases the statistical power of the study to detect an

1 association with formaldehyde that is driven by myeloid leukemia. If the any association
2 between formaldehyde exposure and all leukemia were driven by nonmyeloid leukemias, then
3 this method would have less statistical power.

4 Zhang et al. (2009) also used fixed-effects and random-effect models depending on the
5 degree of heterogeneity among the individual cohorts. Zhang et al. (2009) found the
6 heterogeneity among the studies sufficiently high to warrant use of the random-effects model and
7 reported a summary RR = 1.25 (95% CI: 1.09-1.43) for the 19 studies with data on all types of
8 lymphohematopoietic cancers combined. For the 15 studies reporting data on all leukemias, the
9 summary RR = 1.54 (95% CI: 1.18-2.00). For the six studies of myeloid leukemia, the summary
10 RR = 1.90 (95% CI: 1.31-2.76). The authors concluded that the primary reason that their meta-
11 analytic results differed from those previously reported, was attributable to their differential use
12 of the results from the studies by Hauptmann et al. (2004, Stroup et al. (1986) and Pinkerton et
13 al. (2004) for which they used either the relative risks for myeloid leukemia or the highest
14 exposure category in each study.

15 A sensitivity analysis showed that had Zhang et al. (2009) replaced the results of those
16 three studies with the results used in Bosetti et al (2008) meta-analyses, the summary relative
17 risk for leukemia dropped from 1.54 (95% CI: 1.18-2.00) to RR = 1.10 (95% CI: 0.93-1.31).
18 The authors state that the primary reason for the different results is the use of myeloid-specific
19 data from Hauptmann et al. (2003), Stroup et al. (1986) and Pinkerton et al. (2004). Bosetti et al.
20 (2008) use the SMR results from Hauptmann et al. (2003) while Zhang et al. (2009) use the
21 highest peak exposure results for myeloid leukemia. The use of the result from the internal
22 comparison of Hauptmann et al. (2003) appears to be more appropriate. However, those data
23 from Hauptmann et al. (2003) were missing 1,006 deaths and were reanalyzed by Beane
24 Freeman et al. (2009) and were judged to be similar. The correct RR for the highest exposure
25 level of peak exposure should have been 2.79 rather than the value of 3.46 used by Zhang et al.
26 (2009). Likewise, the value of the SMR used by Bosetti et al. (2008) was 0.93 rather than
27 SMR = 0.85 which they used. In a similar fashion, the Bosetti et al. (2008) meta-analysis used
28 the overall SMR of 1.19 from Stroup et al. (1986) while Zhang et al. (2009) used the myeloid
29 leukemia SMR for longest duration which was 2.19. Zhang et al. (2009) also used the SMR
30 from Coggon et al. (2003) for the highest exposure group which was 0.71 while Bosetti et al.
31 (2008) used the overall SMR of 0.91. The Zhang et al. (2009) also excluded five studies that
32 were included by Collins and Lineker (2004). Had the Zhang et al. (2009) analysis included
33 these five studies, their summary relative risk would have been RR = 1.38 (95% CI: 1.15-1.65).

34 Zhang et al. (2009) also report results of the meta-analysis of formaldehyde and other
35 lymphohematopoietic cancers. The RR for Hodgkin lymphoma was 1.23 (95% CI: 0.67-2.29)

1 and was based on 8 studies. The RR for non-Hodgkin lymphoma was 1.08 (95% CI: 0.86-1.35)
2 and was based on 11 studies while the RR for multiple myeloma was 1.31 (95% CI: 1.02-1.67).

3 The criteria for study inclusion and exclusion applied by Zhang et al. (2009) appear to be
4 appropriate and the methodology for using myeloid-specific results where possible also appears
5 to be appropriate. This study found statistically significant increases in risk of all
6 lymphohematopoietic cancers with RR = 1.25(95% CI: 1.12-1.39), all leukemia with RR = 1.54
7 (95% CI: 1.24-1.91), myeloid leukemia with RR = 1.90 (95% CI: 1.41-2.55) and multiple
8 myeloma with RR = 1.31 (95% CI: 1.02-1.67).

9 Of the three meta-analyses, the study by Zhang et al. (2009) appears to be the most
10 rigorous methodologically. One difference between them is that while Zhang et al. (2009)
11 combined across industry, Collins and Lineker (2003) and Bosetti et al. (2008) stratified by
12 industry which may have reduced the statistical power of those analyses. Nonetheless, both
13 Collins and Lineker (2003) and Bosetti et al. (2008) both reported statistically significant
14 increases in risk of leukemia mortality among the nonindustrial workers. And Bosetti et al.
15 (2008) also reported statistically significant increases in risk of all lymphohematopoietic cancers
16 mortality among the nonindustrial workers.

17 18 **4.1.2.2.1.4. Summary of lymphohematopoietic cancers.**

19 ***All LHP Malignancies***

20 The majority of studies of all LHP malignancies (as a group) have been based on
21 comparison of the risk of cancer incidence or mortality in the studied population compared to the
22 risk in a general population. These external analyses rely on the assumption that cancer
23 incidence or mortality rates are expected to be similar between the general population and the
24 study population in the absence of exposure. However, the ‘healthy worker effect’ is well
25 known to bias rates in workers downwards compared to general populations, and there may be
26 differences in the magnitude of this selection bias by industry or profession.

27 Positive associations between formaldehyde exposure and LHP cancers have been
28 reported based on external comparison groups for chemical workers (Wong, 1983; Bertazzi et
29 al., 1986), embalmers (Walrath and Fraumeni, 1983, 1984; Hayes et al., 1990), anatomists and
30 pathologists (Harrington and Shannon, 1975; Hall et al., 1991; Levine et al., 1984; Stroup et al.,
31 1986; Matanoski et al., 1989). However, associations were not reported in external comparison
32 group analyses for garment workers, iron-foundry workers, and a large US industrial cohort
33 (Pinkerton et al., 2004; Andjelkovich et al., 1995; Beane Freeman et al., 2009; Marsh et al.,
34 1996), although associations were observed in some of these studies when exposure-response
35 relationships were considered.

1 Several published meta-analyses are available which more formally assess the strength of
2 association between formaldehyde exposure and mortality from all LHP cancers. Bosetti et al.
3 (2008) reviewed cohort studies of industry workers and health professionals (i.e., embalmers,
4 anatomists, and pathologists). They used fixed effect models to estimate the relative risk of
5 lymphatic and hematopoietic cancers among four studies of industrial workers exposed to
6 formaldehyde with RR = 0.85 (95% CI: 0.74-0.96) and among eight studies of health
7 professionals with RR = 1.31 (95% CI: 1.16-1.47). A subsequent meta-analysis by Zhang et al.
8 (2009) reports a summary relative risk (RR) of 1.25 (95% CI 1.09-1.43) for both professional
9 and industry workers for all LHP cancers (ICD 9 codes 200-209). These researchers identified
10 19 cohort study analyses, including cohort study updates. Zhang et al. (2009) used the reported
11 RR from the highest exposure category to increase statistical power and reduce uncertainty
12 regarding confounding or other bias. These two summary evaluations of the weight of evidence
13 of a causal association between exposure to formaldehyde and increased risk of mortality from
14 all LHP malignancies both showed statistically significant association. Therefore it is judged
15 that the collective evidence shows a causal association for all LHP as a group.

16 While most of the studies reporting SMRs in the meta-analyses were limited in their
17 exposure assessment to work in an industry known or thought to be exposed to formaldehyde,
18 some of the studies did conduct detailed exposure assessment and were able to compute internal
19 comparison of risk within the group of studied workers. These internal analyses are less likely to
20 be biased by the selection pressures for hiring and retaining healthy workers and represent
21 stronger methodologies.

22 The two individual studies with the relatively strongest exposure assessment reported
23 results that are consistent with the results of the meta-analyses (Hauptmann et al., 2009; Beane
24 Freeman et al. 2009). Hauptmann et al. (2009) conducted extensive interviews with the next of
25 kin and coworkers of the cases and controls provided detailed information on the funeral homes
26 and work practices of the study subjects. The authors noted that since the funeral industry is
27 often a family business, these study subjects' next of kin were believed to be unusually
28 knowledgeable of funeral home work practices. The work history component of these interviews
29 provided data on the frequency and duration of embalmings for jobs held at least five years, as
30 well as information on ventilation of the premises and the frequency of spills. These data were
31 linked to data from an exposure-reconstruction experiment which sought to replicate standard
32 funeral home practices while measuring exposures to formaldehyde.

33 While the results of Hauptmann et al. (2009) are not conclusive of an association on a
34 stand-alone basis, they do show consistently elevated odds ratios for all LHP associated with
35 ever embalming, duration of working in embalming jobs, number of embalmings and cumulative

1 formaldehyde exposure. Of the multiple OR results presented for all LHP, 17 of 19 were
2 elevated but only two were statistically significantly elevated. For the middle category of
3 duration, the OR = 1.5 (95% CI: 0.8-2.8) and for the highest category of duration, the OR = 1.8
4 (95% CI: 1.0-3.4) with a borderline significant test for trend ($p = 0.058$). For the middle
5 category of number of embalming, the OR = 1.9 (95% CI: 1.0-3.6). Based on a binomial
6 distribution assuming that half the ORs would be elevated and half below unity, the probability
7 of 17 OR being elevated out of 19 is $p = 0.0003$. While only two of the OR results were
8 statistically significant elevated, the overall findings were consistent with the results of the meta-
9 analyses. Hauptmann et al. (2009) show elevated risks of all LHP of a somewhat higher
10 magnitude than the summary effects in the meta-analyses that are, however, less statistically
11 precise as they are based on a single study rather than on multiple studies.

12 The individual study with the most detailed and objectively ascertained exposure
13 assessment was that of Beane Freeman et al. (2009). Exposure to formaldehyde was estimated
14 for each individual job category from work histories, with calendar-time and plant-specific
15 estimates based on assessments of job titles and tasks associated with those jobs using plant
16 visits by industrial hygienists and monitoring data (Blair et al. 1986; Stewart et al. 1986; Blair
17 and Stewart 1990). Exposures were categorized by peak exposure, average intensity of exposure
18 and cumulative exposure. The median follow-up time for workers was 42 years, representing
19 998,106 person-years of follow-up among 25,619 workers (Beane Freeman et al., 2009).

20 Internal analyses of exposed workers indicated that peak exposures in the highest
21 exposure category were associated with a significant increase in all lymphohematopoietic deaths
22 comparing death rates among workers with peaks of ≥ 4 ppm to those with > 0 to 2.0 ppm
23 (RR = 1.37, 95% CI: 1.03-1.81). Across the three categories of peak exposure (i.e., exposure
24 >0 ppm), there was also a statistically significant exposure-response trend ($p = 0.02$). The
25 exposure-response trend including the never exposed workers was also statistically significant
26 ($p = 0.04$). However, no association was observed for all lymphohematopoietic cancers for
27 average intensity or cumulative exposure.

28 The results of Beane Freeman et al. (2009) confirm those of the meta-analyses showing
29 statistically significant increased risk at the highest category of peak exposure that was
30 associated with all LHP mortality. These results do not appear to be confounded by any known
31 coexposures as the investigators reported that controlling for duration of exposure to 11
32 potentially confounding coexposures did not meaningfully change results. As these results are
33 based on a large and well-followed occupational cohort, these results are unlikely to be
34 influenced by selection bias. The estimated magnitude of the identified association (OR = 1.37)

1 was approximately equivalent to the summary RR of Zhang et al. (2009) which was 1.25 and the
2 summary RR = 1.31 for health professionals analyzed by Bosetti et al. (2008).

3 Given the consistency and strength of the positive associations for all LHP cancer
4 mortality in professional cohorts (embalmers, anatomists and pathologists) taken together with
5 the strong positive results of the NCI cohort, human epidemiologic evidence are sufficient to
6 conclude that there is a causal association between formaldehyde exposure and mortality from all
7 LHP malignancies (as a group).

9 ***All Leukemia***

10 Like the studies of all LHP (as a group), the majority of studies of all leukemia
11 malignancies (as a group) have been based on comparison of the risk of cancer incidence or
12 mortality in the studies population compared to the risk in a general population and may be
13 influenced by the healthy worker effect which effectively depresses effect estimates. In spite of
14 this potential bias which may mask a true effect of exposure, an association between
15 formaldehyde exposure and leukemia mortality is supported by cohort analyses of embalmers,
16 pathologists and anatomists (Hayes et al., 1990; Walrath and Fraumeni, 1983; Walrath and
17 Fraumeni 1984; Hall et al., 1991; Levine et al., 1984; Stroup et al., 1986; Matanoski et al., 1989).
18 Formaldehyde exposure and formaldehyde-related occupation have also been shown to be
19 associated with leukemia diagnosis in a case-control study (RR = 5.79 (95% CI 1.44-23.25), but
20 not formaldehyde exposure alone (RR = 0.96; 95% CI 0.54-1.71) (Stellman et al., 1998).

21 However, SMR analyses of the large industrial cohorts do not indicate a similar
22 association (Coggon et al., 2000; Beane Freeman et al., 2009, Pinkerton et al, 2004). Although
23 the SMR analysis provided for the NCI cohort does not indicate a positive association for all
24 leukemia using an external reference group (Beane Freeman et al., 2009), the SMR for exposed
25 versus unexposed workers within the cohort suggests all leukemia is elevated 2.13-fold with this
26 type of internal comparison (95% CI 0.99-4.56).

27 Several meta-analyses have been conducted for formaldehyde exposure and all leukemia
28 (as a group) which indicate a positive association. Collins and Lineker (2004) reported an
29 overall RR for 18 available studies of 1.1 (95% CI: 1.0-1.2), suggesting an association of
30 leukemia with formaldehyde exposure. This association was stronger for both
31 pathologists/anatomists (1.4; 95% CI: 1.0-1.9) and embalmers (RR = 1.6; 95% CI: 1.2-2.0) than
32 for industrial workers (RR = 0.9; 95% CI: 0.8-1.0). Study design also impacted the apparent
33 strength of association, with stronger associations seen in case-control studies (RR = 2.4; 95%
34 CI: 0.9-6.5) versus cohort studies (RR = 1.0; 95% CI: 0.9-1.2). Bosetti et al. (2008) reported an
35 association between formaldehyde exposure and leukemia mortality with a pooled RR of 1.39

1 (95% CI 1.15-1.68) for 8 groups of professional workers. In the same analysis, the pooled RR
2 for the 4 industrial cohorts was 0.90 (95% CI: 0.75-1.07). Zhang et al. (2009) reported a pooled
3 RR of 1.54 (95% CI: 1.18-2.00) for all cohorts identified in their meta-analysis, although this
4 pooled RR should be considered with the knowledge that when the source studies specifically
5 identified results for myeloid leukemia those results and not the all leukemia results were
6 included (Zhang et al., 2009). As discussed earlier, this methodology is appropriate if the
7 underlying hypothesis is that formaldehyde causes myeloid leukemia which has the ancillary
8 effect of increasing the risk of all leukemias. By increasing the specificity of the endpoint
9 definition, this meta-analytic method does not create any bias but rather increases the statistical
10 power of the study to detect an association with formaldehyde that is driven by myeloid
11 leukemia. If the association between formaldehyde exposure and all leukemia as a group were
12 driven by nonmyeloid leukemias, then this method would have less statistical power.

13 Of two individual studies with the relatively strongest exposure assessment (Hauptmann
14 et al., 2009; Beane Freeman et al. 2009), Hauptmann et al. (2009) did not report specifically on
15 leukemia (ICD-8: 205-207) but rather on lymphohematopoietic cancers of nonlymphoid origin
16 (ICD-8: 205, 206, 208 or 209) and on myeloid leukemia specifically (ICD-8: 205). Statistically
17 significant increased risk were reported for the larger grouping for ‘ever embalming’ with
18 RR = 3.0 (95% CI: 1.0-9.5), for the highest category of duration with RR = 3.7 (95% CI:
19 1.1–12.2), for the highest category of number of embalming with RR = 3.9 (95% CI: 1.2-12.8)
20 and for the highest category of cumulative exposure with RR = 4.0 (95% CI: 1.2-13.2).
21 However, none of the associated linear tests for trend were significant. Statistically significant
22 increased risks were also reported for the highest category TWA formaldehyde intensity with
23 RR = 3.4 (95% CI: 1.0-11.8) and the highest category of peak formaldehyde exposure with
24 RR = 3.8 (1.1-12.7), both without showing a linear trend. These results were considered to be
25 consistent with the meta-analytic findings of an association between formaldehyde exposure and
26 all leukemia as a group.

27 While not the same as all leukemia as a group, Hauptmann et al. (2009) did look
28 specifically at myeloid leukemia and reported extremely strong associations of each exposure
29 metrics with the majority of RRs greater than 10 and statistically significant as detailed in the
30 following subsection devoted to myeloid leukemia specifically. These myeloid leukemia results
31 are strongly supportive of a causal association with formaldehyde and therefore are, in turn,
32 supportive of a causal relationship with all leukemia as a group. The study by Beane Freeman et
33 al. (2009) did look specifically at all leukemia as a group (ICD-8: 204-207) and reported an
34 elevated RR = 1.42 (95% CI:0.92-2.18) for the highest category of peak exposure that was not
35 statistically significant. Two trend tests were reported for peak exposures with the trend within

1 only the exposure groups having a $p = 0.12$ and the trend across all exposure groups including
2 the unexposed having a significant trend ($p = 0.02$). Results from the analyses of average
3 formaldehyde intensity did not show an association while results from the analyses of cumulative
4 exposure did show some indication of a trend both within the exposed workers ($p = 0.12$) and
5 across all the workers ($p = 0.08$).

6 While the epidemiologic evidence for a causal association between formaldehyde and all
7 leukemia as a group is not as strong as for all LHP as a group, the repeated identification of an
8 association in multiple meta-analyses taken together with the clear causal association between
9 myeloid leukemia demonstrated by Hauptmann et al. (2009) and the consistent evidence reported
10 by Beane Freeman et al. (2009) are sufficient to conclude that there is a causal association
11 between formaldehyde exposure and mortality from all leukemia as a group.

12 13 ***Myeloid Leukemia***

14 The associations between myeloid leukemia and formaldehyde exposure are strong and
15 consistent. Of the six studies which formally assess myeloid leukemia mortality, five are
16 positive, including cohorts of both professional and industrial workers (Hauptmann et al., 2009;
17 Pinkerton et al., 2003; Hayes et al., 1990; Stroup et al., 1986; Walrath and Fraumeni, 1984,
18 Walrath and Fraumeni, 1983; but not Beane Freeman et al., 2009).

19 Walrath and Fraumeni (1983) studied embalmers in NY and reported 6 deaths from
20 myeloid leukemia compared to 4.1 expected based on the US male population stratified by age
21 and calendar time which is equivalent to a SMR = 1.46 (exact 95% CI: 0.54-3.19). Walrath and
22 Fraumeni (1984) studied embalmers in California and reported 6 deaths from myeloid leukemia
23 compared to 4.0 expected based on the U.S. male population stratified by age and calendar time
24 which is equivalent to a SMR = 1.50 (exact 95% CI: 0.55-3.26). While these studies were called
25 proportionate mortality studies by the authors, the reported PMRs are more actually SMRs and
26 should be interpreted as such.

27 Stroup et al. (1986) conducted an historic cohort mortality study of men who were
28 members of the American Association of Anatomists between 1888 and 1969. Only 738 deaths
29 were observed versus 1,133.9 expected, based on U.S. death rates (SMR 0.65), possibly
30 indicating a sizable HWE. However, a slight increase in the risk of lymphatic and hematopoietic
31 cancers (SMR 1.2; 18 observed) and the risk of leukemia (SMR 1.5; 10 observed) was evident.
32 When the leukemia analysis was restricted to the myeloid type, the SMR increased to 8.8, based
33 on five deaths ($p < 0.05$).

34 Hayes et al. (1990) conducted a cohort study of deceased U.S. male embalmers and
35 funeral directors who had died between 1975 and 1985, using records from local licensing

1 boards, state funeral directors' associations in 32 states and the District of Columbia, the
2 National Funeral Directors' Association, and state offices of vital statistics ($n = 894$). Expected
3 deaths by cause were derived from 5-year age- and calendar-year-specific proportions of deaths
4 among appropriate race groups from the U.S. population. No measured exposure data were
5 available. Statistically significant excesses in hematopoietic and lymphatic cancers were found
6 in embalmers and funeral directors. The PMR (actually SMR) was 1.39 (95% CI: 1.15–1.63).
7 The excess risk in all males was higher for myeloid leukemia (ML) (PMR (actually SMR) 1.57
8 [95% CI: 1.01–2.34]; 24 observed).

9 The Pinkerton et al. (2004) occupational cohort study of garment workers also reported
10 SMRs derived from age-, race-, and calendar-time-adjusted U.S. mortality rates. The overall
11 SMR for leukemia was 1.09 (24 deaths) and 1.44 (95% CI: 0.80-2.37; 15 deaths) for ML. After
12 10 years of exposure, the risk for ML was 2.19 (exact 95% CI: 0.95-4.32). Exposure prior to
13 1963 was associated with a risk of 1.61 (exact 95% CI: 0.80-2.88). Among garment workers
14 followed for 20 or more years from initial exposure, the SMR was significantly elevated for ML
15 (1.91 [exact 95% CI: 1.02-3.26]; 13 deaths), as was the SMR for multiple cause leukemia (1.92
16 [95% CI: 1.08–3.17]; 15 deaths) in the subgroup with 10 or more years of exposure to
17 formaldehyde and who were followed for 20 or more years after first exposure. The multiple
18 cause mortality for ML for this subgroup of workers was also significant (SMR 2.55 [95% CI:
19 1.10–5.03]; 8 deaths).

20 The meta-analysis by Zhang et al. (2009) evaluated the studies of formaldehyde exposure
21 and myeloid leukemia available at the time including Hauptmann et al. (2003), Pinkerton et al.,
22 2003; Hayes et al., 1990; Stroup et al., 1986; Walrath and Fraumeni, 1984, Walrath and
23 Fraumeni, 1983. While the findings of Hauptmann et al. (2003) on the NCI cohort have been
24 recently updated by those of Beane Freeman et al. (2009) who updated the cohort, the Zhang et
25 al. (2009) analysis provide the only formal meta-analysis specific to myeloid leukemia. Zhang et
26 al., 2009 reported a statistically significant summary RR of 1.90 (95% CI: 1.31-2.76).

27 As previously mentioned, the two individual studies with the relatively strongest
28 exposure assessment reported results that are also consistent with the results of the meta-analyses
29 (Hauptmann et al., 2009; Beane Freeman et al. 2009). Because the results of Beane Freeman et
30 al. (2009) are based on an update of the Hauptmann et al. (2003) study which was included in the
31 meta-analysis by Zhang et al. (2009) it is not surprising that they are consistent with those of the
32 meta-analyses showing statistically significant increased risk at the highest category of peak
33 exposure that was associated with all LHP mortality. However, Beane Freeman et al. (2009) did
34 not report statistically significant associations between formaldehyde exposure and mortality
35 from myeloid leukemia. At the highest exposure category of peak exposure (peaks ≥ 4 ppm vs.

1 >0 to 2.0 ppm), the RR = 1.42 (95% CI: 0.92-2.18) for myeloid leukemia. Two test for trend
2 were report for myeloid leukemia which provide some support for a concentration-response
3 relationship with peak exposure but neither was statistically significant ($p = 0.13$ and $p = 0.07$).

4 Hauptmann et al. (2009) reported increases in risk for ever embalming with myeloid
5 leukemia (OR = 11.2, 95% CI: 1.3-95.6). Duration of employment in jobs with embalming
6 demonstrated an exposure-response relationship with increased risk of myeloid leukemia
7 ($p = 0.02$). The number of embalming was also significantly associated with increased risk of
8 myeloid leukemia in the middle (OR = 12.7, 95% CI: 1.4-116.7) and highest exposure levels
9 (OR = 12.7, 95% CI: 1.6-119.7). Cumulative exposure was associated with increased risk of
10 myeloid leukemia, with the highest category of exposure showing OR = 13.2 (95% CI:
11 1.5–115.4).

12 Hauptmann et al. (2009) showed that all three categories of average formaldehyde
13 intensity while embalming were very strongly and significantly associated with the risk of
14 myeloid leukemia mortality (OR = 11.1, 14.8, and 9.5), and the test for an exposure-response
15 trend was of borderline statistical significance ($p = 0.058$). The risk of myeloid leukemia
16 mortality was also very strongly and significantly associated with 8-hour TWA exposure for
17 mid-level exposures (OR = 13.6, 95% CI: 1.5-125.8) and high-level exposures (OR = 12.0, 95%
18 CI: 1.3-107.4), as well as for peak exposure in two of three categories (0-7 ppm OR = 15.2, 95%
19 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-
20 116.9). For peak exposures, there was also a statistically significant exposure-response tend
21 ($p = 0.036$).

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

34 Given the consistency of the positive associations for formaldehyde with myeloid
35 leukemia cancer mortality across five of the six studies (Hauptmann et al., 2009; 2009; Pinkerton

1 at al., 2003; Hayes et al., 1990; Stroup et al., 1986; Walrath and Fraumeni, 1984, Walrath and
2 Fraumeni, 1983; but not Beane Freeman et al., 2009), the statistically significant meta analysis
3 by Zhang et al. (2009) and the convincing results from Hauptmann et al. (2009), the human
4 epidemiologic evidence is sufficient to conclude that there is a causal association between
5 formaldehyde exposure and mortality from myeloid leukemia.

6 7 ***Hodgkin Lymphoma***

8 The only meta-analysis to specifically address Hodgkin lymphoma was conducted by
9 Zhang et al. (2009) and included eight studies (Anjelkovich et al., 1995; Coggon et al., 2003;
10 Harrington and Shannon, 1975; Hauptmann et al., 2003; Hayes et al., 1990; Pinkerton et al.,
11 2004; Walrath and Fraumeni, 1983, 1984; and Wong, 1983). Zhang et al. (2009) reported a
12 summary RR = 1.23 (95% CI 0.67-2.29). This elevated, but nonstatistically significant finding is
13 consistent with the large variance on reported results among the individual studies as well as the
14 wide confidence intervals of the results which were based on small numbers of cases—even from
15 the large cohort studies. Six of the eight studies observed three or fewer deaths from Hodgkin
16 lymphoma. Coggon et al. (2003) reported 6 deaths from Hodgkin lymphoma against 8.5
17 expected for an SMT = 0.70 (95% CI: 0.26-1.53) and Hauptmann et al. (2003) reported
18 21 observed deaths with 20 deaths among the exposed workers who has an SMR = 1.26 (95%
19 CI: 0.81-1.95). However, the Beane Freeman et al. (2009) update of the Hauptmann et al. (2003)
20 study had the largest number of observed cases ($n = 27$) and was not included in the Zhang et al.
21 (2009) meta-analysis. In fact, the Beane Freeman et al. (2009) study describes more deaths from
22 Hodgkin lymphoma than all the other studies in Zhang et al. (2009) combined. Excluding the
23 Hauptmann et al. (2003) results from the list of studies in the meta-analysis leaves 19 cases.

24 There is evidence for an exposure-response relationship for Hodgkin lymphoma in the
25 NCI industrial cohort among exposed workers (Beane Freeman et al., 2009). Clear exposure
26 response relationships for Hodgkin lymphoma are defined with all three metrics of exposure,
27 peak average intensity and cumulative exposure ($p = 0.01$, $p = 0.05$ and $p = 0.08$ respectively for
28 mortality through 2004). These associations have been evident from first follow-up through the
29 current publication, and statistically significant for the majority of the follow-up period
30 demonstrating that this is a strong and consistent finding in the NCI cohort (see Figures 4-3 and
31 4-4) (Beane Freeman et al., 2009).

32 As the majority of the studies reported specific data on Hodgkin lymphoma report on just
33 three or fewer cases, the best epidemiologic evidence is obtained from the most recent evaluation
34 of the NCI cohort by Beane Freeman et al. (2009). This cohort study, on its own, reported on
35 more deaths from Hodgkin lymphoma than the remainder of the epidemiologic literature.

1 Hodgkin lymphoma was both shown to be at increased risk associated with peak exposure
2 concentrations. Peak exposures in the highest exposure category were associated with a
3 significant increase in Hodgkin lymphoma deaths comparing death rates among workers with
4 peaks of ≥ 4 ppm to those with > 0 to 2.0 ppm (RR = 3.96, 95% CI: 1.31-12.02). Across the
5 three categories of peak exposure, there was a statistically significant exposure-response trend
6 ($p = 0.01$). The exposure-response trend including the never-exposed workers was also
7 statistically significant ($p = 0.004$). The RR was also elevated for average intensity of
8 formaldehyde exposure with RR = 2.48 (95% CI: 0.84-7.32) and there were significant tests for
9 trend among only the exposed workers ($p = 0.05$) and all workers ($p = 0.03$). Similarly, there
10 were nearly significant tests for trend with cumulative exposure among only the exposed workers
11 ($p = 0.08$) and all workers ($p = 0.06$).

12 The majority of the studies reporting on Hodgkin lymphoma did not have sufficient
13 statistical power describe any potential association with formaldehyde as the numbers of
14 observed and expect cases were small and the resulting effects estimates were imprecise. As the
15 Beane Freeman et al. (2009) study reported on the largest number of cases and was the
16 individual study with the most detailed and objectively ascertained exposure assessment and
17 demonstrated significant exposure-response gradients, it is judged that this epidemiologic
18 evidence is supportive of a causal association between formaldehyde and Hodgkin lymphoma.

19 20 *Non-Hodgkin Lymphoma*

21 The only meta-analysis to specifically address non Hodgkin lymphoma was conducted by
22 Zhang et al. (2009) and included eleven studies. Zhang et al. (2009) reported a summary
23 RR = 1.08 (95% CI 0.86-1.35). Hauptmann et al. (2009) did not specifically report on
24 non-Hodgkin lymphoma. Beane Freeman et al. (2009) did report on 106 deaths from
25 non-Hodgkin lymphoma but did not identify any significant association in their categorical
26 analyses or in their tests for trend for either peak exposure, average intensity of exposure or for
27 cumulative exposure.

28 Wang et al. (2009) assessed the effect of formaldehyde exposure on the risk of
29 non-Hodgkin lymphoma in a population-based case-control study. Semiquantitative exposure
30 metrics included average exposure intensity and average exposure probability which were
31 evaluated individually and together. Analyses use unconditional logistic regression and
32 controlled for age, family history of hematopoietic cancers, alcohol consumption, and race. For
33 the low average exposure intensity category the OR = 1.4 (95% CI: 1.0-1.8), while for the
34 Medium-High category the OR = 1.2 (95% CI: 0.8-1.7). For the Low average exposure
35 probability category the OR = 1.3 (95% CI: 1.0-1.7), while for the Medium-High category the

1 OR = 1.4 (95% CI: 0.9-2.3). The investigators also examined the risk of non-Hodgkin
2 lymphoma among major subtypes. The risk of follicular lymphoma and chronic lymphocytic
3 leukemia/Small lymphocytic lymphoma was slightly elevated but the risk of diffuse large B-cell
4 lymphoma was OR = 1.9 (95% CI: 1.3-2.6) for ever having been exposed to formaldehyde. For
5 Low average intensity exposure, the risk was OR = 2.1 (95% CI: 1.3-2.6) while for
6 Medium-High average intensity exposure, the risk was OR = 1.5 (95% CI: 0.9-2.4). Even so, an
7 exposure-response relationship was demonstrated using the continuous parameterization of
8 average intensity rather than the categorical ($p = 0.03$). Likewise, an exposure-response
9 relationship was demonstrated using the continuous parameterization of average probability of
10 exposure rather than the categorical ($p = 0.01$).

11 The findings of Wang et al. (2009) provide some support for an association between
12 formaldehyde exposure and non-Hodgkin lymphoma. It should be noted that a population-based
13 case-control study, where incidence rather than mortality defines the case—may be more
14 appropriate for cancers with relatively low mortality (i.e., CCL, large B-cell lymphoma, Small
15 lymphocytic lymphoma).

16 Aside from the semiquantitative study by Wang et al (2009), non-Hodgkin lymphoma
17 does not appear to be associated with formaldehyde exposure. There is not sufficient evidence of
18 a causal association between formaldehyde exposure and non-Hodgkin lymphoma.

19

20 ***Multiple Myeloma***

21 The only meta-analysis to specifically address Hodgkin lymphoma was conducted by
22 Zhang et al. (2009) and included nine studies (Boffetta et al., 1989; Coggon et al., 2003; Dell and
23 Teta, 1995; Edling et al. 1987; Hauptmann et al. 2003; Hayes et al. 1990; Heineman et al. 1982;
24 Pottern et al. 1992; Stellman et al. 1998). Zhang et al. (2009) reported a summary RR = 1.31
25 (95% CI 1.02-1.67). This statistically significant finding is consistent with the findings of Beane
26 Freeman et al. (2009) who reported that peak exposures in the highest exposure category were
27 associated with a significant increase in multiple myeloma deaths comparing death rates among
28 workers with peaks of ≥ 4 ppm to those with > 0 to 2.0 ppm (RR = 2.04, 95% CI: 1.01-4.12).
29 Across the three categories of peak exposure, there was also some evidence of an exposure-
30 response trend ($p = 0.08$); however, there was no evidence of an exposure-response trend
31 including the never-exposed workers. The association of multiple myeloma with formaldehyde
32 exposure was also shown throughout the cohort experience (see Figure 4-3 and 4-4) which adds
33 strength to this finding.

34 The epidemiologic evidence for a causal association between formaldehyde and all
35 multiple myeloma as described by the statistically significant increased risk identified in the

1 meta-analysis of Zhang et al. (2009) and the most recently updated analysis of the NCI cohort by
2 Beane Freeman et al. (2009) are considered to be supportive of a causal association between
3 formaldehyde exposure and mortality from multiple myeloma.

4 5 **4.1.2.2.2. *Brain and CNS cancer.***

6 Several studies of professional groups discussed earlier investigated brain and other CNS
7 cancers among those exposed to formaldehyde on the job. Several of these studies found that
8 exposure increased risk two to three times among exposed professionals (Hall et al., 1991;
9 Stroup et al., 1986; Walrath and Fraumeni, 1984), while others found modest or no increase in
10 risk (Hayes et al., 1990; Levine et al., 1984; Walrath and Fraumeni, 1983).

11 None of the industrial cohort worker mortality studies of exposure to formaldehyde found
12 a clear relationship between formaldehyde exposure and risk of brain or CNS cancer (Pinkerton
13 et al., 2004; Coggon et al., 2003; Andjelkovich et al., 1995; Gardner et al., 1993; Stayner et al.,
14 1988; Blair et al., 1987, 1986). To date, no case-control studies of brain and CNS cancer have
15 been completed. In the Hauptmann et al. (2004) study, the authors reported that no clear
16 association was seen for cancer of the brain and CNS and exposure to formaldehyde.

17 18 **4.1.2.2.3. *Pancreatic and other cancers.***

19 Two studies (Kernan et al., 1999; Dell and Teta, 1995) have found increases in the risk of
20 pancreatic cancer in association with possible exposure to formaldehyde. Collins et al. (2001a)
21 conducted a meta-analysis of fourteen studies (Kernan et al., 1999; Andjelkovich et al., 1995;
22 Hansen and Olsen, 1995; Gardner et al., 1993; Hall et al., 1991; Matanoski, 1991; Hayes et al.,
23 1990; Gerin et al., 1989; Stayner et al., 1988; Blair et al., 1986; Stroup et al., 1986; Levine et al.,
24 1984; Walrath and Fraumeni, 1984, 1983) and found a small increase in risk (RR = 1.1 [95% CI:
25 1.0–1.3]).

26 Other sites that have been examined are stomach cancer (Coggon et al., 2003)
27 (SMR = 1.47; $p < 0.05$), intraocular melanoma (Holly et al., 1996) (OR = 2.9 [95% CI:
28 1.2–7.0]), and thyroid cancer among women (Wong et al., 2006) (OR = 8.33 [95% CI:
29 1.16–60.0]; 2 cases). However, without further substantiation, it is difficult to infer causation
30 based on these isolated results alone.

31 32 **4.1.2.3. *Summary: Carcinogenic Hazard in Humans***

33 The weight of the epidemiologic evidence at this time supports a link between
34 formaldehyde exposure and NPC in humans. This conclusion is based on the longitudinal cohort
35 study of Hauptmann et al. (2004) as well as the case-control studies of NPC and formaldehyde

1 exposure completed by Vaughan et al. (2000), West et al. (1993), Vaughan et al. (1986b) and
2 several additional case-control studies described in the text. With the exception of Hauptmann et
3 al. (2004), most of the other cohort studies found little or no increased risk of NPC from
4 exposure to formaldehyde. However, Hauptmann et al. (2004) employed different exposure
5 metrics and based their analyses on conservative internal comparisons that limited the potential
6 for the HWE to obscure true effects. The case-control studies that provide additional evidence of
7 an association between NPC and formaldehyde have more power and generally rely on better
8 diagnoses of NPC. Better ascertainment of histologic types of tumors can sometimes also be
9 obtained if the cases are taken from cancer registries. The NPC risk is also supported by
10 experimental evidence in animals in which formaldehyde induces nasal cancers (see
11 Section 4.2.2). Since the physiology of the rat nasal passage is somewhat different from that of
12 humans, it is not possible to obtain a direct site-specific correspondence between the species.
13 However, in both species, the tumors are found within the same area of the URT where
14 maximum exposure can be expected to occur.

15 Several researchers have challenged the conclusion of a relationship between
16 formaldehyde and NPC. Those critical of the link argue that, given the wide variability in results
17 across studies and competing explanations, conclusions about any link from the existing studies
18 are premature. The difficulty in attaining consensus on whether formaldehyde influences the risk
19 of NPC in humans arises from several limitations inherent in epidemiologic methods and
20 exposure assessment, as well as from the characteristics of the disease. The most prominent of
21 these limitations are the rarity of the cancer and imprecise estimates of exposure. Because NPC
22 is a very rare cancer with an incidence of less than 1 per 100,000, it is difficult to obtain precise
23 estimates of risk from cohort studies. Although case-control studies are better suited for
24 studying rare conditions, they are limited in obtaining valid and precise exposure assessments. A
25 further problem with exposure assessment is isolating formaldehyde exposure from other
26 potential chemical or particulate exposures that may influence risk of NPC. Imprecise exposure
27 assessment and the inability to isolate formaldehyde exposure from other exposures are largely
28 the bases on which Marsh and coworkers have challenged the NCI cohort study (Marsh et al.,
29 2007a, b, 2002, 1996; Marsh and Youk, 2005). Marsh and coworkers (Marsh et al., 2007a) show
30 that subjectively assessed exposure to silversmithing is tentatively associated with NPC. Given
31 that there were no prior citations of an association between silversmithing exposures and NPC in
32 the medical literature and given the many post hoc reexaminations of alternative hypotheses to
33 explain the original NCI findings, it is more likely that silversmithing is an artifactual potential
34 confounder.

1 It may be expected that, without new approaches for obtaining more accurate and precise
2 estimates of exposure, further follow-up of current cohorts and future epidemiologic studies of
3 formaldehyde and NPC will face the same limitations and criticisms found with existing studies.
4 These limitations notwithstanding, the epidemiologic studies reviewed here represent what may
5 be currently discernable about a formaldehyde-NPC link in humans by using rigorous
6 observational methods. As such, concluding any influence of formaldehyde must be made on the
7 weight of all human and animal evidence in the face of known and expected limitations in study
8 design and exposure assessment.

9 The results of two well-designed cohort studies found a positive association between
10 formaldehyde-exposed professionals, such as pathologists, embalmers, and funeral directors, and
11 LHP cancer, particularly ML. The largest cohort study of formaldehyde has the most extensive
12 exposure assessment (Blair et al., 1986; Stewart et al., 1986), and the cohort was followed for a
13 median duration of 35 years (Hauptmann et al., 2003). By using cumulative exposure measures
14 not previously used and by using internal comparison groups, significant increases in the risk of
15 cancer of the LHP system, particularly ML, were reported. This study demonstrated that
16 formaldehyde was a risk factor for LHP cancers, independent of other risk factors, such as
17 benzene and smoking. Hauptmann et al. (2003) found statistically significant dose trends for
18 peak exposure and AIE. Pinkerton et al. (2004) also found a significant increase in the risk of
19 ML in garment workers 20 years after their initial exposure and in workers with 10 or more years
20 of exposure. Additionally, several studies of pathologists, embalmers, and other medical
21 workers reported greater numbers of observed deaths from leukemia than expected although
22 many studies of these groups suffer from a substantial HWE based on comparisons with external
23 death rates. Two of these studies, Hayes et al. (1990) and Stroup et al. (1986), also report a
24 significantly excess risk of ML in embalmers, funeral directors, and anatomists.

25 There is a range of biological plausibility for an agent whose primary action is at the
26 POE. Acute leukemias (ALL and AML), believed to arise from transformation of stem cells in
27 the bone marrow, are less plausible. In contrast chronic lymphatic leukemia, lymphomas,
28 multiple myelomas (from plasma B cells), and unspecified cancers may involve an etiology in
29 peripheral tissues to include cells, cell aggregates, germinal centers, and lymph nodes. An
30 association of these cancers to an exogenous agent acting at the POE is biologically plausible.

31 It is the conclusion of this assessment that the weight of the epidemiologic evidence at
32 this time supports a link between formaldehyde exposure and carcinogenicity in humans.

33

1 4.2. ANIMAL STUDIES

2 This section discusses the available laboratory animal data on the toxicity of inhalation,
3 oral, and dermal exposures to formaldehyde. An extensive database of laboratory animal studies
4 is available for formaldehyde, including numerous 2-year bioassays by both the inhalation and
5 oral exposure routes. Although a large portion of the literature reports studies focused on toxic
6 effects at the site of contact or portal of entry (POE), general systemic effects as well as
7 neurobehavioral effects, reproductive and developmental effects, immunologic changes, and
8 sensitization are represented in the literature as well. The organization and general content of the
9 chapter is discussed below.

10 The first subject addressed for the animal studies is the occurrence of reflex bradypnea
11 (RB) observed in rodents exposed to reactive gases, including formaldehyde (see Section 4.2.1).
12 RB is a reduction in ventilation rate, minute volume, and other physiological parameters
13 experienced by rodents exposed to an irritant/reactive gas. Although humans and nonhuman
14 primates do not exhibit the same change in respiratory rate, these studies are included in order to
15 better understand the effects on RB in interpreting rodent studies presented in the balance of the
16 chapter. Additionally, although binding to the trigeminal nerve and subsequent downstream
17 events do not result the acute signs of RB in humans, the mechanism itself may play a role in
18 understanding other adverse health effects observed in humans including more subtle changes in
19 pulmonary function, sensitization, and sensory irritation.

20 The available data for the inhalation and oral exposures confirm direct formaldehyde-
21 induced toxicity in tissues present at the POE. These observations are consistent with the
22 physicochemical characteristics, reactivity, and metabolic pathways of formaldehyde as
23 discussed in Chapter 3. Indications of cell damage, cell proliferation, and inflammatory
24 responses are similar for each route of exposure, therefore effects at the POE for inhalation and
25 oral exposures are described first (see Sections 4.2.2 and 4.2.3, respectively). Given the well-
26 established nature of these health effects and the wealth of literature for inhalation exposures,
27 complete study summaries for respiratory tract effects are provided. Studies are organized by
28 study duration—acute, subchronic and chronic—where some of the chronic bioassays were
29 designed to address carcinogenic potential.

30 Although a majority of the oral and inhalation studies focus on health effects at the
31 POE—respiratory tract and GI tract—the general systemic toxicity of formaldehyde is addressed
32 where it was integral to the study. Therefore, body weight and organ weight changes, gross
33 pathology, organ histopathology outside of the POE, blood and urine chemistry, and other
34 biochemical measures may be included in these study summaries. An overview of general
35 systemic findings is provided in Section 4.4 for all routes of exposure.

1 Studies addressing immune function, neurobehavioral effects, sensitization, and
2 reproductive and developmental effects are addressed across routes of exposure. The specialized
3 nature of these studies requires discrete treatment, and inclusion of data across routes of
4 exposure allows for a synthesis of the available information, to better understand the toxic
5 potential of formaldehyde exposure on these endpoints.

6 7 4.2.1. Reflex Bradypnea

8 Reflex bradypnea (RB), which is believed to be a protective response, is often observed
9 in rodents exposed to reactive gases. It is primarily characterized by marked decreases in
10 activity, respiratory rate, body temperature, and metabolic rate. RB is not seen in humans and
11 nonhuman primates. An understanding of the RB is important to the interpretation of many of
12 the animal bioassays examining formaldehyde-induced health effects. Of chief concern is that
13 the physiological effects of RB, described below, may interfere with appropriate interpretation of
14 adverse effects noted with formaldehyde exposure. It is important to distinguish between an
15 effect directly related to RB versus formaldehyde exposure. Additionally the effects of RB may
16 mask or alter formaldehyde-induced health effects. Secondly, differential respiratory effects of
17 RB due to species and strain will result in differential inhaled doses at the same exposure level.
18 This needs to be considered both when comparing the results of animal studies and in
19 extrapolation to humans. Finally, although humans do not experience RB, the mechanism of RB
20 as a reflex response to trigeminal nerve stimulation assists in understanding human health related
21 to localized and reflex responses due to trigeminal nerve stimulation.

22 Irritant gases have been shown to decrease body temperature, heart rate, and blood
23 pressure as well as alter blood chemistry in rodents (Pauluhn, 2003, 1996; Jaeger and Gearhart,
24 1982). Because of their small size, mice can rapidly lower their body temperatures and thus their
25 metabolic rate and ventilation rate. The hypothermia that results from RB can directly affect
26 nearly all biological processes (Gordon et al., 2008). Formaldehyde exposure can dramatically
27 lower ventilation rate and reduce body temperature in mice by as much as 4°C, and it has been
28 posited that decreased oxygen supply is likely to have profound effects on organisms with
29 substantial oxygen demands (Jaeger and Gearhart, 1982). The effects of RB are reversible,
30 though it may take several minutes to several hours to return to pre-exposure conditions
31 (Pauluhn, 1996; Jaeger and Gearhart, 1982).

32 The literature on sensory irritation is broad; many studies have investigated species
33 differences, dose response relationships, tolerance, and cross-tolerance to other sensory irritants
34 (see Tables 4-8 and 4-9). This discussion focuses on the changes in respiratory rate and minute
35 volume during formaldehyde exposure. Sensory irritation is often quantified as the statistically

1 derived exposure concentration that results in a 50% reduction in respiratory rate (RD₅₀) in
 2 rodents (ASTM, 2000; Kane et al., 1979). Kane and Alarie (1977) evaluated various aspects of
 3 sensory irritation, including establishing the RD₅₀, exploring the reproducibility of response,
 4 investigating the effect of tracheal cannulation, and determining the potential for tolerance with
 5 repeated exposure or pre-exposure in male Swiss-Webster mice, caused by formaldehyde and
 6 acrolein. The RD₅₀ was established by exposing four mice for 10 minutes at each concentration
 7 across a range representing approximately 10 to 80% reduction in respiration and calculated by
 8 using least squares regression. The RD₅₀ and its 95% CI for formaldehyde were calculated to be
 9 3.1 (2.1–4.7) ppm (3.8 [2.58–5.77] mg/m³). The tracheal cannulation experiments demonstrated
 10 that the effect on respiratory rate was caused by URT sensory irritation.

11
 12 **Table 4-8.** Respiratory effects of formaldehyde-induced reflex bradypnea in
 13 various strains of mice
 14

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)

15 ^aExposure concentration that results in a 50% reduction in respiratory rate.

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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 naïve 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 RD₅₀ 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 RD₅₀ values ranging from 10 ppm in male Wistar rats to 31.7 ppm in male F344 rats. No reported RD₅₀ 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 (CO₂) production (exhaled to air) as a reflection of total metabolism, and core body temperature in male Charles River CD rats and male C57BL6/F1

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1 mice. Animals (three/concentration) were exposed to 15 ppm (18.4 mg/m³) formaldehyde for up
2 to 2 hours. Mice exhibited a greater decrease in respiratory frequency and minute volume
3 compared with the rats. CO₂ production and body temperature were also affected to a greater
4 extent in the mice (see Table 4-8). The authors postulated that the decreased body temperature
5 in mice would likely lead to decreased biologic action of formaldehyde in the tissue.

6 7 **4.2.1.1. Tolerance**

8 Tolerance is defined as an increase in the concentration required to elicit the same degree
9 of RB response and was evaluated by Kane and Alarie (1977). In the first set of experiments,
10 mice (four/concentration) were exposed 3 hours/day for 4 days at the concentration associated
11 with either a 30 or 50% decrease in respiratory frequency (specific concentrations not given)
12 (Kane and Alarie, 1977). Naïve animals served as controls for each day. The maximum
13 response increased with each additional day of exposure, and the diminution of response that was
14 typically exhibited after 60 minutes of exposure in naïve animals was markedly delayed. In the
15 second set of experiments, mice were exposed to a formaldehyde concentration at one-tenth the
16 RD₅₀ (i.e., 0.3 ppm) 3 hours/day for 3 days. On the fourth day the animals underwent a similar
17 exposure protocol to identify the concentration that resulted in an RD₅₀, following the above
18 protocol. No change in the RD₅₀ was demonstrated. Both of these experiments indicate no
19 change in tolerance with either type of pretreatment in Swiss-Webster mice.

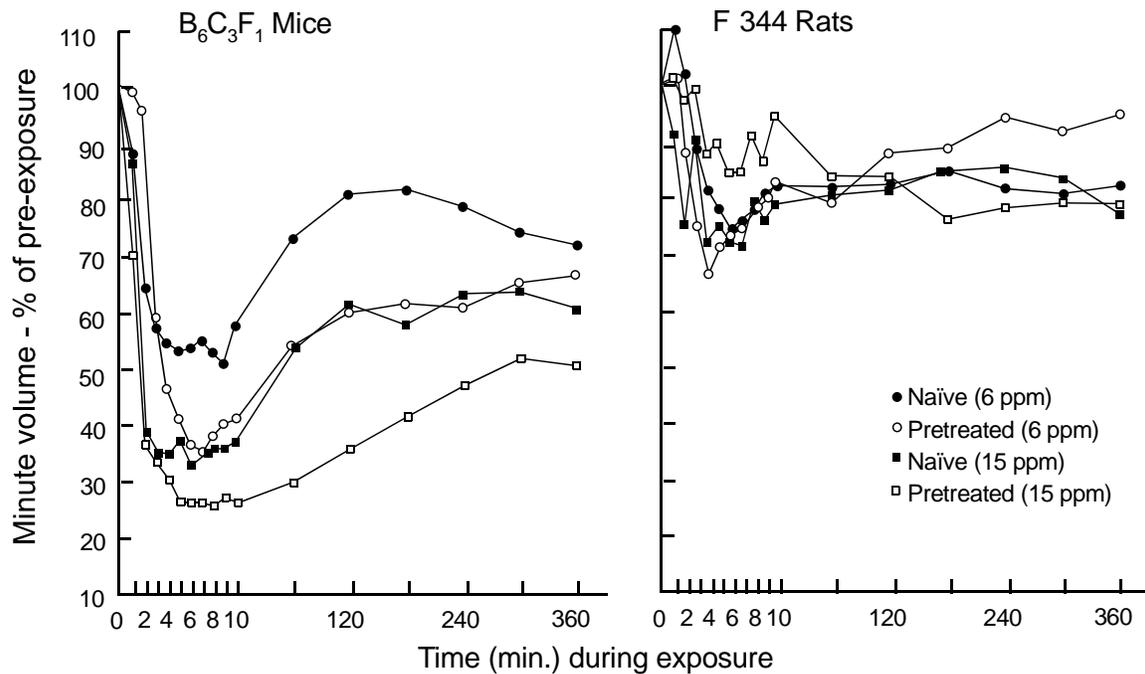
20 Chang and Barrow (1984) tested whether tolerance would develop in male F344
21 (CDF[F344]CrI/Br) rats exposed to formaldehyde. Exposure to formaldehyde at 15 ppm
22 (18.4 mg/m³) for 6 hours/day, 5 days/week failed to induce tolerance. However, tolerance was
23 observed following exposure to 28 ppm (34.4 mg/m³) formaldehyde for 4 days. The
24 concentration-response curve in these animals was significantly different than that of naïve
25 animals, with an increase in the RD₅₀ estimate for this exposure duration from 31.7 to 70.2 ppm.

26 27 **4.2.1.2. Cross-Species Differences in Inhaled Dose**

28 Formaldehyde-induced RB lowers both respiratory rate and tidal volume and thus
29 reduces the inhaled dose of formaldehyde at a given exposure concentration. Chang et al. (1983)
30 and Barrow et al. (1983) evaluated the species differences and the effective inhaled dose between
31 rats and mice, since mice seem to be more sensitive to formaldehyde-induced RB and do not
32 exhibit tolerance as shown in F344 rats. Groups (four/concentration) of male F344 rats and male
33 B6C3F1 mice were exposed to formaldehyde concentration ranges of 6.2–48 ppm
34 (7.6–59 mg/m³) or 0.78–14.0 ppm (0.96–17.2 mg/m³), respectively, for 10 minutes. Pretreated
35 animals used in the tolerance experiments were exposed to formaldehyde at 2, 6, or 15 ppm

1 (2.45, 7.36, or 18.4 mg/m³) 6 hours/day for 4 days prior to determination of the RD₅₀ and
2 concentration response across the same ranges.

3 A concentration-dependent decrease in respiratory rate was seen in both naïve and
4 pretreated rats during formaldehyde exposure. Tolerance (defined as a decrease in respiratory
5 rate followed by a subsequent return to control values) occurred after 4 minutes of exposure and
6 was more pronounced at concentrations above 4 ppm. Concentration-response relationships
7 were very similar for naïve and pretreated rats, and the RD₅₀s were similar for both groups
8 (naïve = 13.1 ppm [95% CI: 10.6–17.5]; pretreated = 10.8 ppm [95% CI: 7.6–16.9]). In contrast,
9 naïve or pretreated mice did not develop tolerance during exposures. An examination of
10 concentration-response relationships for mice showed similar RD₅₀ values (naïve = 4.4 ppm
11 [95% CI: 0.9–5.0] and pretreated = 4.3 ppm [95% CI: 3.4–5.5]) compared with rats, although the
12 slopes of the concentration-response regressions were statistically different (see Figure 4-5).
13



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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).

22 Exposure of naïve or pretreated rats resulted in an increased (compensatory) tidal
23 volume. However, the increase in tidal volume did not compensate entirely for the decrease in
24 ventilation rate and was only concentration dependent in pretreated rats. Comparison of tidal

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1 volume from naïve and pretreated mice exposed to formaldehyde showed a slight increase in
 2 naïve animals but a decrease in pretreated ones. The effect of formaldehyde exposure on tidal
 3 volume was concentration dependent in both groups of mice. These results indicate that tidal
 4 volume does not compensate entirely for the decrease in respiratory rate and that the
 5 compensation is slightly greater in rats than in mice.

6 These studies (Barrow et al., 1983; Chang et al., 1983) showed that B6C3F1 mice sustain
 7 RB, whereas F344 rats develop tolerance more readily both during exposure and with
 8 pretreatment. Thus, these results suggest that the rat may be the more sensitive species for the
 9 effects of inhaled formaldehyde due in part to the difference in sensitivity between mice and rats
 10 as evidenced by an RD₅₀ of 4.9 versus 31.7 ppm and the ability of rats to develop tolerance while
 11 mice appear to sustain RB. Barrow et al. (1983) used the results of these experiments to estimate
 12 an inhaled dose equivalent to the exposure concentration of 15 ppm for the strains of mice and
 13 rats used in the chronic formaldehyde bioassays by Kerns et al. (1983) and Monticello and
 14 Morgan (1994) described in Section 4.1.2 as follows:

15
 16 Inhaled dose ($\mu\text{g}/\text{min}\text{-cm}^2$) =

$$\frac{\text{HCHO concentration } (\mu\text{g}/\text{L}) \times \text{minute volume } (\text{L}/\text{min})}{\text{Nasal cavity surface area } (\text{cm}^2)} \quad (5-1)$$

17
 18
 19
 20
 21 As shown in Table 4-10, because mice were observed to be able to decrease their minute
 22 volume by approximately 75% as compared with 45% in rats, a twofold higher inhaled dose
 23 would be expected in rats versus mice. This difference may be relevant to the increased
 24 incidence of SCC in the nasal cavity seen in F344 rats when compared with B6C3F1 mice.

25
 26 **Table 4-10. Inhaled dose of formaldehyde to nasal mucosa of F344 rats and**
 27 **B6C3F1 mice exposed to 15 ppm**
 28

Parameter	F344 rats	B6C3F1 mice
HCHO concentration ($\mu\text{g}/\text{L}$)	18.4	18.4
Minute volume (L/min)	0.114	0.012
URT surface area (cm^2)	13.44	2.89
Inhaled dose ($\mu\text{g}/\text{min}/\text{cm}^2$)	0.156	0.076

29
 30 Source: Barrow et al. (1983).
 31
 32

1 **4.2.1.3. Cross-Tolerance**

2 Cross-tolerance of chemically-induced reflex responses has been examined in several
 3 systems in order to better understand the specificity and nature of the interaction of reactive
 4 chemicals (such as formaldehyde with chlorine) with the trigeminal nerve involved in the RB.
 5 Development of cross-tolerance to formaldehyde following preexposure to chlorine or to
 6 chlorine following preexposure to formaldehyde was shown to be a function of the duration of
 7 the pretreatment in male F344 rats (Chang and Barrow, 1984) (see Table 4-11). A 7-day
 8 recovery period resulted in only a slight loss of cross-tolerance from a 4-day pre-exposure to
 9 either chlorine or formaldehyde (data not shown). The cross-tolerance between formaldehyde
 10 and chlorine demonstrated in the Chang and Barrow (1984) study suggests that these chemicals
 11 may act via a common mechanism and may involve the trigeminal nerve. In rats, cross-tolerance
 12 was induced after chlorine exposure but not after formaldehyde exposure, which suggests that
 13 the trigeminal nerve may have different reactive sites that are differentially activated, depending
 14 on the stimulus.

16 **Table 4-11. Exposure regimen for cross-tolerance study**

17

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	Naïve
Chlorine	10 ppm, 6 hours/day	1 day	64.5 ppm	31.7
		4 days	66 ppm	
		10 days	115 ppm	

18 Source: Chang and Barrow (1984).

19
 20
 21
 22 Babiuk et al. (1985) evaluated the potential for formaldehyde pretreatment to cause cross-
 23 tolerance with various other inhaled aldehydes, including acetaldehyde and acrolein. Male F344
 24 rats were pretreated with 15 ppm (18.4 mg/m³) formaldehyde 6 hours/day for 9 days and
 25 challenged on the 10th day with the second aldehyde for 10 minutes at various concentrations
 26 (four rats/concentration) to establish an RD₅₀. Exposure to acetaldehyde and acrolein, the two
 27 smallest molecules in the series of aldehydes tested, resulted in cross-tolerance. The RD₅₀ and

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1 its 95% CI for acetaldehyde were estimated at 2,991 (95% CI: 2,411–3,825) ppm in the naïve
2 rats, and this was increased by approximately 3.5-fold to 10,601 (95% CI: 7,902–15,442) ppm in
3 the rats pretreated with formaldehyde. With acrolein, the RD₅₀ increased approximately fivefold,
4 from 6.0 (95% CI: 3.5–18.1) ppm to 29.6 (95% CI: 15.6–93.0) ppm. Cross-tolerance with
5 formaldehyde has only been demonstrated with acetaldehyde, acrolein, and chlorine (Babiuk et
6 al., 1985; Chang and Barrow, 1984), suggesting that it is not a generalized phenomenon.

7 Whether the phenomenon of tolerance involves modulation of specific trigeminal nerve
8 receptors or whether it results from less specific chemical injury of the nasal mucosa has not
9 been determined. For example, different mechanisms lead to stimulation of the trigeminal nerve
10 and are likely to control the decrease in respiratory rate. In particular, acetaldehyde might
11 interact with sensory nerves via an amino group (Steinhagen and Barrow, 1984; Schauenstein et
12 al., 1977), whereas the receptor-binding site for formaldehyde and acrolein is believed to be a
13 thiol group. Furthermore, different binding sites exist on the trigeminal nerve for different
14 irritants (Nielsen, 1991). Thus, Bos et al. (1992) concluded that the data on tolerance or
15 “desensitization” versus “sensitization” (as defined strictly on the basis of the respiratory apneic
16 response) may be the result of adaptation or reversible/irreversible adverse changes. The
17 mechanisms underlying sensitization or desensitization are not well characterized.

18 19 **4.2.1.4. Formaldehyde Binding and Activation of Trigeminal Nerve Afferent Activity**

20 Kane and Alarie (1978) evaluated the effect of 11 combinations of acrolein and
21 formaldehyde on respiratory rate in outbred specific-pathogen-free male Swiss-Webster mice.
22 Exposure concentrations ranged from 0.12–8.97 ppm (0.28–21 mg/m³) for acrolein and
23 0.37–9.73 ppm (0.45–11.9 mg/m³) for formaldehyde. The data were evaluated using a simple
24 model of competitive antagonism. Comparing the observed and predicted responses indicated no
25 apparent differences, and paired t-tests showed no statistical significance. The authors concluded
26 that acrolein and formaldehyde acted at the same receptor site and acted as competitive
27 antagonists when exposure occurred simultaneously.

28 Kulle and Cooper (1975) investigated the effects of formaldehyde on trigeminal nerve
29 afferent activity in adult male Sprague-Dawley rats. The authors isolated both the ethmoid and
30 nasopalatine branches of the trigeminal nerve and recorded afferent signaling as electrical
31 activity while reactive gases (formaldehyde, ozone, and amyl alcohol) were passed through the
32 nasal passages of the anesthetized animals. The authors reported that both branches of the
33 trigeminal nerve responded similarly to all three chemicals, and they therefore conducted the
34 balance of their experiments on the nasopalatine branch of the nerve. Nerve response was
35 calculated as the difference between exposed and control activity, and the threshold for a positive

1 response was arbitrarily defined as an increase of 0.1 spikes per second. The sensory threshold
2 was determined by extrapolation from the measured nerve response to a range of formaldehyde
3 concentrations (0.5–2.5 ppm) or ozone (5.0–29 ppm) for an exposure duration of 2 minutes.
4 Amyl alcohol exposure (0.3–10.0 ppm) lasted for 25 seconds. Threshold was arbitrarily defined
5 as an increase of 0.1 spikes per second. The mean thresholds were 0.25 ppm for formaldehyde,
6 5.0 ppm for ozone, and 0.30 ppm for amyl alcohol, suggesting that the trigeminal nerve is highly
7 sensitive to formaldehyde and amyl alcohol compared with ozone exposure.

8 In a second set of experiments, Kulle and Cooper (1975) investigated the effects of
9 prolonged formaldehyde-exposure on the odor response to amyl alcohol. Rats were pre-exposed
10 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 11.9, 24, or 36 mg/m³]) then a 1-hour continuous formaldehyde exposure (0, 0.5, 1.0, 1.5, or
12 2.0 ppm [0, 0.61, 1.23, 1.84, or 2.45 mg/m³]). There was a progressive decrease in odor
13 response to amyl alcohol with increasing stimulus of formaldehyde concentration ($p < 0.01$,
14 analysis of variance [ANOVA]). The response to formaldehyde concentration was described by
15 a power function $Y = 0.741 \times X^{1.47}$, where X is the formaldehyde concentration. The effects of
16 exposure to 2.0 ppm were similar, regardless of whether it was presented immediately as a
17 separate exposure or as the final concentration of a progressively increasing series. The response
18 to amyl alcohol did not fully recover within the 1-hour extended recovery period. Thus, it
19 appeared that the afferent function depression was not due to receptor adaptation or insufficient
20 time for formaldehyde diffusion away from receptor sites.

21 In an attempt to elucidate the basis of the differential effects of various types of
22 aldehydes on sensory irritation, Tsubone and Kawata (1991) recorded the afferent activity of the
23 surgically isolated ethmoidal nerve (a branch of the trigeminal nerve) during delivery of
24 0.32–4.7 ppm (0.39–5.77 mg/m³) formaldehyde, 0.18–7.2 ppm (0.41–16.5 mg/m³) acrolein, and
25 134–2,232 ppm (241–4,021 mg/m³) acetaldehyde into the cannulated URT of male Wistar rats
26 (six/aldehyde) at a flow rate of 200 mL/minutes for 22 seconds. Only one aldehyde was used in
27 each animal and each exposure was repeated two to four times at different concentrations. The
28 activity of the nerve was recorded as the number of electrical discharges for a total period of
29 100 seconds, including preinhalation (30 second), inhalation (22 second), and postinhalation
30 (48 second) periods. Nitrogen was used as the control gas and as the vehicle to dilute the
31 aldehyde gases in order to not interfere with the gas chromatography used to analyze the
32 exposures. The vapor concentrations associated with a 50% increase in nerve activity over the
33 level of control gas were calculated as approximately 1.8, 1.2, and 908 ppm for formaldehyde,
34 acrolein, and acetaldehyde, respectively. These results are consistent with the findings of

1 Steinhagen and Barrow (1984) and the hypothesis that the differences in RD₅₀ are due to
2 differences in chemical reactivity in the tissue.

3 In summary, RB is a phenomenon observed in rodents exposed to reactive gases,
4 believed to be a protective response to the irritant properties of the gas. In comparative studies,
5 mice have a more pronounced RB response to irritant gases than rats and generally respond at a
6 lower concentration than rats. Interestingly, only rats appear to develop tolerance to irritant
7 gases, while mice sustain an RB response. When formaldehyde exposure is studied in concert
8 with other reactive gases like chlorine and other aldehydes like acetaldehyde and acrolein, cross-
9 tolerance developed. However, the mechanism underlying this response is unknown. It is
10 thought that RB may occur as a result of stimulation of the trigeminal nerve. Thus, although RB
11 appears to be a phenomenon specific to rodents, the mechanism by which it occurs, trigeminal
12 nerve stimulation, may be applicable to understanding MOAs in other species, such as primates
13 and humans, particularly in regard to sensitization.

14 15 4.2.2. Respiratory Tract Pathology

16 The database for evaluating the POE toxicity in the respiratory tract of inhaled
17 formaldehyde is robust, with well-designed studies that span a duration range of a few hours to
18 chronic 2-year bioassays. Toxicity testing has been performed in various species, including
19 mice, rats, hamsters, guinea pigs, dogs, and nonhuman primates. Although a few studies include
20 examination of tissues outside of the URT, the majority of studies focus on changes in cell
21 proliferation and cell pathology in the nasal mucosa. Both mice and rats are well-defined animal
22 models with standard histologic sections established to evaluate various regions of the nasal
23 passages, divided into Levels 1 to 5 and illustrated in Figure 4-6. Pathology of the nasal mucosa
24 will be discussed with reference to these sections, and the region examined will be stipulated
25 (e.g., nasoturbinates, maxilloturbinates, or ethmoid turbinates [ETs]). Additionally, pathology of
26 the respiratory epithelium will be distinguished from effects on the olfactory epithelium,
27 although the nature of the lesions is similar.

28 Direct effects of formaldehyde exposure on mucociliary clearance are presented first—as
29 this may be the first interaction of the reactive chemical with elements of the upper respiratory
30 tract. A discussion of changes in cell proliferation follows, as increased cell proliferation at low
31 levels of exposure may be a more sensitive indicator of effects on the underlying epithelium.
32 This balance of this section summarizes studies that have investigated cellular pathology in the
33 URT and in the lung. Below, full study descriptions are provided for both short-term, subchronic
34 and chronic duration studies (including, where appropriate, how cell proliferation relates to the
35 observed formaldehyde-induced pathology).

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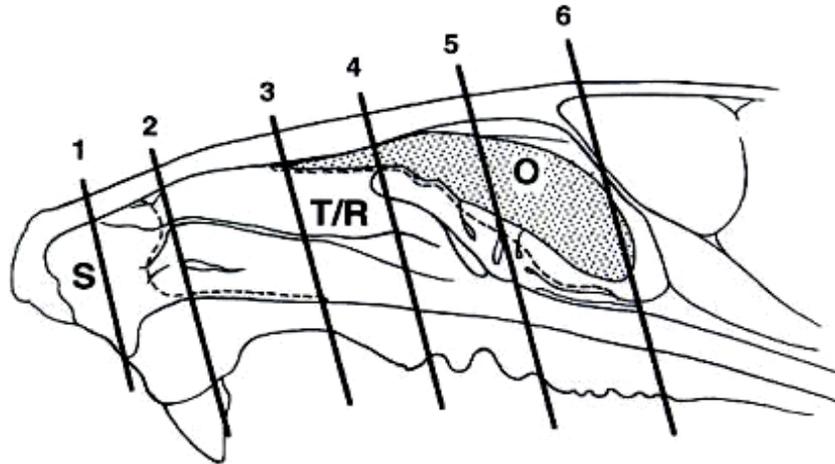


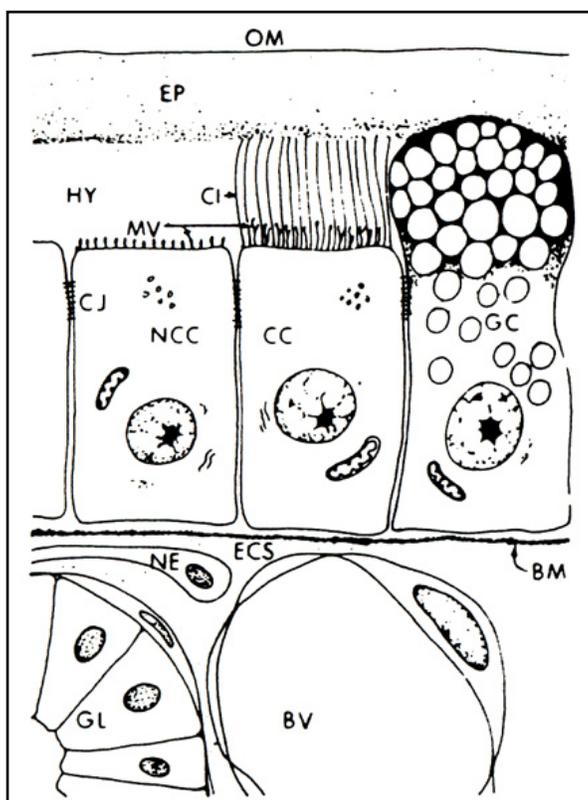
Figure 4-6. Sagittal view of the rat nose (nares oriented to the left).

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.



1
2 **Figure 4-7. Main components of the nasal respiratory epithelium.**

3
4 Note: OM = osmiophilic membrane; EP = epiphase; HY = hypophase; CI = cilia;
5 MV = microvilli; CJ = cell junction; CC = ciliated cell; NCC = nonciliated cell;
6 GC = goblet cell; NE = nerve; GL = gland; BV = blood vessel; ECS =
7 extracellular space; BM = basement membrane.
8

9 Source: Morgan et al. (1986d).

10
11
12 Mucus flow slows upon formaldehyde exposure, despite an increase in the ciliary
13 beat of the underlying epithelial cells, which propel the mucus across the nasal epithelium
14 (Morgan et al., 1986a, c, d; 1983). These findings are consistent with other studies since
15 airborne pollutants and reactive gases have been shown to decrease mucus flow rates in
16 several animal models (Mannix et al., 1983; Irvani, 1974; Carson et al., 1966; Dalhamn,
17 1956; Cralley, 1942). In addition to slowing flow, the mucus layer has been observed
18 breaking up as it floats on the epiphase, creating gaps in the epiphase and revealing the
19 hydrophase below (Morgan et al., 1986c, d). Formaldehyde reacts with glycoproteins in

1 the mucus of the epiphase, creating cross-links between these large molecules; this is
2 believed to increase the viscosity of the mucus.

3 In their first experiments, Morgan et al. (1983) describe progressive mucostasis (slowing
4 of mucous flow) and ciliastasis (disruption of ciliary beat) with increasing days of exposure to
5 formaldehyde in male F344 rats (15 ppm; 6 hours/day for 1, 2, 4, or 9 days). Ciliastasis occurred
6 with greater frequency and across more regions of the nasoturbinate with subsequent days of
7 exposure. After 9 days, mucostasis was recorded in all but two regions evaluated. Although the
8 severity and time course of these changes varied across regions of the nose, the process followed
9 a similar pattern: decreased flow, increased ciliary action, mucostasis, and ciliastasis. Since the
10 formaldehyde-induced deficits in mucociliary function increased with days of exposure, activity
11 did not fully recover between exposures (18 hours) (Morgan et al., 1983). Therefore, the
12 severity and extent of adverse effects are dependent on both the concentration of exposure and
13 duration (in this case, days of repeated exposures).

14 In subsequent studies, Morgan et al. (1986c) examined the exposure-response
15 relationship of formaldehyde effects on mucociliary function and functional recovery 18 hours
16 after exposure ceased. Exposure regimens similar to the above experiment included additional
17 exposure concentrations (0.5, 2, and 6 ppm) and an additional time point of 15 days duration.
18 Exposure at 2 and 6 ppm resulted in the same progression of effects on mucus flow and ciliary
19 beat. Considering both severity and extent of effects a clear exposure-response relationship was
20 demonstrated. Additionally, within each exposure group, effects progressed both in severity and
21 extent by duration of exposure to formaldehyde (from 1 to 4, 9, and 15 days of exposure)
22 (Morgan et al., 1986c).

23 Flow and ciliary beat were not reduced, but rather increased, in epithelium from rats
24 exposed to 0.5 ppm formaldehyde. Mucus flow in 2 of 10 areas assessed was clearly increased
25 (275 and 200% of controls) after 4 days of exposure to 0.5 ppm formaldehyde. Two other
26 epithelial regions showed a similar trend (150% of controls), but this change was not statistically
27 significant. Interestingly, measurements made in corresponding areas after 9 days of exposure
28 did not show an increase, and measurements in one region were reduced to 37% of control.
29 Although it is not known whether the observed increase in mucus flow rate is a subtle indication
30 of an adaptive response to a low level irritant, the increase appears to be transient. It is not
31 known if flow rate would continue to decrease below control levels for repeated exposures at
32 0.5 ppm for longer than 9 days.

33 The regions affected at 15 ppm generally included the lateral aspects of the nasoturbinate
34 and both the dorsal and medial aspects of the maxilloturbinate. In general there was an anterior
35 to posterior effect with increasing concentration and time. Additionally, impaired mucociliary

1 function was more extensive with greater concentration and length of exposure. Nasal lesions
2 were seen on the nasal epithelium and correlated with those areas where some inhibition of
3 ciliary function was measured. Areas without mucus flow but that still retained ciliary function
4 did not develop epithelial lesions. Morgan et al. (1986c) reported “coagulated mucus,” viewed
5 as a “continuous membrane” over the epithelium after 6 hours of exposure to 15 ppm
6 formaldehyde. Minor cell damage and infiltrating neutrophils and monocytes were also seen in
7 these areas. The coagulated mucus was not seen in similarly exposed rats that were allowed
8 18 hours of recovery before sacrifice. However, ciliated cells were damaged, and there was a
9 greater presence of neutrophils and macrophages (MPs) after this recovery period. The authors
10 noted that, as the exposure continued, these areas exhibited increased signs of inflammation and
11 epithelial damage, eventually resulting in “severe degenerative changes.”

12 Morgan et al. (1986a) refined their study design to implement a nose-only exposure to
13 formaldehyde in order to better examine the progression of changes in mucociliary function
14 during short-term exposure, allowing examination of mucus flow immediately following
15 exposure. Three F344 rats/group were exposed to 15 ppm (18.4 mg/m³) formaldehyde for 10,
16 20, 45, or 90 minutes or 6 hours. Two groups of rats were exposed to 2 ppm to determine a no
17 effect level for 90 minutes or 6 hours. The extent and severity of mucostasis and ciliastasis seen
18 after a 6-hour 15 ppm (18.4 mg/m³) formaldehyde exposure and a 1-hour recovery period were
19 similar to the earlier study (Morgan et al., 1986a), indicating that similar exposure conditions
20 were reached with this nose-only apparatus. Ciliastasis and mucostasis were both less severe and
21 less extensive in a time-dependent manner and at the earlier time points of 10, 20, 45, and
22 90 minutes. Significant recovery was seen in mucociliary function by allowing a 1-hour
23 recovery between exposure and sacrifice. Regions of both the nasal septum and lateral wall,
24 which exhibited no mucus flow when examined immediately after a 6-hour exposure, had
25 measurable flow after the 1-hour recovery period. Similar recovery was seen at all durations of
26 exposure. No decreases in mucociliary function were seen after exposure for either 90 minutes
27 or 6 hours at 2 ppm formaldehyde. However, given evidence of recovery (Morgan et al., 1986a)
28 and the time taken to dissect and view the tissues ex vivo may have obscured more subtle effects.

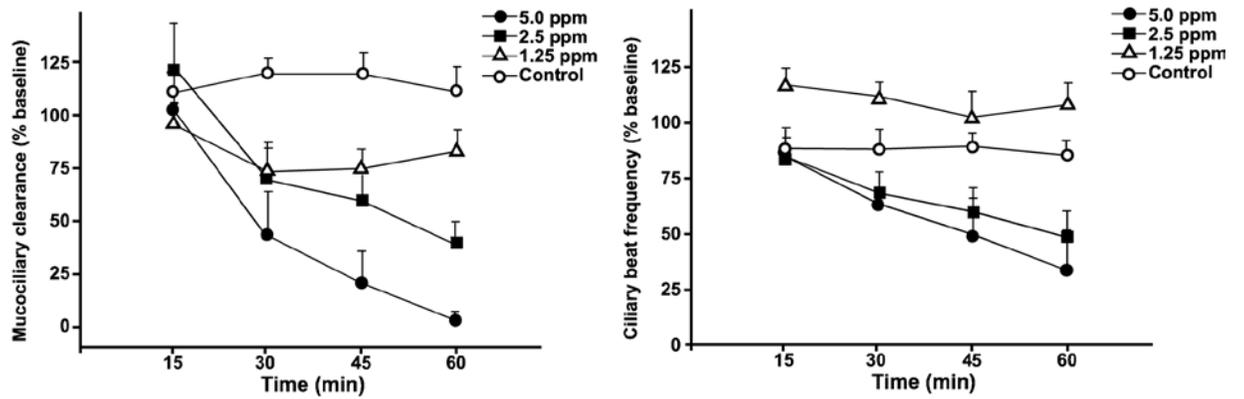
29 To assess more immediate effects on mucociliary apparatus, Morgan et al. (1984a) have
30 examined formaldehyde effects on the mucociliary apparatus of isolated frog palates. This
31 system allowed observation of mucociliary function during exposure. Unexposed frog palates
32 were covered by a continuous sheet of mucus of variable thickness, which was observed to flow
33 in streams across the palette, exhibiting a wave-like form in some areas of the epiphase. The
34 authors reported particle movement in a lower, less viscous layer that was consistent with a less
35 viscous underlying hydrophase, similar to that described in rat mucosa. The basal mucus flow

1 rate was 0–4 mm/minute, with localized ciliary activity. Short periods of increased mucus flow
2 were associated with seemingly spontaneous increases in ciliary beat.

3 Formaldehyde exposure resulted in an initial increase in ciliary beat and mucus flow rate
4 in all palates exposed at 1.37, 4.36, and 9.58 ppm formaldehyde (but not 0.23 ppm). With
5 increasing formaldehyde concentration and time of exposure, mucostasis was evident as mucus
6 became stiff and eventually rigid. Ciliary beat continued after mucostasis was reached until
7 palates were exposed to 4.36 and 9.48 ppm formaldehyde, when ciliastasis was reached. The
8 time course to peak mucus flow rate, mucostasis, and ciliastasis was concentration dependent,
9 with mucostasis reached in less than 3 minutes at 9.48 ppm. In contrast, increased mucus flow
10 peaked at 8 minutes in palettes exposed at 1.52 ppm formaldehyde, which, though declining,
11 remained above basal levels after 25 minutes with no mucostasis or ciliastasis noted at this level.

12 Fló-Neyret et al. (2001) demonstrated reduced mucociliary clearance and decreased
13 frequency of ciliary beats by using a similar isolated frog palette mucociliary apparatus.
14 However the palates were exposed by formaldehyde in the Ringer’s solution in which the palates
15 were placed (0, 1.25, 2.5, or 5 ppm). Also, mucus was removed from the palettes and did not
16 come into direct contact with the formaldehyde. Despite these differences, formaldehyde caused
17 mucociliary clearance to decrease in a time- and concentration-dependent manner; mucostasis
18 occurred after 60 minutes of exposure to 5 ppm formaldehyde (see Figure 4-8). Ciliary beat was
19 decreased in a time-dependent manner at 2.5 and 5 ppm exposure but increased at 1.25 ppm
20 formaldehyde (see Figure 4-8). Reduced mucociliary clearance at 2.5 and 5 ppm was consistent
21 with the reduced ciliary beat. However, clearance decreased at 1.25 ppm formaldehyde, where
22 there was an apparent increase in ciliary beat. The authors suggest this may be a result of
23 disrupting the harmonic movement of the cilia, impairing effective mucociliary clearance. Based
24 on study results, the authors hypothesize that changes in ciliary beat, including excitation at
25 lower exposures, are likely to be a direct effect of formaldehyde on epithelial cells or other
26 cellular components of the mucosa.

27 In summary, numerous studies have identified impaired mucociliary clearance activity
28 associated with formaldehyde exposure (see Table 4-12). Although low-dose and short-term
29 exposures first increase ciliary beat, impaired mucus flow, slowed ciliary beat, and eventual
30 mucostasis and ciliastasis have been demonstrated in both in vivo and in vitro exposure systems.
31 These effects are both concentration and duration dependent and can be seen in as few as
32 15 minutes from exposure. Repeated inhalation exposures in rats indicate the effect does not
33 fully recovery in an 18-hour period between exposures, contributing to greater impairment over
34 extended periods of exposure.



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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. After 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.

1 Morgan et al. (1983) suggested that the initial stimulation of ciliary activity may be a
2 defensive response to the irritant gas, possibly indicating some penetration of formaldehyde to
3 the underlying epithelial cells. Later effects of mucostasis may be a result of cross-linking of
4 mucus glycoproteins by formaldehyde, creating a rigid mucus that is not able to flow even with a
5 rigorous ciliary beat. It is unknown if the eventual cessation of ciliary beat is a result of
6 compound-related effects on ciliated epithelium as formaldehyde diffuses through the mucus or
7 an indirect effect associated with mucostasis. However, in vitro experiments by Fló-Neyret et al.
8 (2001) indicate that formaldehyde in solution, supporting isolated frog palates without mucus,
9 resulted in the same sequence of effects, including increased ciliary beat at the lowest exposure.
10 These data suggest a role of formaldehyde beyond its ability to form protein cross-links in
11 mucociliary proteins.

12 13 **4.2.2.2. Cell Proliferation**

14 Formaldehyde-induced cell proliferation has been demonstrated under range of exposure
15 conditions in vivo and in vitro as well (see Chapter 3). Formaldehyde-induced mitogenesis may
16 be a primary effect (as demonstrated in the in vitro work) or secondary to adaptive responses and
17 tissue remodeling (Swenberg et al., 1983). This section provides a comprehensive discussion of
18 formaldehyde effects on cell proliferation in the epithelial tissues in the respiratory tract. The
19 majority of the work discussed investigates cell proliferation with in vivo labeling of
20 proliferating cells, although additional methods, such as flow-cytometry, have been employed in
21 some instances.

22 Swenberg et al. (1986) conducted a series of experiments in rodents to assess cell
23 proliferation in the nasal mucosa after formaldehyde inhalation. Radiolabeled thymidine
24 [³H]-thymidine was injected intraperitoneally (I.P.) into male F344 rats and B6C3F1 mice after
25 formaldehyde exposure to assess the extent of in vivo incorporation into proliferating cells. Two
26 hours later, animals were sacrificed and the nasal passages were fixed, embedded, and sectioned
27 to examine the nasal mucosa. Slides were exposed for 12 weeks and developed to identify cells
28 that incorporated the radiolabeled thymidine. The percentage of labeled cells, as indicated by the
29 presence of five or more grains over the nucleus, was determined by visual count. A total of
30 4,000 or 1,500 cells were counted per section for rats and mice, respectively.

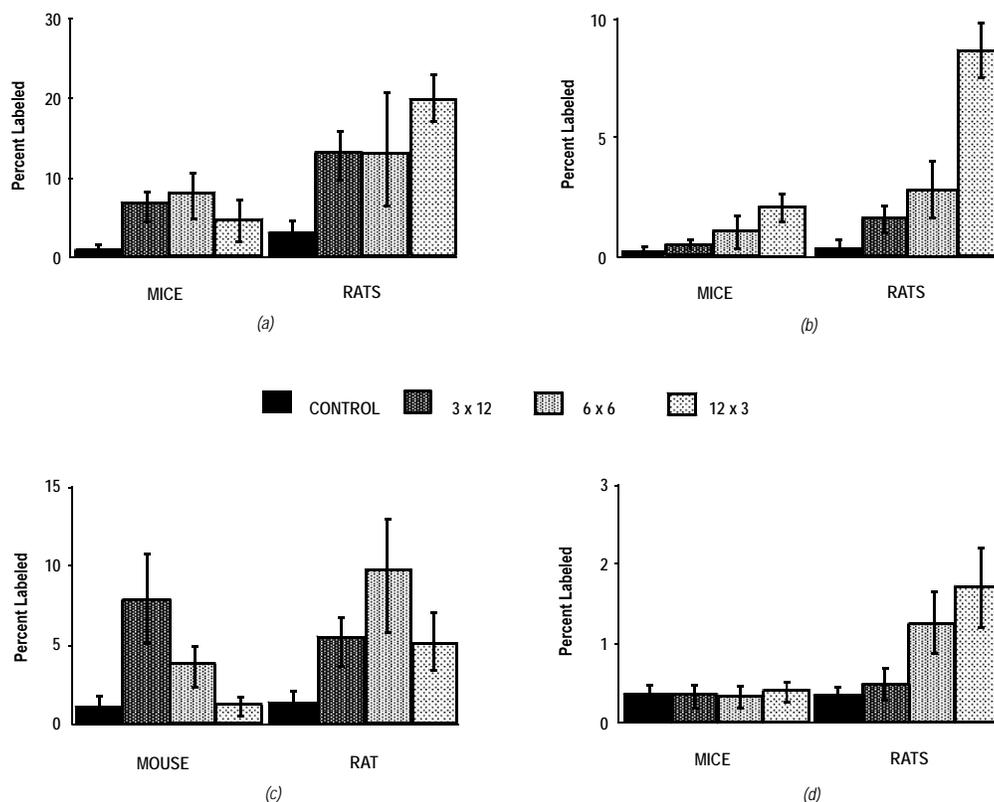
31 The first set of studies reported by Swenberg et al. (1986) compared the dose response of
32 rats and mice. Animals were exposed to 0, 0.5, 2, 6, or 15 ppm (0, 0.61, 2.45, 7.36, or
33 18.4 mg/m³) formaldehyde 6 hours/day for 3 days. Tritiated thymidine for cell labeling was
34 injected 2 hours after the end of exposure. No change in the percentage of labeled cells was seen
35 after 0.5 or 2 ppm formaldehyde exposure. However, the nasal passages of rats exposed at 6 and

1 15 ppm showed 10- to 20-fold increases over controls in LI at level 2. A similar cell
2 proliferation response was seen in mice treated with 15 ppm formaldehyde, although no increase
3 over control was seen in mice exposed to 6 ppm formaldehyde. These findings are consistent
4 with other data that indicate rats are more sensitive to formaldehyde exposure than mice. This
5 may be due to differences in the reflex apneic response between the two species. As discussed in
6 Section 4.2.1.1, mice maintain decreases in minute volume in response to formaldehyde, which
7 results in a lower overall effective internal dose to the mice.

8 Comparing cell proliferation rates after 2 versus 18 hours of exposure, Swenberg et al.
9 (1986) found that the longer exposure duration gave twice the cell proliferation rates after
10 repeated exposures. Therefore, these researchers conducted a second dose-response study to
11 examine cell proliferation 18 hours after exposure instead of the shorter exposure duration. The
12 dose-response study varied dose as well as duration of treatment. Rats were exposed 6 hours/day
13 to either 0.5, 2, or 6 ppm (0.61, 2.45, or 7.36 mg/m³) formaldehyde over periods of 1, 3, or
14 9 days. Formaldehyde exposure at 0.5, 2, or 6 ppm for 1 day increased cell proliferation in the
15 nasal epithelium. However, these increases were transient, and cell proliferation was not
16 increased after 3 or 9 days of exposure to 0.5 ppm or 2 ppm formaldehyde. Although still
17 elevated after a 3-day exposure to 6 ppm formaldehyde, cell proliferation returned to control
18 values after 9 days of exposure to 6 ppm formaldehyde (Swenberg et al., 1986). Therefore,
19 although concentration is a major determinant of cell proliferation, duration of exposure also
20 influenced formaldehyde-induced cell proliferation in the nasal epithelium.

21 Swenberg et al. (1986) directly tested the effects of cumulative exposure versus
22 concentration for both mice and rats. Animals were treated with one of three regimens, resulting
23 in the same C × t product: 3 ppm × 12 hours, 6 ppm × 6 hours, or 12 ppm × 3 hours, each
24 exposure resulting in 36 ppm-hours. The animals were exposed once a day for either 3 or 9 days.
25 Tritiated thymidine was injected 18 hours after exposure to label of proliferating cells. Tissue
26 sections from levels 1 and 2 of the nasal passages were examined in each case, and the
27 percentage of cells labeled was reported as the percentage of proliferating cells (see Figure 4-9).

28 Cell proliferation at level 1 in the nasal cavity was much greater than at level 2 for all
29 C × t combinations of formaldehyde exposure in both mice and rats (see Figure 4-9). The
30 authors noted that level 1 is more anterior and lacks significant defense from the mucociliary
31 apparatus, which may account for the observed greater sensitivity to formaldehyde. At all C × t
32 exposure products, 3 days of exposure resulted in greater cell proliferation than 9 days of
33 exposure. This was true for both species and for both examined levels of the nasal cavity. The
34 decrease in cell proliferation by day 9 is consistent with data on rats labeled 18 hours
35 postexposure (Swenberg et al., 1986).



2

3

4 **Figure 4-9. Effect of formaldehyde exposure on cell proliferation of the**
 5 **respiratory mucosa of rats and mice.**

6

7 Note: *a* and *b* are data following 3 days of exposure; *c* and *d* are for 9 days of
 8 exposure. *a* and *c* are from level 1 (most anterior); *b* and *d* are from level 2.
 9 [³H]-thymidine was administered 18 hours after the last exposure.

10

11 Source: Swenberg et al. (1986).

12

13

14 When comparing C × t exposures for a single species and location, the findings are more

15 complex. Cell proliferation in level 2 of the nasal passages appeared to be more dependent on

16 concentration than on duration or cumulative exposure, with the strongest response seen for

17 12 ppm formaldehyde in combination with the shortest exposure period, 3 hours (see Figure 4-9).

18 This pattern was observed in both rats and mice after 3 days of exposure and in rats after 9 days

19 of exposure. No increases in cell proliferation at level 2 were seen for any C × t combination in

20 mice after 9 days. In contrast, increases in cell proliferation at level 1 of the nasal passages were

21 not strictly concentration dependent. After a 3-day exposure, no clear differences were seen

22 among different C × t treatments for either mice or rats, suggesting cumulative exposure may be

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1 the important metric. Therefore, it may be concluded that cell proliferation for level 1 of the
2 nasal passages, where there is less protection of the epithelium, is influenced by concentration,
3 time, and duration of exposure. Cell proliferation at level 2 appeared to be more dependent on
4 concentration than time of exposure (Swenberg et al., 1986).

5 Cassee and Feron (1994) reported a qualitative increase in histochemical staining for
6 proliferating cell nuclear antigen (PCNA) in the respiratory epithelium of the nasoturbinates,
7 maxilloturbinates, septum, and lateral wall at levels 2 and 3 of rat nasal passages after repeated
8 exposures to 3.5 ppm (4.29 mg/m³) formaldehyde 22 hours/day for 3 days. While no increases
9 were seen in olfactory epithelium, frank necrosis, squamous metaplasia, and hyperplasia of both
10 ciliated and nonciliated epithelium were noted at these section levels.

11 Quantitative cell proliferation studies have been conducted by several researchers in the
12 same laboratory (Reuzel et al., 1990; Wilmer et al., 1989; Zwart et al., 1988; Wilmer et al., 1987;
13 Woutersen et al., 1987) (see Summary Table 4-17). These studies build off of those of Swenberg
14 et al. (1986), who labeled proliferating cells with [³H]-thymidine in assessing cell proliferation
15 within the nasal mucosa. The studies, all performed in male albino Wistar rats and using a
16 similar experimental design, provide the basis for comparing different exposure levels and dose
17 regimens across studies. Wilmer et al. (1987) demonstrate a concentration-dependent increase in
18 cell proliferation after 3 days of repeated 8-hour exposures at 5, 10, or 20 ppm (6.13, 12.3, or
19 24.6 mg/m³) formaldehyde, regardless of continuous versus interrupted exposure conditions
20 (2.83, 8.87, and 19.8 versus 0.86% proliferation in controls). Similar trends were seen when the
21 repeated continuous exposures were extended for 4 weeks, but cell proliferation was not
22 maintained at the same levels. As observed by Swenberg et al. (1986), these results suggest that
23 duration of repeated exposures may be an important determinant of cell proliferation rates.

24 Woutersen et al. (1987) reported that the majority of the dose-dependent increases in cell
25 proliferation seen at section level 3 after 3 days of repeated 6-hour exposures to 10 and 20 ppm
26 (12.3 and 24.6 mg/m³) formaldehyde occurred in areas of the epithelium showing “clear
27 squamous metaplasia and hyperplasia.” Cell proliferation rates in metaplastic epithelium of
28 29.5 and 33.2% were much higher than the 1.4 to 2.8% proliferation in the visibly unaffected
29 respiratory epithelium from rats exposed at 10 ppm formaldehyde. Although there was a slight
30 trend towards increased cell proliferation in the visibly unaffected epithelium of exposed animals
31 compared with unexposed controls, the majority of increased cell proliferation resulting from
32 exposure to 10 and 20 ppm formaldehyde was attributed to the metaplastic epithelium.

33 Similarly, dose-dependent increases in cell proliferation seen at level 3 after 3 days of
34 repeated 6-hour exposures at 0.3, 1, and 3 ppm (0.37, 1.23, and 3.68 mg/m³) formaldehyde
35 ($p < 0.001$) corresponded to focal basal cell hyperplasia and loss of cilia (Woutersen et al., 1987).

1 No necrosis or focal erosion was noted at these levels of formaldehyde exposure. Cell
2 proliferation was not sustained at this location, and no lesions were noted after 13 weeks of
3 repeated 6-hour exposures. The authors hypothesized that defensive mechanisms, such as the
4 mucociliary apparatus, may have provided greater protection of the mucosa at level 3. Swenberg
5 et al. (1986) drew a similar conclusion when evaluating extended exposures, suggesting that
6 more posterior sections had a greater adaptive ability than those anterior sections with little
7 mucociliary function. Both Woutersen et al. (1987) and Swenberg et al. (1986) reported
8 sustained cell proliferation and development of lesions in the more anterior cross section.
9 Repeated exposures to 3 ppm formaldehyde (6 hours/day) resulted in significant increases in cell
10 proliferation in the epithelial cells at level 2, with accompanying disarrangement, focal
11 hyperplasia, and squamous metaplasia (Woutersen et al., 1987). Although no cell death was
12 observed at level 2 when viewed by light microscopy, “strongly indented and disarranged nuclei”
13 were seen by electron microscopy, which may be consistent with apoptosis (Woutersen et al.,
14 1987). However, later work in the same laboratory indicated no increased cell proliferation at
15 levels 2 or 3 in male Wistar rats exposed to formaldehyde at 1 or 2 ppm (1.23 and 2.45 mg/m³)
16 (8-hour repeated exposures for 3 days or 13 weeks) and only minimal response in rats exposed at
17 4 ppm formaldehyde (interrupted 8-hour exposures for 3 days or 13 weeks) (Wilmer et al.,
18 1989).

19 Reuzel et al. (1990) published the only report in which formaldehyde effects on cell
20 proliferation were studied for longer daily exposure durations: 22 hours/day versus
21 6–8 hours/day. Male Wistar rats were exposed to formaldehyde, ozone, or the combination of
22 the two 22 hours/day for 3 consecutive days. The concentrations of formaldehyde were 0.3, 1.0,
23 or 3.0 ppm (0.37, 1.23, or 3.68 mg/m³). Rats were injected with [³H]-thymidine 2 hours rather
24 than 18 hours after the last exposure. Cell proliferation was quantified by enumerating the
25 percentage of labeled cells in fixed and stained tissue sections. Cell proliferation on the
26 nasoturbinates, maxilloturbinates, lateral wall, and septum at levels 2 and 3 were quantified and
27 reported separately. Cell proliferation was increased at all locations in level 2 at 3 ppm
28 formaldehyde exposure ($p < 0.05$) but not at 0.3 or 1 ppm exposures (see Summary Table 4-17).
29 Whereas proliferation of cells in the nasoturbinate, maxilloturbinate, and septum was nearly
30 undetectable in control animals, 4, 5, and 3% proliferation was reported after repeated 22-hour
31 exposures to 3 ppm formaldehyde. Basal proliferation in the lateral wall was greater than in
32 other areas, approximately 1% increasing to 6% after exposure to 3 ppm formaldehyde.
33 Although basal levels of cell proliferation were slightly higher in all areas of level 3,
34 formaldehyde had no significant effects on cell proliferation in the level 3 areas evaluated. There
35 was a slight trend for increases at 3 ppm, but all proliferation rates were below 1%. Exposure to

1 3 ppm formaldehyde also damaged the respiratory epithelium at levels 2 and 3, where cell
2 disarrangement and hyperplastic and metaplastic lesions were reported.

3 Roemer et al. (1993) investigated the effects of formaldehyde exposure on cell
4 proliferation in the trachea and lung in addition to nasal mucosa. Male Sprague-Dawley rats
5 were exposed head only to 2, 6, or 20 ppm (2.45, 7.36, or 24.5 mg/m³) formaldehyde 6 hours/day
6 for either 1 or 3 days. Proliferating cells were labeled with 5-bromodeoxyuridine (BrdU), the
7 label injected 16–22 hours after formaldehyde exposure ended. Free lung cells were harvested
8 by tracheal lavage, and the majority of isolated cells were MPs (>97%). Epithelial cells were
9 isolated from the nasal and tracheal mucosa by dissection, physical disaggregation, and enzyme
10 treatment to release epithelial cells. All cells were fixed and stained with fluorescent dyes to
11 detect BrdU and total DNA. Flow cytometry was used to determine the percentage of BrdU-
12 labeled cells as a measure of cell proliferation. Cells undergoing unscheduled DNA synthesis
13 (e.g., DNA repair) were excluded by cell cycle analysis.

14 The proportion of BrdU-labeled cells from the nose and trachea increased two- to
15 threefold above control values after a single 6-hour exposure to formaldehyde (see Table 4-13).
16 The lowest effective dose for increased cell proliferation was 2 ppm for nose and tracheal cell
17 proliferation ($p < 0.05$). However, increased proliferation in the nasal mucosa at the lowest dose
18 was transient, returning to control levels after a 3-day exposure. Cell proliferation remained
19 increased in the nasal mucosa after exposure to 6 or 10 ppm (7.36 or 12.3 mg/m³) formaldehyde
20 for 3 days. In contrast, proliferation of tracheal cells appeared to be reduced as a result of a
21 3-day exposure to 2 or 6 ppm formaldehyde. A similar trend was seen in free lung cells, but the
22 differences were not statistically significant.

23
24 **Table 4-13. Cell proliferation in nasal mucosa, trachea, and free lung cells**
25 **isolated from male Wistar rats after inhalation exposures to formaldehyde**
26

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

27 ^aExposures were 6 hours/day.

28 ^bProliferation is measured as the percent of BrdU-labeled cells.

29 ^cStatistically different from controls ($p < 0.05$).

30 ^dThe majority of free lung cells were MPs (97%).

31 Source: Roemer et al. (1993).

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1 The flow cytometry employed by Roemer et al. (1993) allowed for subtle changes in
2 proliferation rates to be measured with good discrimination. However, the method of cell
3 isolation did not allow examination of proliferation rates in discrete regions of the mucosa,
4 which may have attenuated the magnitude of the response. Additionally, proliferation rates
5 represent a mix of cell types that were not separated in this analysis, making the findings difficult
6 to interpret. This may be especially noteworthy in the free lung cells that were reportedly
7 primarily MPs.

8 Monticello et al. (1990) investigated whether changes in cell proliferation rate correlated
9 with areas of cell injury or with areas that developed tumors due to formaldehyde exposures by
10 using a unique metric of cell proliferation. They hypothesized that treatment-related effects on
11 cell populations could influence the apparent cell proliferation measured as LI, even though no
12 proliferative effect had occurred. For example, cell death could give an apparent increased
13 proliferation as a LI (% cells proliferating) by reducing the total number of cells present. This
14 would be especially true for a stratified epithelium, where the number of basal cells in active
15 proliferation may not change but cells above the basal layer might die or slough off, thereby
16 reducing the overall number of population of cells counted. The unit length labeling index
17 (ULLI) metric was developed to normalize proliferation rates against length of basal membrane
18 rather than cell population. However, application of a ULLI to the pseudostratified epithelium of
19 the nasal mucosa introduced additional complexities. First, undamaged mucosa has a single
20 layer of epithelial cells that have the capability for cell proliferation. Second, cells only become
21 layered in response to cell damage as a protective measure. Therefore, the total cells present and
22 the linear cell density should be considered, as well as the number and density of proliferating
23 cells, in developing an understanding of the proliferative response of these tissues to toxic insult.

24 Monticello et al. (1990) directly compared the apparent effects of formaldehyde exposure
25 on cell proliferation when quantified as an LI or as a ULLI. Male F344 rats were divided into
26 groups ($n = 6$) and exposed to 0, 2, 6, or 15 ppm (0, 2.45, 7.36, or 18.4 mg/m³) formaldehyde
27 6 hours/day, 5 days/week for 12 weeks. Rats were administered [³H]-thymidine continuously for
28 the last 5 days of exposure by surgically implanted osmotic pumps. After sacrifice, nasal
29 passages were fixed, and sections from standard level 3 were prepared for examination. Cell
30 proliferation was quantified at the midseptum and the lateral meatus at this level. Basement
31 membrane length, total number of cells present, and number of labeled proliferating cells were
32 recorded for each location. Each of these areas also was scored for the presence of nasal lesions.

33 The formaldehyde-related lesions included epithelial hyperplasia, squamous metaplasia,
34 and acute inflammation. These lesions were most severe in animals exposed to 15 ppm, mild at
35 6 ppm, but absent at 2 ppm. Cell proliferation, measured either as LI or ULLI, was increased in

1 the level 3 septum and lateral meatus after 13 weeks of exposure to 15 ppm formaldehyde but
2 not to 6 or 2 ppm (see Table 4-14). There was a slight increase in both cell number and labeled
3 cells in the lateral meatus of rats exposed to 6 ppm formaldehyde, but both measures of
4 proliferation were unchanged from controls. The increased proliferation in the lateral meatus at
5 15 ppm was entirely due to an increased number of labeled cells. Total cells were unchanged at
6 15 ppm; therefore, both LIs demonstrated a similar increase over control. In addition to
7 increased labeled cells in the septum at 15 ppm, total cells were increased from 470 to 640
8 ($p < 0.05$). Where the total cells and linear cell density were increased, the ULLI was
9 proportionally increased over the LI. These observations are consistent with the development of
10 squamous metaplasia and hyperplasia seen at 15 ppm. However, while both LI and ULLI
11 showed an eightfold increase in cell proliferation in the lateral meatus, they gave different results
12 in the septum where cell number was increased by formaldehyde treatment. LI increased 19-fold
13 and ULLI 25-fold with repeated exposures to 15 ppm formaldehyde. Although these data are
14 based on only 5–6 animals/group, and only in an extended study, the results suggest that the
15 ULLI and LI may not be proportional under all conditions studied. In similar experiments the LI
16 and ULLI provided different indices of proliferation in the olfactory epithelium after methyl
17 bromide exposure (Monticello et al., 1990). Methyl bromide exposure decreased cell
18 number/mm of basement membrane in a time-dependent manner, and the LI and ULLI were not
19 proportional across these changes. At day 3 there was an increase in labeled cells but a decrease
20 in total cells; therefore, the LI was increased greater than 20-fold, where the ULLI was only
21 increased eightfold. The authors endeavored to explain why the ULLI and LI yielded different
22 findings. Where ULLI is a more time-efficient method of assessing cell proliferation, the authors
23 suggested that representative areas should be quantified by LI to better understand the nature of
24 increased ULLI.

25 Monticello et al. (1990) reported similar results in a contemporary abstract; although
26 treatment groups were slightly different than in the above experiments, the findings were similar.
27 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/m³)
28 formaldehyde 6 hours/day for 4 days, 6 weeks, or 3 months. ULLIs were determined in the
29 septum and lateral meatus (methods not detailed). It is not stated whether [³H]-thymidine
30 labeling was carried out by injection or continuous infusion. Significant increases in cell
31 proliferation were reported after repeated exposures to 6, 10, and 15 ppm for 4 days and 6 weeks.
32 After 3 months of exposure, cell proliferation was still increased in rats exposed to 10 and
33 15 ppm formaldehyde. The authors noted that, although increased cell proliferation was seen at
34 earlier time points, sustained increased cell proliferation was only seen at 10 and 15 ppm, which
35 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

^aDifferent from control, $p < 0.05$.

^bBM is basal membrane length in mm.

^cCalculated from group averages: LI = (labeled cells)/total cells.

^dCalculated 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/m³) formaldehyde 6 hours/day for 1, 4, or 9 days and for 6 weeks, using a 5 days/week regimen. Rats were injected with [³H]-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.

1 As discussed above, proliferating cells were visually identified by the number of grains
2 over the nucleus, 10 grains indicating a proliferating cell. Cell proliferation was quantified as the
3 number of proliferating cells per length of basement membrane (cells/mm) and reported as a
4 ULLI. The report does not indicate the length of membrane viewed for each section as an
5 indication of how representative the counts are for each region. Lesions associated with
6 formaldehyde exposure may change the density of cells/mm of basement membrane (Monticello
7 et al., 1990). Areas of disarranged cells, erosion, metaplasia, or layering of epithelial cells may
8 exhibit different cell profiles. These processes would alter cell density, and therefore the ULLI,
9 independent of differential proliferation rates. As such, it is not expected to be proportional to
10 cell proliferation rates across conditions that have the potential to change cell density
11 (Monticello et al., 1990).

12 No formaldehyde-induced epithelial lesions or increases in the ULLI were seen in rats
13 exposed to 0.7 or 2.0 ppm formaldehyde, regardless of duration (see Table 4-15).
14 Formaldehyde-induced lesions were present in all regions of the nasal epithelium after exposures
15 to 10 and 15 ppm formaldehyde, regardless of duration (Monticello et al., 1991). Incidence and
16 severity of the lesions increased with concentration and duration of treatment and were
17 correlated to areas with increased cell proliferation. Rats exposed to 6 ppm formaldehyde
18 developed lesions in the level 2 nasal passages, where the ULLI was clearly elevated, but not in
19 the deeper level 3 passages. For example, no formaldehyde-related lesions were seen at the
20 lateral meatus and septum of level 3 at 1, 4, and 9 days of repeated exposure at 6 ppm, although
21 cell proliferation was increased. This transient increase in ULLI returned to near-control levels
22 after 6 weeks of repeated exposure (see Table 4-15). Monticello et al. (1991) suggested that cell
23 proliferation is a more sensitive indicator of cellular response and not necessarily dependent on
24 cellular necrosis.

25 The sustained cell proliferation at the lateral meatus and midseptum in rats exposed to 10
26 and 15 ppm formaldehyde, locations where SCCs are known to arise, supports a role for
27 compensatory cell proliferation in tumor development. However, Monticello et al. (1991) noted
28 that regional differences in sustained cell proliferation do not always correspond to the
29 occurrence of nasal tumors, primarily SCCs, in formaldehyde-exposed rats. Where sustained
30 cell proliferation has been demonstrated in the medial maxilloturbinate (MMT) at level 2
31 (Monticello et al., 1991), SCCs have not been found to originate in this area at similar exposures
32 (Monticello et al., 1996; Woutersen et al., 1989). Monticello et al. (1991) suggested that the
33 findings of Bermudez and Allen (1984), indicating that the epithelial cells of the maxilloturbinate
34 are more resistant to the genotoxic effects of DEN, support the possibility that differences in
35 regional tissue susceptibility may contribute to site specificity of formaldehyde-related SCCs.

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2
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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

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^aULLI is expressed as the number of labeled cells/mm of basement membrane.

^bIndicates significantly different from control, $p < 0.05$.

^cIndicates a location where epithelial lesions were not seen by light microscopy.

Source: Monticello et al. (1991).

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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/m³) formaldehyde for up to 24 months with interim sacrifices at 3, 6, 12,

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1 and 18 months. Before each interim sacrifice [³H]-thymidine was continuously injected for the
 2 last 5 days of exposure through a surgically implanted pump. Nasal passages were prepared, and
 3 six standard sections were taken and developed as above for [³H]-thymidine-labeled cells.
 4 Stained tissue sections were viewed in order to map all nasal tumors. A ULLI was determined
 5 for each region (details not provided). The total cell population of each nasal region was
 6 estimated from control animals sacrificed at 3 months (see Table 4-16). Cell profiles were
 7 counted across 0.5 mm of basement membrane length at two locations for each region (site not
 8 specified). Total cells per region were estimated from these counts and the modeled surface area
 9 expected in each region (Fluid Dynamics Analysis Package version 7.0). It is unclear if one or
 10 more rats were used to quantify cell population. Cell counts and variability were not reported.

11
 12 **Table 4-16. Cell population and surface area estimates in untreated male**
 13 **F344 rats and regional site location of squamous cell carcinomas in**
 14 **formaldehyde-exposed rats for correlation to cell proliferation rates**
 15

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

16
 17 ^aTotal cell number determined in unexposed rats as a product of representative cell counts and expected surface area
 18 of the region.

19 ^bModeled surface area of the defined region by FDIP version 7.0.

20 ^cThe number of animals bearing a tumor located in the region. Animals were exposed 6 hours/day for 24 months
 21 prior to sacrifice.

22
 23 Source: Monticello et al. (1996).

24
 25
 26 ULLIs were quantified by region of the nasal passages in order to correlate with regional
 27 localization of tumors. For example, the anterior midseptum included cells from the midseptum
 28 from approximately standard section levels 2 to 3. An anterior to posterior pattern of
 29 formaldehyde effects, especially differences in cell proliferation rates, has been well established.
 30 As such, cell proliferation rates would be expected to vary across the nasal regions used in this

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1 analysis. Areas considered to possibly be preneoplastic were not quantified for this work.
2 Monticello et al. (1996) reported increased ULLIs in the ALM and the MMT of rats exposed to
3 10 or 15 ppm at all time points (3, 6, 12, and 18 months) but provided no indication of variability
4 or a statistical analysis, making it difficult to determine where true differences may exist. Some
5 caution should be used in interpreting the ULLI counts assigned for each region.

6 The PWULLI was calculated by multiplying the reported ULLIs by the calculated cell
7 populations by region. SCC incidence by region had a greater correlation to the calculated
8 PWULLI than the ULLI, $R^2 = 0.88$ versus $R^2 = 0.46$, respectively. The authors noted that the
9 relative lack of correlation with the ULLI was influenced by findings at the maxilloturbinate
10 where cell proliferation was high but SCC incidence was low. Other tumor types were not
11 included in the analysis (polypoid adenomas, adenocarcinomas, and rhabdomyosarcomas).
12 Additionally, 54 of the SCC tumors could not be accurately localized and were excluded from
13 the analysis, resulting in exclusion of 30 and 39% of animals with SCCs in the 10 and 15 ppm
14 treatment groups, respectively. The authors cautioned that the absence of these data might have
15 skewed the regional analysis of tumor location. Although the purpose of weighting the ULLIs
16 by total population of cells available in each region is to better represent the chance of a tumor
17 arising in each region, the cancer incidence was represented by the number of animals, not the
18 number of tumors, per region. Based on the exclusion of location data (up to 40% of the
19 animals), lack of variability and significance reported for the ULLI for cell counts, and SCC
20 incidence considered by animal rather than by tumor, the significance of a greater correlation by
21 PWULLI versus ULLI is of questionable value.

22 Monticello et al. (1989) also assessed formaldehyde-induced cell proliferation and
23 regional site location of lesions in the respiratory tract of rhesus monkeys (see Section 4.2.2.2,
24 Figures 4-11 and 4-12 for a full study description). LIs from the histoautoradiograms indicated
25 increased cell proliferation in transitory, respiratory, and olfactory epithelial cells after the 6-
26 week formaldehyde exposure. Similar trends were seen after only 1 week but were statistically
27 significant only in the respiratory epithelium. Although increased proliferation in the trachea and
28 carina was statistically significant after 1 week of exposure, the greater increases seen after
29 6 weeks of exposure were not statistically significant. A small sample size ($n = 3$) and high
30 variability may have contributed to the lack of statistical significance. The authors noted that
31 increased cell proliferation was seen in locations with minimal histologic changes, indicating
32 proliferation may be a more sensitive predictor of adverse health effects of formaldehyde
33 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 (<i>p</i> < 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)

^aN = number of animals per treatment group.

^bTreatment is given as the concentration of formaldehyde, duration of exposure each day, and length of the experiment in days and weeks.

^cStandard section levels of the nasal passages as shown in Figure 4-6 are given for experiments in rats or mice.

^dNR = not reported; I.P. = intraperitoneally.

^eStudy is described in full in Section 4.2.1.2.2.4.

^fDifferent from control, $p < 0.05$.

^gIntermittent exposures were 30 minutes per hour for 8 hours.

^hData from one animal only.

1 4.2.2.3. *Short-Term Studies*

2 Inhalation of formaldehyde for a few hours has been shown to result in damage of the
3 nasal mucosa, depending on the exposure concentration. Bhalla et al. (1991) observed changes
4 in cell morphology in male Sprague-Dawley rat nasal epithelia after a single 4-hour exposure to
5 10 ppm (12.3 mg/m³) formaldehyde. Three exposed rats were sacrificed 1 hour and 24 hours
6 after exposure, with two control rats at each time point. Noses were fixed, decalcified, and sliced
7 along the midsagittal plane through the nasal septum. The exposed turbinates were examined by
8 scanning electron microscopy. Transverse sections through the hard palate, at the level of the
9 incisive papillae, were prepared for light microscopy from similarly exposed rats (*n* = 10). The
10 authors provided detailed descriptions of cell epithelial organization in untreated rat turbinates
11 and changes observed in formaldehyde-treated rats, as set forth below. No statistical analysis
12 was provided.

13 Scanning electron microscope examination of nasoturbinates showed increased mucus,
14 erythrocyte infiltration, swelling of microvillus cells, and some cell separation in formaldehyde-
15 treated rats. Nasoturbinates examined 1 day after exposure showed greater effects, including cell
16 damage, matted cilia, and blebbing of cell membranes. Damage to microvillus cells of the
17 maxilloturbinate included deformed cilia, cell swelling and rupture, and lack of typical microvilli
18 on the cell margins. As in the nasoturbinates, damage was more marked 24 hours after exposure.
19 The epithelium of the ETs exhibited less cell damage than in the nasal and maxillary regions,
20 with the slight lesions noted in the upper (ET1) portion and little to no damage noted on the mid
21 and lower (ET2 and ET3) regions. Examination of transverse tissue sections revealed swollen
22 goblet cells and stretched epithelial cells that formed an epithelial lining approximately 40%
23 taller than the lining seen in control rats. There was also a patchy loss of ciliated cells in the
24 respiratory epithelium, where columnar cells were present.

25 Buckley et al. (1984) investigated the respiratory tract lesions associated with several
26 sensory irritants. As part of this investigation, male Swiss-Webster mice were exposed to
27 3.13 ppm (3.85 mg/m³) formaldehyde 6 hours/day for 5 days. A total of nine chemicals were
28 tested in parallel. The report indicates there were 24–34 mice in each group, although not
29 detailed for each chemical. One-half of the treatment group and unexposed controls were
30 sacrificed immediately after the last exposure. The remaining exposed mice were sacrificed
31 72 hours later. The head, trachea, and lungs were fixed and heads decalcified. Five sections
32 were taken of each nose at levels equivalent to standard levels 2–6 (see Figure 4-6) and were
33 examined by light microscopy. Details on lung and trachea sections were not given.
34 Formaldehyde induced lesions in the respiratory epithelium of exposed mice, including
35 inflammation, exfoliation, erosion, ulceration, necrosis, and squamous metaplasia. The section

1 level for these effects was not given. No effects were reported in the squamous epithelium,
2 olfactory epithelium, trachea, or lungs of formaldehyde-exposed mice.

3 Monteiro-Riviere and Popp (1986) evaluated damage to the respiratory epithelium due to
4 acute formaldehyde exposures. Male F344 (CDF [F344]/CrIBr) rats (three to five per group)
5 were exposed at 0.5, 2.0, 6.0, or 15 ppm (0.62, 2.5, 7.4, or 18.5 mg/m³) formaldehyde
6 6 hours/day for either 1, 2, or 4 days. Rats were sacrificed either immediately after exposure or
7 18 hours later (see Table 4-18). After fixation and decalcification, blocks of tissue were
8 collected from transverse sections of the skull. The first block of tissue, 1 µm thick, was taken
9 just posterior of the incisor teeth. The second block was taken halfway between the first block
10 and the incisive papillae. The dorsal nasal conchae, lateral wall, and ventral nasal conchae were
11 microdissected, postfixed, and viewed by transmission electron microscopy (Monteiro-Riviere
12 and Popp, 1986).

13
14 **Table 4-18. Concentration regimens for ultrastructural evaluation of male**
15 **CDF rat nasoturbinates**
16

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.

17
18 ^aNumber of exposed rats is shown in parentheses.

19 ^bFive control rats were examined for each experiment.

20
21 Source: Monteiro-Riviere and Popp (1986).

22
23
24 No lesions were observed at either 0.5 or 2.0 ppm formaldehyde for either 1 day or
25 4 days, evaluated 18 hours after exposure. However, an unusual altered ciliary configuration,
26 including blebbing of the cell membrane, was observed in almost all formaldehyde-treated rats,
27 whereas it was only “occasionally noted” in control rats. Focal lesions in the dorsal and ventral

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1 conchae and lateral wall were seen in rats exposed at 6 and 15 ppm for 1 day and sacrificed
2 immediately after exposure. These lesions included cytoplasmic and autophagic vacuoles, loss
3 of microvilli, and hypertrophy. Lesions increased in severity with both exposure concentration
4 and duration. Neutrophil infiltration and intercellular edema were seen after 1 day at 6 and
5 15 ppm. Nonkeratinized squamous metaplasia was noted after 4 days at 6 and 15 ppm in treated
6 rats. Cell death and sloughing were noted after only 2 days of exposure at 6 ppm formaldehyde.

7 As described above, Cassee and Feron (1994) examined the effects of intermittent
8 exposure to formaldehyde (3.5 ppm [4.3 mg/m³]), ozone (0.44 ppm [0.86 mg/m³]), or a
9 combination of the two on changes to the rat nasal epithelium. Exposure occurred through six
10 consecutive 12-hour cycles in which rats were exposed for 8 hours and then not exposed for a
11 further 4 hours. Rats were weighed before the first and after the last exposure periods and
12 sacrificed immediately after the last exposure. To collect tissue for biochemical analysis, skulls
13 were split sagittally and the respiratory epithelium collected. Tissues from six rats were pooled
14 and homogenized to enable the measurement of glutathione (GSH) and the activities of the
15 following enzymes: glutathione S-transferase (GST), glutathione peroxidase (GPX), glucose-6-
16 phosphate dehydrogenase (G6PDH), glutathione reductase (GR), alcohol dehydrogenase (ADH),
17 and formaldehyde dehydrogenase (FALDH). The remaining heads were fixed, decalcified, and
18 sectioned (standard cross sections [see Figure 4-6]).

19 All groups, including controls, lost weight during the course of treatment. Rats exposed
20 to formaldehyde, ozone, or both lost more weight than controls ($p < 0.05$, $p < 0.01$, and
21 $p < 0.001$, respectively). Formaldehyde treatment alone increased GPX from 48.6 to
22 64.0 $\mu\text{mole/minute-mg protein}$ ($p < 0.05$) (see Table 4-19). Formaldehyde exposure, in
23 conjunction with ozone, decreased GST from 490 to 389 $\mu\text{mole/minute-mg protein}$ ($p < 0.05$).
24 No other enzyme activities or tissue GSH levels were affected by formaldehyde exposure.

25 Formaldehyde-exposed rats exhibited lesions in the nasal epithelium at levels 2 and 3 of
26 the nose, with effects slightly more severe in level 2. Lesions observed include necrosis,
27 hyperplasia accompanied by squamous metaplasia, and rhinitis. Exposure to formaldehyde in
28 the presence of ozone resulted in more severe squamous metaplasia (statistics not given). These
29 findings are similar to those of Monteiro-Riviere and Popp (1986), indicating that single or
30 repeated exposures can result in cell damage and death. Cell death and increased cell
31 proliferation were seen here after 3 days of repeated exposures to 3.5 ppm formaldehyde. While
32 no increases were seen in olfactory epithelium, frank necrosis, squamous metaplasia, and
33 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)

^aValues shown are the means and SDs of three measurements of a pooled sample. Units are $\mu\text{mole/minute/mg}$ of cytosolic protein.

^bRats were exposed intermittently, 12-hour cycles of 8 hours exposed and 4 hours unexposed, for 3 days.

^cDifferent 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

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1 mucosal epithelium were seen in levels 2, 3, 4, and 5 of the nasal cavity, with more severe
2 changes noted in the nasal septum. Increased secretion and desquamation of mucosal cells
3 occurred in the trachea, and a slight hyperplasia of the alveolar wall was noted in rats exposed to
4 145.6 ppm formaldehyde (Kamata et al., 1996b)

5 Hester et al. (2003) carried out a transcriptional analysis of the nasal epithelium of male
6 F344 rats 24 hours after nasal instillation of 40 μ L of 400 mM formaldehyde. Immediately after
7 sacrifice, cells were harvested from the nasal cavity for RNA extraction. The authors found
8 several phase I and II enzymes, indicative of oxidative stress, to be elevated. They also reported
9 the greatest increase in inflammatory genes, such as iNOS and neuropeptides. In an effort to
10 phenotypically link any gene changes to pathology, Hester et al. (2003) noted that this exposure
11 scenario has been demonstrated to induce regenerative hyperplasia with minimal cytotoxicity. In
12 this regard, they observed no significant change in nine genes involved in three apoptotic
13 pathways.

14 In an expansion of their earlier study, Hester et al. (2005) carried out a transcriptional
15 analysis of the nasal epithelium of male F344 rats that had been exposed to formaldehyde by
16 nasal instillation for a single exposure, 5 days of exposures, or 28 days of exposure. In addition,
17 this study also attempted to characterize the comparative toxicity of glutaraldehyde with
18 structurally similar formaldehyde (van Birgelen et al., 2000). Thus, four animals per group were
19 instilled with 40 μ L of deionized water (control group), 40 μ L of 400 mM formaldehyde, or
20 40 μ L of 20 mM glutaraldehyde. Phenotypically, both aldehydes induced similar
21 histopathologic changes.

22 Both aldehydes induced similar changes in DNA repair and apoptotic pathways initially,
23 but the patterns of gene changes were different after about 5 days of exposure. Eight genes were
24 differentially expressed between formaldehyde and glutaraldehyde that indicated different
25 pathways for DNA repair, including recombination, base excision repair, and nucleotide excision
26 repair. Within this group, replication protein 70 and DNA excision repair ERCC1 showed a
27 twofold induction by formaldehyde compared with glutaraldehyde. Since both of these genes
28 and their products function by recognizing and removing damaged DNA bases, Hester et al.
29 (2005) hypothesized that formaldehyde-exposed cells may remove damaged bases more
30 efficiently than glutaraldehyde-exposed cells

31 In addition to nasal pathology, several researchers specifically investigated
32 formaldehyde-induced effects in the trachea, bronchi, and pulmonary tissues of the deep
33 respiratory tract in a variety of species (Lino dos Santos Franco et al., 2006; Kamata et al.,
34 1996a, b; Schreibner et al., 1979; Ionescu et al., 1978).

1 Ionescu et al. (1978) described progressive damage in pulmonary tissue of adult male
2 rabbits exposed to an aerosol of 3% formaldehyde solution 3 hours/day for up to 50 days
3 (method of aerosol generation or particle size were not provided). An equivalent air
4 concentration was not reported and cannot be derived from the information given. Animals were
5 sacrificed at several time points (3, 7, 15, 20, 30, and 50 days), and fragments of the caudal lobes
6 of both lungs were taken to examine bronchi (intrapulmonary and distal) and lung parenchyma.
7 Enzymatic activity was characterized in frozen sections for β -galactosidase, adenosine
8 triphosphatase (ATPase), adenosine monophosphatase (AMPase), lactate dehydrogenase (LDH),
9 malate dehydrogenase, succinate dehydrogenase (SDH), acid phosphatase, Tween-60 esterase,
10 naphthol-AS-D-acetate esterase, proline oxidase, hydroxyproline epimerase, leucyl
11 aminopeptidase, and β -glucuronidase. A portion of the lung was fixed and sectioned and viewed
12 by light microscopy to determine changes in cell populations and tissue pathology.

13 In addition, biochemical analysis revealed that enzymatic activity of β -galactosidase,
14 ATPase, AMPase, LDH, malate dehydrogenase, and SDH were all unchanged by formaldehyde
15 exposure across the course of treatment (Ionescu et al., 1978). The activities of several enzymes
16 were increased through the course of exposure, including acid phosphatase, Tween-60 esterase,
17 naphthol-AS-D-acetate esterase, proline oxidase, and hydroxyproline epimerase. Although no
18 details were reported, the authors described the changes as progressive, with the increase in
19 proline oxidase and hydroxyproline epimerase seen only in the second half of the treatment
20 course. The activities of two enzymes, leucyl aminopeptidase and β -glucuronidase, were
21 observed to decrease rapidly (time frame not provided) (Ionescu et al., 1978)

22 Histologic changes in the lung tissue were noted after only 3 days of exposure and were
23 generally progressive throughout the course of treatment. Early changes in the bronchial
24 epithelium included increased mucus secretion, hyperplasia, and hypertrophy of epithelial cells.
25 Lymphocyte infiltration was noted in many areas, and a limited thickening of the alveolar walls
26 was reported after 3 days of exposure. Epithelial cell lesions, thickening of the alveolar, and
27 infiltration of lymphocytes increased as exposure continued. Mucus cells increased as much as
28 40% after 40 days of treatment. After 40 days of treatment, Ionescu et al. (1978) observed
29 “destructive and fibrotic lesions” and provided a detailed description of progressive lesions.

30 Schreiber et al. (1979) also examined histologic changes in lung tissue after high
31 formaldehyde exposures. Syrian golden hamsters (34, sex not stated) were exposed to 250 ppm
32 (308 mg/m³) formaldehyde 1 hour/day for 1, 2, 5, or 15 days. Five hamsters in each treatment
33 group were sacrificed 2 days after exposure was ended. Three hamsters in each group were
34 sacrificed 1, 2, or 6 weeks after exposure ended to determine if formaldehyde-induced changes
35 regressed over time. Tracheal washing was carried out to collect cytologic samples in each

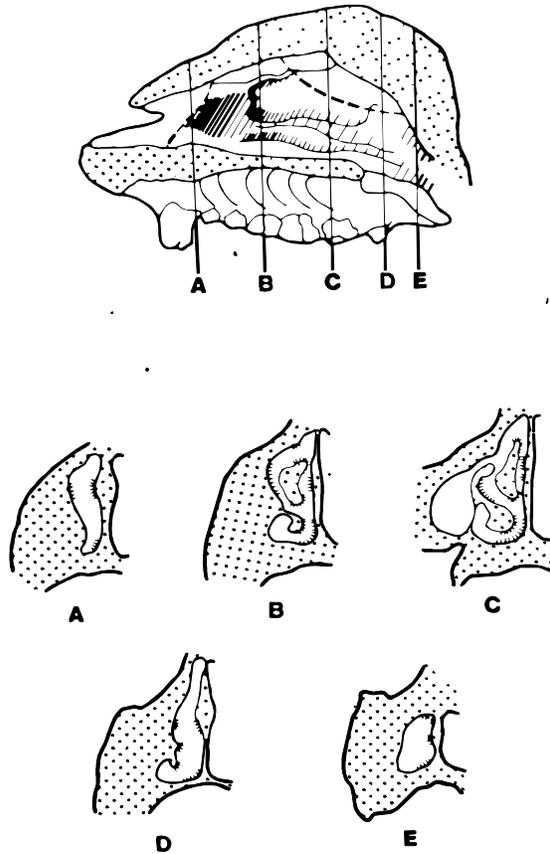
1 animal prior to sacrifice. Samples were fixed, stained, and examined by light microscopy.
2 Lungs and tracheae were removed en bloc and fixed, and 20, 1 µm thick cross sections were
3 taken (location not detailed). The remaining respiratory tissue was sectioned at 200 µm
4 intervals. Sections were stained and viewed by light microscopy.

5 Abnormal epithelial cells were found in tracheal washings from formaldehyde-exposed
6 hamsters. Schreiber et al. (1979) described cells with lobulated nuclei and a coarse chromatin
7 pattern, especially in cells showing signs of degeneration (e.g., vacuolization of nuclei and
8 cytoplasm) (Schreiber et al., 1979). Cell number and damage were not quantified, and there was
9 no discussion of the effects of exposure duration on treatment, if any, on these observations.
10 Tracheal washing was normal 2 and 6 weeks after the end of exposure, indicating that the
11 cytological changes were reversible (Schreiber et al., 1979).

12 Formaldehyde exposure caused multifocal lesions in the mucociliary epithelium in the
13 trachea and larger bronchi. Dysplastic and poorly differentiated squamous metaplastic foci
14 replaced ciliated epithelium (Schreiber et al., 1979). Abnormal nuclear membranes, tonofibrils
15 around the nuclei, the appearance of nucleoli, and heterochromatin condensation were distinct in
16 the formaldehyde-treated hamsters. These changes, observed 2 days after formaldehyde
17 exposure, were reversible over time and not seen 2 and 6 weeks later.

18 Because of the similarity of form and physiology of rhesus monkey URTs to the human
19 respiratory tract, the effects of short-term formaldehyde exposure were evaluated in both nasal
20 and lung tissue in these monkeys by Monticello et al. (1989). Male rhesus monkeys (nine/group)
21 were exposed to 6 ppm formaldehyde (7.4 mg/m³) 6 hours/day for 5 days/week for either 1 or
22 6 weeks. Control animals were exposed to the same regimen of filtered air for 1 week. Monkeys
23 were weighed during the course of exposure and observed for clinical signs of irritation or
24 sickness. Monkeys were intravenously injected with [³H]-thymidine 18 hours after the last
25 formaldehyde treatment to evaluate induced cell proliferation. Sections of the nasal passages,
26 trachea, larynx, lung carina, and duodenum were processed for histoautoradiography. Tissues
27 fixed and sectioned for examination by light microscopy included nose, adrenal, sternum (bone
28 marrow), duodenum, esophagus, eyes, gallbladder, heart, kidney, liver, lymph nodes, pancreas,
29 stomach, spleen, and tongue. The nose was cut into a series of transverse sections, 3 µm thick,
30 and sections from five levels were examined (see Figure 4-10). Lung lobes were trimmed
31 midsagittally and sectioned with care to include airway bifurcations. Sections of the nasal
32 passages, trachea (cross section), larynx (cross section), lung carina (frontal section), and
33 duodenum were also processed for histoautoradiography.

34



1
2 **Figure 4-10. Diagram of nasal passages, showing section levels chosen for**
3 **morphometry and autoradiography in male rhesus monkeys exposed to**
4 **formaldehyde.**

5
6 Source: Redrawn from Monticello et al. (1989).

7
8
9 There were no significant changes in body weight over the course of the experiment.
10 Oronasal breathing was noted in the first 15 minutes of formaldehyde exposure (Monticello et
11 al., 1989). Monkeys did experience eye irritation (mild lacrimation and conjunctival hyperemia)
12 during exposure.

13 Formaldehyde-related lesions were reported in the nasal passages, tracheas, and in the
14 larynx of treated animals (see Figure 4-11) (Monticello et al., 1989). Nasal epithelium from
15 treated animals exhibited many of the histologic lesions described in rodent studies, including
16 loss of goblet cells, loss of cilia, epithelial hyperplasia, squamous metaplasia, and neutrophilic
17 inflammatory response in the respiratory epithelium. The lesions were more severe after
18 6 weeks of exposure and were present over a greater percentage of the epithelium compared with
19 the 1-week exposure group ($p < 0.05$) (see Figure 4-11).

20
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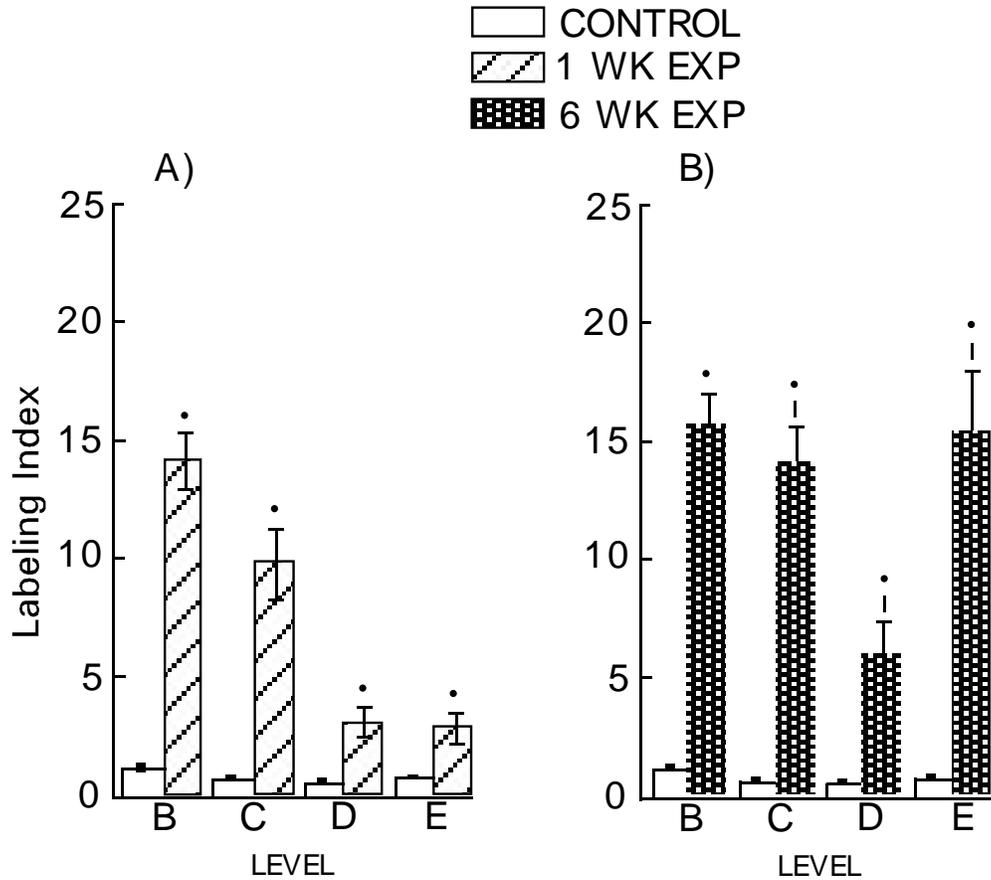


Figure 4-11. Formaldehyde-induced cell proliferation in male rhesus monkeys exposed to formaldehyde

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

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1 formaldehyde exposure (see Figure 4-12) (Monticello et al., 1989). Similar trends were seen
 2 after only 1 week but were statistically significant only in the respiratory epithelium. Although
 3 increased proliferation in the trachea and carina was statistically significant after 1 week of
 4 exposure, the greater increases seen after 6 weeks of exposure, compared with controls, were not
 5 statistically significant. A small sample size ($n = 3$) and high variability may have contributed to
 6 the lack of statistical significance. Monticello et al. (1989) noted that increased cell proliferation
 7 was seen in locations with minimal histologic changes, indicating proliferation may be a more
 8 sensitive predictor of adverse health effects of formaldehyde exposure.

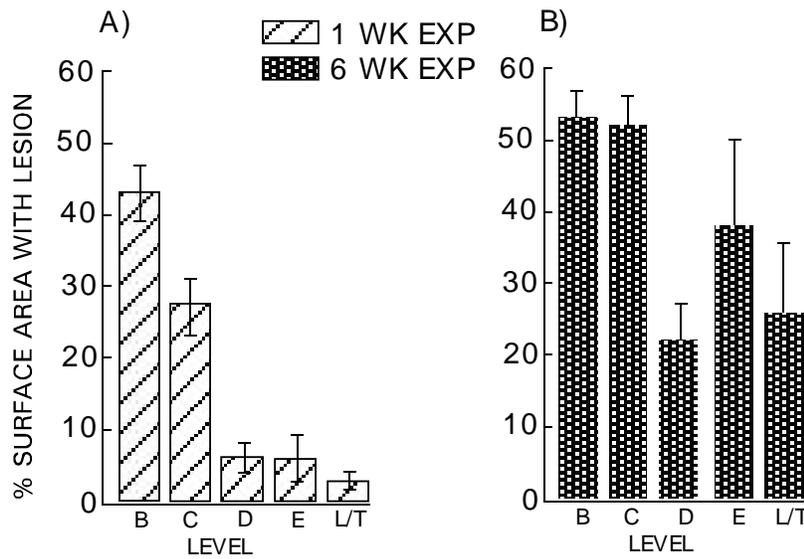


Figure 4-12. Formaldehyde-induced lesions in male rhesus monkeys exposed to formaldehyde.

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

1 rats to 0, 128.4, or 294.5 ppm (0, 158, or 362 mg/m³) formaldehyde for 6 hours. Lung lavage
 2 samples were collected and the fluid analyzed for the lipids, free cholesterol, phosphatidyl
 3 ethanolamine, phosphatidyl choline, sphingomyelin, and triglyceride.

4 The bronchoalveolar lavage (BAL) was analyzed for triglycerides, cholesterol, and
 5 phosphatidyl choline. As in the first experiment (Kamata et al., 1996a), triglyceride
 6 concentration was reduced in the lavage of treated animals, in this case, to 16% of controls in
 7 lavage in those rats exposed to 145.6 ppm formaldehyde (see Table 4-20). Cholesterol
 8 concentration was unchanged and phosphatidyl choline was increased to 220% of that of control
 9 rats as a result of exposure to 145.6 ppm formaldehyde. However, BAL lipids were unchanged
 10 in 15 ppm exposed rats. Triglycerides were reduced in unwashed lung tissue from
 11 formaldehyde-treated rats in a concentration-dependent manner and free fatty acids were reduced
 12 in rats exposed to 145.6 ppm formaldehyde. Neither triglyceride nor sphingomyelin was
 13 detected in lung lavage fluid from the high treatment group.

14
 15 **Table 4-20. Lipid analysis of lung tissue and lung lavage from male F344**
 16 **rats exposed to 0, 15, or 145.6 ppm formaldehyde for 6 hours**
 17

	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

18 ^aSD given in parentheses.

19 ^bSignificant difference from controls ($p < 0.05$).

20 ^cSignificant difference from controls ($p < 0.01$).

21 Source: Kamata et al. (1996b).

22
 23
 24
 25 Concentration-dependent decreases were seen in nonprotein sulfhydryl (SH) groups and
 26 lipooxygenase in nasal mucosa homogenate and nonprotein SH groups in lung tissue

1 homogenate (see Table 4-21). Increases in both lipooxygenase and LDH activities were found in
2 lung tissue homogenate from formaldehyde-exposed rats.
3

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

^aSD given in parentheses.

^b5 or 10% nasal mucosa homogenates.

^cnmol malonaldehyde/g tissue.

^dUnits per gram tissue.

^e20% lung homogenates.

^fSignificant difference from controls ($p < 0.01$).

^gSignificant 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/mm³). These values are means \pm standard error of

1 the mean (SEM) for six animals/group. The effect appeared to reflect changes in the
 2 mononuclear cell population ($1.1 \pm 0.02 \times 10^4$ versus $0.6 \pm 0.003 \times 10^4$ cells/mm³) rather than
 3 peripheral blood neutrophils ($0.2 \pm 0.003 \times 10^4$ cells/mm³ in test animals and controls). There
 4 was also an apparently compound-related increase in the total cell count in the spleen
 5 ($112.7 \pm 4.4 \times 10^6$ versus $94.2 \pm 5.5 \times 10^6$ cells). However, a change in the number of cells
 6 eluted from bone marrow did not reach statistical significance ($54.6 \pm 1.3 \times 10^6$ versus
 7 $45.0 \pm 4.3 \times 10^6$ cells). Lino dos Santos Franco et al. (2006) provided data on dose-dependent
 8 changes in methacholine-induced contractions in isolated tracheae and bronchi obtained from
 9 formaldehyde-exposed and control rats. Although the maximal contractile response induced by
 10 methacholine in tracheae of formaldehyde-treated rats was unchanged compared with controls,
 11 contractions in isolated bronchi were significantly weaker than those observed in controls.

12 The authors examined the effect of formaldehyde inhalation on rat lung mast cells.
 13 Degranulation and significant neutrophil infiltration were features of the response to
 14 formaldehyde (see Table 4-22).
 15

16 **Table 4-22. Mast cell degranulation and neutrophil infiltration in the lung of**
 17 **rats exposed to formaldehyde via inhalation**
 18

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

19
 20 ^aValues are means ± SEM; n = 6.
 21 ^b4.2 ± 0.6 cells/mm² intact mast cells were found in the lungs of controls.
 22 ^cNo statistical analysis was provided by the authors for these changes.
 23

24 Source: Lino dos Santos Franco et al. (2006).
 25
 26

27 Selected pharmacological agents were used to explore the mechanism by which exposure
 28 to formaldehyde might have brought about the observed lung infiltration and bronchial
 29 hyporesponsiveness. Lino dos Santos Franco et al. (2006) provided data showing that separate
 30 pretreatment of the animals with compound 48/80, sodium cromoglycate (SCG), and
 31 indomethacin reduced the formaldehyde effect on neutrophil release into BAL but had no effect
 32 on mononuclear cell counts. Compound 48/80 and SCG also reversed the formaldehyde-induced
 33 reduction in bronchial response to methacholine, but indomethacin had the opposite effect
 34 (causing an additional decrease in bronchial responsiveness). In broad terms, these findings
 35 were thought to implicate mast cells as a possible mediator of the toxicological effects of

1 formaldehyde. Histologically, a significantly increased number of degranulated mast cells were
2 evident in the pulmonary tissue of rats that were exposed to formaldehyde.

3 Lino dos Santos Franco et al. (2006) also examined the regulatory role of NO on
4 formaldehyde-induced bronchial activity. Nitrites generated by cultured cells of BAL from
5 formaldehyde-treated rats increased about threefold compared with those from controls.
6 However, pretreatment with the NO synthase inhibitor, *N*-nitro-L-arginine methyl ester,
7 prevented the formaldehyde-induced bronchial hyporesponsiveness to methacholine but had no
8 effect on pulmonary leukocyte recruitment. These data implicate the existence of distinct
9 mechanisms for the induction of lung inflammation versus bronchial hyporeactivity. Further
10 support for this concept came from an experiment in which rats were pretreated with capsaicin to
11 examine the involvement of sensory fibers in lung inflammation and the bronchial
12 hyporesponsiveness induced by formaldehyde inhalation. Although the treatment did not
13 influence formaldehyde-induced bronchial hyporesponsiveness to methacholine, the number of
14 leukocytes recovered in the BAL fluid were reduced compared with those of rats exposed to
15 formaldehyde alone.

16 17 ***Extrapulmonary effects***

18 Kamata et al. (1996a) exposed male F344 rats to 0, 128.4, or 294.5 ppm (0, 158, or 362
19 mg/m³) formaldehyde for 6 hours. In addition, blood samples were monitored for hematology
20 and clinical chemistry parameters, including red blood cell (RBC) count, hemoglobin (Hb),
21 packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin
22 concentration (MCHC), white blood cell (WBC) count, and plasma levels of total protein (TP),
23 albumin (ALB), blood urea nitrogen (BUN), glucose, phospholipids, triglycerides, total
24 cholesterol, cholinesterase, and LDH. Male rats exposed to 294.5 ppm formaldehyde had
25 increased RBC count, Hb, hematocrit (HCT), MCV, and serum glucose ($p < 0.05$) compared
26 with controls (Kamata et al., 1996a). There were concentration-related decreases in serum
27 measures of TP, ALB, and phospholipids ($p < 0.05$). BUN was decreased in rats exposed to
28 128.4 ppm but increased in the higher treatment group ($p < 0.05$). Phospholipid analysis of the
29 lung surfactant indicated a decrease in the production in formaldehyde-treated animals ($p <$
30 0.05). Total free cholesterol, phosphatidyl ethanolamine, and phosphatidyl choline were reduced
31 to 60, 55, and 38% of controls for rats treated with 294.5 ppm formaldehyde ($p < 0.05$).
32 Sphingomyelin was reduced to 32% of controls in the low treatment group ($p < 0.05$).

33 In a subsequent study in the same laboratory (Kamata et al., 1996b), male F344 rats were
34 exposed to either 0, 15, or 145 ppm (0, 18.5, or 178 mg/m³) formaldehyde nose only for 6 hours
35 (Kamata et al., 1996b). Fifteen animals were treated at each level and separated into subgroups

1 of five animals each for tissue collection and the determination of other endpoints. Blood
2 samples were collected from one subgroup to determine such hematological and clinical
3 chemistry parameters as RBC count, Hb, PCV, MCV, MCHC, WBC count, and plasma levels of
4 TP, ALB, BUN, glucose, phospholipids, triglycerides, total cholesterol, LDH, alkaline
5 phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and
6 G6PDH. BAL was collected from five animals of each group and analyzed for phospholipids.
7 Lung homogenate from five animals in each treatment group was analyzed for nonprotein SH
8 groups, lipid peroxides, and total lipids. The 20,000 × g supernatant of the lung homogenate was
9 assayed for the activities of GR, G6PDH, and LDH. Similarly, nonprotein SH groups and lipid
10 peroxidase were measured in homogenates of excised nasal mucosa. At autopsy, organs (brain,
11 heart, lung, liver, kidney, spleen, and testis) were weighed and tracheae and nasal turbinates
12 examined. After fixation and decalcification, five sections across the nose were taken,
13 corresponding to standard sections 1–5 (see Figure 4-6).

14 Several blood parameters were affected after these acute exposures. The WBC count was
15 slightly increased, from 4.7×10^3 cells/mm³ in control rats to 5.1×10^3 cells/mm³ and 6.1×10^3
16 cells/mm³ at 15 and 145.6 ppm formaldehyde, respectively (Kamata et al., 1996b). Serum levels
17 of AST and LDH decreased in an apparent concentration-dependent manner (AST 68 and 54%
18 of controls and LDH 48 and 28% of controls, respectively). Serum levels of G6PDH and ALT
19 were decreased similarly across exposure groups at 45 and 78% of controls, respectively.

20 A synopsis of respiratory pathology findings following short-term exposure to
21 formaldehyde is presented in Table 4-23.

22

23 **4.2.2.4. Subchronic Studies**

24 In a study by Maronpot et al. (1986), female and male B6C3F1 mice (10/group) were
25 exposed at 0, 2, 4, 10, 20, or 40 ppm (0, 2.46, 4.92, 12.3, 24.6, or 49.2 mg/m³) formaldehyde 6
26 hours/day, 5 days/week for 13 weeks. Clinical observations were made daily, and mice were
27 weighed weekly. At autopsy, tissue sections from each organ system (approximately 50 tissues
28 per mouse) were fixed, stained, and examined by light microscopy. Noses were fixed,
29 decalcified, and transversely trimmed at three levels: the incisor teeth, midway between the
30 incisor teeth and first molar teeth, and the second molar teeth (corresponding to sections 2, 3, and
31 4 in Figure 4-6).

32 Although control mice gained weight, mice exposed to 40 ppm formaldehyde lost weight
33 during the 13-week exposures. Expressed by the authors as a percent of weight gain in controls,
34 the weight losses were –235% in males and –168.6% in females. Early mortality for both male
35 and female mice exposed to 40 ppm was 80%. Although gross and histochemical effects in
36 excised pieces from each organ system were evaluated, endometrial hypoplasia in mice treated

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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
<i>Nasal pathology</i>					
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
<i>Tracheal and lung pathology</i>					
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)
<i>Extrapulmonary effects</i>					
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.

1 with 40 ppm was the only effect noted outside the respiratory system. The authors considered
2 this effect secondary to the observed respiratory tract lesions and frank toxicity at 40 ppm
3 formaldehyde.

4 While no statistical comparison was provided, respiratory tract lesions showed an
5 increased incidence with concentration, as well as an increased distribution throughout the
6 respiratory tract (see Table 4-24). No lesions were seen in the nasal cavity, larynx, trachea, or
7 lung of control mice or mice treated with 2 ppm formaldehyde. Minimal squamous metaplasia in
8 the nasal cavity was noted in 1 of 10 male mice treated with 4 ppm formaldehyde, but none were
9 observed in the female mice. However, squamous metaplasia was observed in all mice in the
10 higher treatment groups (10, 20, and 40 ppm). Lesions became more severe and penetrated more
11 deeply into the respiratory tract as exposure concentration increased. Where lesions were present
12 in the nasal cavities of all mice exposed to 10 ppm, similar lesions were reported in the larynx
13 and trachea of some animals exposed to 20 ppm and all animals exposed to 40 ppm
14 formaldehyde. Mice exposed to 40 ppm formaldehyde exhibited lesions as deep as the lung,
15 including squamous metaplasia, submucosal fibrosis inflammation, and epithelial hyperplasia.

16 The findings of Maronpot et al. (1986) indicated a no-observed-adverse-effect level
17 (NOAEL) of 4 ppm and a LOAEL of 10 ppm in mice, based on squamous metaplasia in the
18 nasal epithelium. Although a LOAEL of 10 ppm was observed, there was 80% mortality for
19 both sexes at 40 ppm, indicating a very narrow range between the first observed adverse health
20 effects and frank effect concentrations in mice for this 13-week treatment.

21 In a study by Woutersen et al. (1987), male and female albino SPF Wistar rats (10/group)
22 were exposed to 0, 1, 10, or 20 ppm (0, 1.23, 12.3, or 24.6 mg/m³) formaldehyde 6 hours/day,
23 5 days/week for 13 weeks. Rats were checked daily and weighed weekly. Three longitudinal
24 sections of lungs, trachea, and larynx and six standard cross sections of the nose were taken for
25 microscopic examination. Two rats per exposure group were similarly treated for 3 days and
26 sacrificed 18 hours later, and nasoturbinates were dissected to measure cell proliferation.
27 Woutersen et al. (1987) noted that the majority of the dose-dependent increases in cell
28 proliferation seen at section level 3 after 3 days of repeated 6-hour exposures to 10 and 20 ppm
29 (12.3 and 24.6 mg/m³) formaldehyde occurred in areas of the epithelium showing “clear
30 squamous metaplasia and hyperplasia.” Cell proliferation rates in metaplastic epithelium of
31 29.5 and 33.2% were much higher than the 1.4 to 2.8% proliferation in the visibly unaffected
32 respiratory epithelium from rats exposed at 10 ppm formaldehyde. Although there was a slight
33 trend towards increased cell proliferation in the visibly unaffected epithelium of exposed animals
34 compared with unexposed controls, the majority of increased cell proliferation resulting from
35 exposure to 10 and 20 ppm formaldehyde was attributed to the metaplastic epithelium
36 (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	—

^aDash indicates no lesions recorded in that treatment group.

^bND = no data.

Source: Maronpot et al. (1986).

1 Statistically significant increases were seen in focal respiratory epithelial hyperplasia and
2 keratinization in both male and female rats at the highest treatment level (20 ppm) (see
3 Table 4-25). Male rats also had statistically significant increases in observed respiratory
4 epithelial squamous metaplasia, focal olfactory epithelial thinning, and rhinitis. Both male and
5 female rats treated with 10 ppm formaldehyde showed statistically significant increases in
6 squamous metaplasia, hyperplasia, and keratinization of the respiratory epithelium (Woutersen et
7 al., 1987).

8 Disarrangement of the respiratory epithelium was only significantly increased in female
9 rats, but this change was observed at both the 10 and 20 ppm treatment levels. Although some
10 lesions were observed in animals treated with 1 ppm formaldehyde, their incidences were not
11 statistically significant and the findings were equivocal.

12 Feron et al. (1988) examined recovery of formaldehyde-induced nasal lesions after
13 subchronic exposures. Male albino SPF Wistar rats (50–55/group) were exposed to 0, 10, or
14 20 ppm (0, 12.3, or 24.6 mg/m³) formaldehyde 6 hours/day, 5 days/week for either 4, 8, or
15 13 weeks. All groups were observed for a total of 130 weeks, including treatment and recovery.
16 Rats were weighed weekly for the first 13 weeks and monthly thereafter. Rats (five/group) were
17 sacrificed immediately after the end of exposure (4, 8, or 13 weeks). The balance of the rats
18 were sacrificed after 130 weeks, inclusive of exposure time. At sacrifice, noses were fixed and
19 sectioned by using standard section levels.

20 Formaldehyde exposure (20 ppm) was associated with reduced body weight throughout
21 the exposure period (4, 8, or 13 weeks). However, body weight in these groups matched that of
22 controls after 8, 40, and 100 weeks, respectively. Rats exposed to 10 ppm for 8 or 12 weeks had
23 slightly decreased body weight (further details not given).

24 Nonneoplastic lesions were reported in the nasal mucosa of rats exposed to either 10 or
25 20 ppm formaldehyde and examined immediately after exposure was discontinued (4, 8, or
26 13 weeks). Lesions increased in severity with both exposure duration and concentration (details
27 of severity and incidence were not provided). Rhinitis, hyperplasia, and squamous metaplasia of
28 the respiratory epithelium were seen in rats from both dose groups, but changes in olfactory
29 epithelia were only seen in rats exposed to 20 ppm, where cell disruption, thinning of the
30 epithelium, and simple cuboidal or squamous metaplasia were also reported. Changes in the
31 dorsomedial region, at the junction of the respiratory and olfactory epithelium, were similar to
32 those seen in the olfactory epithelium of rats exposed to 20 ppm formaldehyde. A similar
33 concentration- and duration-dependent increase in histopathologic changes in nasal epithelium
34 was observed after the full 130 weeks, which included 126, 122, or 117 weeks of recovery for
35 the three duration groups, 4, 8, and 13 weeks, respectively (see Table 4-25).

1
2
3
4

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
<i>Respiratory epithelium</i>	<i>Severity</i>	<i>Males</i>				<i>Females</i>			
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	–	–	–	–
<i>Olfactory epithelium</i>									
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	–	–	–	–
<i>Rhinitis</i>		–	2	5 ^b	10 ^c	–	–	3	2
<i>Larynx</i>									
Squamous metaplasia	Very slight	–	–	–	3	–	NE ^d	NE	–
	Slight	–	–	–	1	–	NE	NE	–
	Moderate	–	–	–	1	–	NE	NE	–
Keratinization	Slight	–	–	–	2	–	NE	NE	–

5 ^a Dash indicates no lesions reported.

6 ^b Different from control, $p < 0.05$.

7 ^c Different from control, $p < 0.01$.

8 ^d NE = not evaluated.

9 Source: Woutersen et al. (1987).

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1 Feron et al. (1988) did not provide a direct comparison among lesions reported at the
 2 interim sacrifice and terminal sacrifice after the extended recovery period. However, similar
 3 lesions were reported after the recovery period, including focal hyperplasia and stratified
 4 squamous metaplasia of the respiratory epithelium, stratified cuboidal or squamous metaplasia in
 5 the dorsomedial area, and replacement of olfactory epithelium. The incidence and severity of
 6 these lesions in rats exposed to 20 ppm formaldehyde were statistically different from control
 7 animals, regardless of exposure duration (see Table 4-26).

8
 9 **Table 4-26. Formaldehyde-induced nonneoplastic histopathologic changes in**
 10 **male albino SPF Wistar rats exposed to 0, 10, or 20 ppm formaldehyde**
 11 **(6 hours/day, 5 days/week) and examined at the end of 130 weeks inclusive of**
 12 **exposure**
 13

Formaldehyde, ppm	4 Weeks			8 Weeks			13 Weeks		
	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

14
 15 ^aSignificantly different from control, $p < 0.05$.

16 ^bSignificantly different from control, $p < 0.01$.

17
 18 Source: Feron et al. (1988).
 19

1 Although a slight increase in changes to the olfactory epithelium and dorsomedial area
 2 was seen in rats treated with 20 ppm formaldehyde for only 4 weeks, these differences were
 3 significant and more severe in the 8- and 13-week treatment groups. Replacement of olfactory
 4 epithelium by respiratory epithelium was described as slight after 8 weeks of exposure and slight
 5 to moderate after 13 weeks of exposure in the 20 ppm treatment groups. Therefore,
 6 formaldehyde-induced lesions were not resolved after a considerable nonexposure recovery
 7 period of up to 126 weeks (Feron et al., 1988).

8 Feron et al., (1988) derived a correlation between the development of nonneoplastic
 9 changes in nasal epithelium and the development of nasal tumors as a result of these subchronic
 10 formaldehyde exposures. Two SCCs were reported in rats exposed to 10 ppm formaldehyde but
 11 were not considered to be formaldehyde related because of their locations (nasolacrimal duct,
 12 incisor tooth). Six tumors were observed in the 20 ppm, 13-week exposure group (see
 13 Table 4-27) of which three of the tumors were SCCs similar to those observed as a result of
 14 chronic formaldehyde exposure. Two polypoid adenomas also were reported in rats exposed to
 15 20 ppm formaldehyde. Feron et al. (1988) concluded that subchronic exposures to 20 ppm
 16 formaldehyde could result in an increase in nasal tumors, an effect that followed observation of
 17 cellular proliferation.

18
 19 **Table 4-27. Formaldehyde-induced nasal tumors in male albino SPF Wistar**
 20 **rats exposed to formaldehyde (6 hours/day, 5 days/week for 13 weeks) and**
 21 **examined at the end of 130 weeks inclusive of exposure**
 22

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

23
 24 ^aTumor considered to be associated with formaldehyde exposure.
 25

26 Source: Feron et al. (1988).

1 A companion study from the same laboratory examined the effects of lower concentration
2 formaldehyde exposures (Zwart et al., 1988). Male and female albino Wistar rats (50/group)
3 were exposed to 0, 0.3, 1, or 3.0 ppm (0, 0.37, 1.2, or 3.7 mg/m³) formaldehyde 6 hours/day,
4 5 days/week for 13 weeks. Body weight, general condition, and behavior were recorded weekly.
5 No effects of formaldehyde exposure on body weight changes were noted, and growth was
6 considered comparable among different exposure groups and controls. Rats were sacrificed
7 during week 14, and noses were fixed and sectioned (exact time after exposure ended not given).
8 Six standard cross sections were examined for each animal by light microscopy, anterior to
9 posterior. Noses were fixed and decalcified, and six standard cross sections were taken and
10 developed.

11 No formaldehyde-related lesions were reported in the respiratory epithelium at section
12 level 3 after 13 weeks of formaldehyde exposure (0.1 ppm, 1 ppm, or 3 ppm). Signs of
13 inflammation (rhinitis, sinusitis, mononuclear cell infiltrates) were observed in
14 formaldehyde-treated rats, but there was no concentration-response relationship (data not
15 provided). Formaldehyde-related pathology in the anterior part of level 2 epithelium was
16 reported in 37/50 males and 21/50 female rats exposed to 3.0 ppm for 13 weeks. Both
17 keratinized and unkeratinized squamous metaplasia were present, and disarranged cells and
18 hyperplastic respiratory epithelium were found in the transitional zone between squamous and
19 pseudostratified epithelium at level 2. Foci of keratinized squamous epithelium, glandularization
20 of goblet cells, and deciliated epithelium were observed by electron microscopy in anterior
21 sections of level 2 of rats exposed to 3 ppm formaldehyde. Epithelial cells with irregularly
22 shaped and strongly indented nuclei were described at level 2 in animals exposed to 0.3 and
23 1 ppm formaldehyde and were considered to be disarranged as well at 3 ppm formaldehyde
24 exposures.

25 Although early cell proliferation at level 3 corresponded to basal cell hyperplasia at
26 3 days, neither effect persisted for the course of the exposure. The authors speculate that this is
27 an indication of an adaptive response, perhaps through increased function of the mucociliary
28 apparatus present at level 3. In contrast, the early changes at section level 2 were less dramatic
29 but persisted through 13 weeks, including clear formaldehyde-related pathology.

30 Concentration times time ($C \times t$) issues have been investigated for histopathology as well
31 as for cellular proliferation, outlined above. Specifically, Wilmer et al. (1989, 1987) compared
32 the effects of 8-hour continuous and 8-hour intermittent formaldehyde exposure in two studies.
33 Fifty male albino Wistar rats (10/group) were exposed to different exposure regimens to achieve
34 similar compound-related $C \times t$ products. A $C \times t$ product of 40 ppm-hours (49.2 mg/m³-hours)
35 was attained by an 8-hour exposure to 5 ppm (6.2 mg/m³) or a 4-hour exposure to 10 ppm (12.3

1 mg/m³) (Wilmer et al., 1987). Similarly, an 80 ppm-hours (98.4 mg/m³-hours) C × t product was
2 attained from continuous 10 ppm exposure or intermittent 20 ppm (24.6 mg/m³) exposure. Rats
3 were exposed to one of these regimens 8 hours/day for either 3 days (two/group) or 4 weeks
4 (eight/group). Eighteen hours after exposure ended, rats were injected with [³H]-thymidine and
5 sacrificed 2 hours later. Noses were fixed and decalcified, and six standard cross sections were
6 taken and developed.

7 Thinning and disarrangement of the respiratory epithelium, squamous metaplasia, basal
8 cell hyperplasia, and rhinitis were seen in formaldehyde-treated rats. Lesions were most severe
9 in group 4 (20 ppm intermittent). Groups 2 and 3 had similar lesions (10 ppm intermittent and
10 continuous). Rats in group 1 had mild lesions. Formaldehyde concentration was the major
11 determinate in severity of nasal lesions. Formaldehyde effects were less severe in group 1 than
12 in group 3, even though the C × t product was the same, indicating concentration rather than
13 duration or cumulative exposure correlates to severity. Epithelial lesions in group 3 rats were
14 similar among rats exposed to 10 ppm, regardless of duration (groups 2 and 3).

15 In a follow-up study, Wilmer et al. (1989) assessed both cellular proliferation and
16 histologic lesions in Wistar rats exposed to formaldehyde in groups that differed by
17 concentration and time. Group A served as a control group (0 ppm). Group B was exposed to
18 1 ppm for 8 hours, group C to 2 ppm for 8 hours, group D to 2 ppm for 4 hours (30 minutes for
19 8 hours), and group E to 4 ppm for 4 hours (30 minutes for 8 hours). The experimental design
20 and cellular proliferation results are illustrated in Table 4-28. Intermittent exposures at 2 and 4
21 ppm resulted in formaldehyde-related histopathologic lesions similar to those reported by Zwart
22 et al. (1988). Disarrangement and squamous metaplasia in respiratory epithelium were observed
23 at 4 ppm (see Table 4-28). Disarrangement, nest-like infolds, goblet cell hyperplasia, and rhinitis
24 were observed at 2 ppm. Rats exposed continuously for 8 hours at 2 ppm formaldehyde had
25 fewer lesions than rats intermittently exposed to 2 ppm and were not statistically different from
26 controls. Although lesions were noted in rats given the continuous 1 ppm, 8-hour treatment,
27 their incidence was not significantly different from the controls (see Table 4-28). It should be
28 noted that the control rats in this study were reported to have a higher frequency of lesions than
29 controls in two previous studies from this laboratory employing the same techniques (Zwart et
30 al., 1988; Woutersen et al., 1987). For example, lesions noted in the respiratory epithelium of 25
31 control rats included 13 disarrangements, 13 basal cell hyperplasia, and 5 each of goblet cell
32 hyperplasia, nest-like infolds, and squamous metaplasia. This is in contrast to the data of
33 Woutersen et al. (1987), who reported no lesions in the respiratory epithelium of 20 control rats
34 (male and female). Although Zwart et al. (1988) discussed inflammatory lesions in control rats,
35 no mention was made of the other scored lesions in control animals. Overall, Wilmer et al.

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2
3

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

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^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).

10
11

(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.

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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

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1 the next reexposure and do not represent a true continuous exposure. This research group has
2 speculated that defensive adaptation of the nasal mucosa may include the function of the
3 mucociliary apparatus (Feron et al., 1989). Morgan et al. (1986a) have shown formaldehyde
4 effects on mucus flow and ciliary beat in F344 rats to result from hourly exposures to 15 ppm
5 formaldehyde. However, effects seen in repeated 8-hour exposures may not correspond to those
6 effects resulting from shorter duration exposures to higher formaldehyde concentrations.

7 Rusch et al. (1983a, b) performed a comparative study of formaldehyde effects on the
8 nasal epithelium in F344 rats, Syrian golden hamsters, and cynomolgus monkeys. Groups of
9 animals were exposed at 0, 0.2, 1, or 3 ppm (0, 0.25, 1.2, or 3.7 mg/m³) formaldehyde
10 22 hours/day, 7 days/week for 26 weeks. Six male monkeys, 10 male and 10 female hamsters,
11 and 20 male and 20 female rats were exposed at each exposure level. The experiment was run in
12 two trials, each with its own control group: trial 1 at 0.2 or 1 ppm and trial 2 at 3 ppm. Animals
13 were weighed weekly and physically assessed (details not given). At sacrifice, organ weights
14 were recorded for the kidney, adrenals, heart, and liver. Tissue sections of the lung (4), trachea,
15 and nasal turbinates (4) of each animal were examined by light microscopy (section locations not
16 given). Additionally, sections were examined by electron microscopy for rats in the control and
17 1 ppm treatment groups (five rats per group).

18 Body weights of both male and female rats in the 3 ppm treatment group were depressed
19 by 20% between week 2 and the end of the 26-week exposure. Absolute liver weights were
20 decreased in these animals as well (26% lower in males and 12% lower in females, $p < 0.05$).
21 This decrease in liver weight remained significant for male rats when normalized for body
22 weight (a ratio of 2.9 in treated versus 3.16 in controls) but not for female rats. No significant
23 body weight or organ weight changes were seen in hamsters or monkeys. Increased incidences
24 of congestion (36/156), hoarseness (32/156), and nasal discharge (62/156) were observed in
25 monkeys in the 3.0 ppm treatment group versus no hoarseness or congestion and only five
26 observations of nasal discharge in 156 observations for control monkeys. Increased nasal
27 congestion was noted in the two lower treatment groups of monkeys: 30/156 and
28 45/156 observations, respectively, versus 9/156 observations in nasal discharge in the controls.
29 The authors reported an increase in nasal discharge and lacrimation in treated hamsters but no
30 increases in symptoms in rats. However, observations of adverse symptoms in the control rats
31 were greater than 10% on some measures.

32 Rhinitis increased in rats in the 3 ppm treatment group, and the incidence in controls was
33 notable (see Table 4-29). All groups of monkeys showed some rhinitis, and no treatment effects
34 were observed in either monkeys or hamsters. Monkeys and rats in the high treatment group
35 (3 ppm) had a greater incidence of lesions in the nasoturbinate epithelium (see Table 4-30).

1 Rusch et al. (1983a, b) noted that most lesions were mild to moderate but were “somewhat more
 2 severe” in the high treatment group. Hamsters did not exhibit a similar increase, with few
 3 lesions noted in the nasal epithelium. Overall, these studies show a clear increase in adverse
 4 health effects at 3 ppm for rats and monkeys, with no adverse effects seen in hamsters at this
 5 treatment level or rats and monkeys at the lower concentrations (0.2 ppm and 1 ppm).

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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

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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	

18
19
20
21

Source: Rusch et al. (1983a, b).

22 Andersen et al. (2008) examined the effect of formaldehyde exposure at several
 23 concentrations and durations. This study comprised histopathology and cell proliferation data, as
 24 well as genomic analyses at Level II of the nasal cavity. Toxicogenomics analysis was
 25 performed only at Level II because this was the region where the most severe lesions have been

1 reported in chronic bioassays (Andersen et al., 2008; Monticello et al., 1991; Kerns et al., 1983).
2 More specifically, Andersen et al. (2008) stated that the histopathologic and cell proliferation
3 effects at Levels II and III (with similar tissue structure) (Monticello et al., 1991) provided
4 phenotypic anchoring for the genetic analysis. Table 4-31 summarizes many of the broad
5 phenotypic findings.

6 The primary conclusions of this study with regard to the histopathology and cell
7 proliferation are as follows:

- 8
- 9 • The presence of inflammatory cell infiltrates in the nasal epithelial tissue of F344 rats is
10 highly variable and provides no coherent pattern with dose or duration at levels below
11 6 ppm.
- 12 • Hyperplasia was observed following exposure to ≥ 2 ppm.
- 13 • Metaplasia was observed at 6 ppm on day 5, but not before or after.
- 14 • Cell proliferation (as measured by labeling indices) was significantly elevated in
15 Levels I–III at 6 ppm on day 5 and Level I on day 15, leading to the conclusion that
16 significant changes in cell proliferation may not occur at exposures to ≤ 2 ppm.
- 17 • A significant decrease in cell density was observed at Level I in animals exposed to
18 6 ppm formaldehyde for 15 days, which was posited to be related to tissue remodeling in
19 response to this concentration.

20

21 Based on their analysis of the microarray data, Andersen et al. (2008) concluded that no
22 genes were significantly altered by exposure to 0.7 ppm from 1 to 15 days. Exposure to 2 ppm
23 primarily resulted in gene changes at 5 days of exposure, but not thereafter. One gene was
24 significantly increased on day 1, but the authors did not identify that gene. At 6 and 15 ppm,
25 42 and 745 genes were altered at day 1, respectively. After 5 days, gene changes were only
26 observed at 6 ppm (15 ppm was not examined after day 1). These findings support conclusions
27 reached by their laboratory in an earlier analysis. Thus, the primary conclusion in the Andersen
28 et al. (2008) study is that genomic changes, including those suggestive of mutagenic effects, did
29 not temporally precede or occur at lower doses than phenotypic changes in the tissue. The
30 implications of this finding will be examined later in Section 4.5.

31 Studies have also investigated the ability for formaldehyde to induce pathology in the
32 trachea, bronchi, and lung tissue. These studies have reported tracheal tissue changes, lung
33 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

Response	ppm					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																					
P2											–	–	–	a																					
P3											–	–	–	a																					
CD											321±30	336±64	377±141	400±61																					
G	–	0	1	42	745	–	0	0	0	–	–	0	15	28	–	–	0	0	9	–	–	–	–	–	–	–	–	–	–	–					

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.

^aSignificantly elevated ULLI and LI at Level I on day 5 or significantly elevated ILII 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.

^bStatistically significant difference from control ($p < 0.05$).

Source: Andersen et al. (2008).

1 of species. Özen et al. (2003a) noted changes in zinc concentration in the lung tissue following
 2 exposure for formaldehyde. Dallas et al. (1989) and Dinsdale et al. (1993) observed changes in
 3 P450 enzyme activity in the lung associated with formaldehyde exposure.

4 Özen et al. (2003a) measured zinc, copper, and iron content in lung tissue from
 5 formaldehyde-exposed Wistar rats. Adult male rats were exposed to 0, 5, or 15 ppm (0, 6.2, or
 6 18.5 mg/m³) formaldehyde 8 hours/day, 5 days/week for either 4 or 13 weeks. Rats were
 7 checked daily and weighed weekly. At sacrifice, rats were autopsied and examined for gross
 8 pathological changes. Lung tissue was homogenized and analyzed for zinc, copper, and iron.

9 Body weight gain was depressed in all treatment groups in a concentration-dependent
 10 manner ($p < 0.001$) (see Table 4-32). Formaldehyde-exposed rats consumed less food and water
 11 than controls and showed unsteady breathing, increased nose cleaning, excessive licking,
 12 frequent sneezing, and nasal mucosa hemorrhages. Significant decreases were seen in the zinc
 13 content of lungs after either 5 or 10 ppm formaldehyde exposure (see Table 4-33). Copper
 14 content was unchanged from controls in all treatment regimens, whereas iron content was
 15 increased after 4 weeks of 5 ppm exposure and after 13 weeks of either 5 or 10 ppm
 16 formaldehyde exposure (Özen et al., 2003a).

17
 18 **Table 4-32. Percent body weight gain and concentrations of iron, zinc, and**
 19 **copper in cerebral cortex of male Wistar rats exposed to formaldehyde via**
 20 **inhalation for 4 and 13 weeks**
 21

Exposure (mg/m ³)	Weight gain (%) ^a	Zinc (mg/kg) ^a	Copper (mg/kg) ^a	Iron (mg/kg) ^a
<i>4-week data</i>				
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
<i>13-week data</i>				
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

22
 23 ^aValues are means ± SDs ($n = 7$).

24
 25 Statistical significance of differences versus controls, as calculated by the authors:

26 ^b $p < 0.05$.

27 ^c $p < 0.02$.

28 ^d $p < 0.002$.

29 ^e $p < 0.001$.

30
 31 Source: Özen et al. (2003b).

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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

^aRats were exposed 8 hours/day, 5 days/week for the number of weeks indicated.

^bConcentrations are expressed as moles/mg of tissue, wet basis.

^cValues 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/m³) 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/m³) 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

1 **Table 4-34. Total lung cytochrome P450 measurements of control and**
 2 **formaldehyde-treated male Sprague-Dawley rats**
 3

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) ^c	87 (11) ^d	35 (7)	172 (12) ^c	38 (9)
3.0 ppm	ND	ND	357 (10) ^e	278 (100) ^c	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

4
 5 ^aRats were exposed 6 hours/day, 5 days/week for the duration shown.

6 ^bCytochrome P450 expressed as pmol P450/mg of protein. Values are means (SDs) ($n = 3$).

7 ^cDifferent from control, $p < 0.05$.

8 ^dDifferent from control, $p < 0.01$.

9 ^eDifferent from control, $p < 0.001$, as calculated by the authors.

10 ND = not detected above the limit of detection, approximately 10 pmol/mg protein.

11
 12 Source: Dallas et al. (1989).

13
 14
 15 was generated by the thermal depolymerization of paraformaldehyde as was done by Dallas et al.
 16 (1989). The concentration of P450 and activity of several P450 isozymes were measured in lung
 17 microsomes (pentoxyresorufin O-dealkylase, benzyloxyresorufin O-dealkylase, ethoxyresorufin
 18 O-dealkylase, and 2-aminofluorene *N*-hydroxylation). ALP and γ -glutamyl transpeptidase
 19 activity were measured in BAL fluid collected from each animal. No changes were seen in BAL
 20 enzyme activity or the activity of lung microsomes for the P450 substrates tested. Cytochrome
 21 P450 levels were unchanged in experiment 1, where formaldehyde was generated from formalin.
 22 Cytochrome P450 levels were increased in experiment 2 with formaldehyde generated from
 23 paraformaldehyde (see Table 4-35).

24
 25 **Table 4-35. Cytochrome P450 levels in formaldehyde-treated rats**
 26

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

27
 28 ^aValues are means ± SDs ($n = 3-5$).

29 ^bDifferent from controls, $p < 0.05$.

30
 31 Source: Dinsdale et al. (1993).

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1 ***Extrapulmonary toxicity***

2 Several studies have investigated toxicity in organs other than those associated with the
3 respiratory tract. An earlier cross-species study examined changes in lung tissue resulting from
4 continuous exposure (Coon et al., 1970). Animals were exposed to 3.7 ppm (4.6 mg/m³)
5 formaldehyde for 90 days. Five species of animals were studied: male and female Sprague-
6 Dawley and Long-Evans derived rats (15), male and female Princeton-derived guinea pigs (15),
7 male New Zealand albino rabbits (3), male squirrel monkeys (*Saimiri sciureus*) (3), and purebred
8 male beagle dogs (2). Blood samples were taken for Hb concentration, HCT, leukocyte counts,
9 and serum levels of BUN, AST, ALT, ALP, and LDH. Sections of heart, lung, liver, kidney, and
10 spleen were fixed and examined from each species (details of method not provided). Brain,
11 spinal cord, and adrenal tissue also were examined in monkeys and dogs as well as thyroid from
12 dogs. Liver and kidney sections were stained for reduced nicotinamide adenine dinucleotide,
13 lactate, isocitrate, and β-hydroxybutyrate. Tissue sections of the nasal mucosa were not
14 examined in this study.

15 Hematological parameters were unaffected by formaldehyde treatment. The lung tissue
16 of all species exhibited interstitial inflammation after 90 days of formaldehyde exposure
17 (detailed description not provided). Formaldehyde-treated rats and guinea pigs also had focal
18 chronic inflammation in heart and kidney tissue sections. However, the authors were uncertain
19 whether the observed changes to heart and kidney were due to formaldehyde exposure.

20 As mentioned above, Woutersen et al. (1987) exposed male and female albino SPF
21 Wistar rats (10/group) to 0, 1, 10, or 20 ppm (0, 1.23, 12.3, or 24.6 mg/m³) formaldehyde
22 6 hours/day, 5 days/week for 13 weeks. Rats were checked daily and weighed weekly. During
23 week 13, blood samples were taken for Hb, PCV, RBC count, and a differential count of
24 leukocytes. Urine samples were also analyzed. At sacrifice, blood samples were analyzed for
25 ALB, creatinine, glucose, TP, BUN, and the enzyme activities (AST, ALT, and ALP). GSH and
26 protein content were determined in liver homogenates. Organs were examined and weighed:
27 adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, spleen, testes, thymus, and thyroid.

28 No gross pathological changes were seen upon autopsy, but body weights decreased in
29 both male and female rats at the 20 ppm treatment level. Of the organs weighed, 6 of 11 had
30 significantly increased relative rates in male rats exposed to 20 ppm formaldehyde. Relative
31 brain weight was increased in female rats at the same treatment level (Woutersen et al., 1987).

32 Clinical chemistry parameters of liver and kidney function and hematological parameters
33 were also measured after the 13-week treatment by Woutersen et al. (1987). Compared with
34 those of controls, activities of AST, ALT, and ALP were significantly elevated in plasma from
35 the 20 ppm treated male rats (by 124, 132, and 126%, respectively; *p* < 0.05). Total plasma

1 protein was reduced to 95% of controls in the same animals. Although there was an observed
2 increase in BUN in male rats treated with 1 ppm, this was not considered a treatment effect.
3 Furthermore, no statistically significant differences were seen for these parameters in female rats
4 at any concentration level (Woutersen et al., 1987).

5 Sul et al. (2007) exposed Sprague-Dawley rats to 0, 5, and 10 ppm formaldehyde for
6 6 hours/day (5 days/week) for 2 weeks and collected lung samples for tissue damage and
7 genomic analysis. According to their results, 21 genes were altered in a dose-dependent manner
8 by microarray analysis; 2 were up regulated and 19 were down regulated in the lung tissue of
9 animals exposed to formaldehyde. However, six of the nine genes further analyzed by PCR did
10 not show dose dependency (authors did not comment). Although the authors briefly describe the
11 functions and potential implications for changes in the expression of some of the altered genes,
12 there is no discussion of the relationship between these altered genes (i.e., there is no pathway
13 analysis).

14 In 2006, Im et al. (2006) published a proteomic analysis using the same exposure
15 protocols (possibly using the same animals as in the Sul et al. [2007] study, although neither
16 study makes reference to the other). Im et al. (2006) examined DNA damage in lymphocytes
17 and liver tissues, as well as protein and lipid oxidation in plasma and liver samples. Similar to
18 changes reported in the lung (discussed elsewhere), using two-dimensional electrophoresis and
19 matrix-assisted laser desorption ionization time-of-flight mass spectrometry, the authors also
20 reported dose-dependent changes in the levels of 32 proteins in plasma (19 up, 13 down). None
21 of the changes in plasma proteins correspond to the changes in lung reported by Sul et al. (2007).
22 Again, no pathway analysis was provided. Interestingly, Im and colleagues (2006) also
23 demonstrated a dose-dependent increase in plasma IL-4 and dose-dependent decrease in IFN γ ,
24 perhaps indicative of Th-2-mediated inflammatory response. An overview of formaldehyde
25 exposure-related pathology in the respiratory system of laboratory animals is presented in
26 Table 4-36.

27 28 **4.2.2.5. Chronic Inhalation Bioassays**

29 The respiratory pathology observed in chronic bioassays is consistent with the subchronic
30 studies. As exposure concentration and duration of exposure are increased, the pathology
31 becomes more severe and penetrates more deeply into the respiratory tract. These effects are
32 progressive over time. Tumors are reported in several bioassays, primarily SCCs. Experimental
33 results regarding both the severity of respiratory tract pathology as well as the tumor incidence
34 vary by species strain and experimental design. As discussed above rodents experience RB, and
35 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
<i>Nasal pathology</i>					
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)
<i>Tracheal and lung pathology</i>					
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 <i>n</i> = 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)
<i>Extrapulmonary effects</i>					
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)

1 absorbed dose in the respiratory tract, given the same exposure concentration (Chang and
2 Barrow, 1983). Additionally, differences in the nasal architecture result in species-dependent
3 variation of formaldehyde absorption (flux) within the respiratory tract (see Section 3.4).
4 Therefore, chronic studies are discussed by species for greater clarity.

6 **4.2.2.5.1. Mice.**

7 Early experiments by Horton et al. (1963) subjected mice (C3H, sex unspecified) to
8 extreme formaldehyde concentrations (0, 0.05, 0.1, and 0.2 mg/L or 41–163 ppm) in an attempt
9 to simulate lung pathology reported in humans exposed to cigarette smoke. The mice were
10 exposed 1 hour/day, 3 days a week for up to 35 weeks. The authors did not note the effects of
11 RB or provide any information on pathology of the URT. There was a clear increase in
12 histologic changes in the tracheobronchial epithelium by exposure, including basal-cell
13 hyperplasia, stratification squamous cell metaplasia and atypical metaplasia. Subsequent
14 exposures to various combinations of formaldehyde and coal tar did result in squamous cell
15 tumors. The findings of Horton et al. (1963) suggest a role for formaldehyde in lung cancer
16 under some conditions. However, the exposure design and early deaths in the treatment groups
17 severely limit the usefulness of these data in human health risk assessment.

18 In a comprehensive study conducted by Swenberg et al. (1980) (also reported in Kerns et
19 al. [1983]) in conjunction with Chemical Industry Institute of Toxicology (CIIT) and Battelle
20 Columbus Laboratories, male and female C57BL/6 × C3H F₁ (B6C3F₁) mice (approximately
21 120/sex/concentration) were exposed to 0, 2.0, 5.6, or 14.3 ppm (0, 2.45, 6.87, or 17.5 mg/m³)
22 formaldehyde 6 hours/day, 5 days/week for 24 months. This exposure period was followed by
23 up to 6 months of nonexposure to evaluate recovery. Interim sacrifices were conducted at 6, 12,
24 18, 24, 27, and 30 months (due to unscheduled deaths, no male mice were sacrificed at 18 or
25 27 months). Exposure generation was accomplished by sublimation of paraformaldehyde, and
26 exposures were conducted in whole-body chambers. Detailed sectioning and examination of the
27 nasal passages were conducted at each interim sacrifice, beginning at 12 months, and for all
28 unscheduled deaths. Gross organ pathology was noted for all animals and complete
29 histopathologic examination was conducted on all animals in the control and high-exposure
30 groups. There were no differences in survival in any exposure group compared with controls.
31 Generally, poor survival in all groups of male mice was attributed to fighting and infections of
32 the urogenital tract associated with group housing; 78, 77, 81, and 82 unscheduled deaths were
33 recorded before 24 months in the 0, 2.0, 5.6, and 14.3 ppm treatment groups, respectively (
34 $n = 119, 120, 120, \text{ and } 119$ males, respectively). After the interim sacrifices (6 and 12 months)
35 only 17–22 male mice survived to the 24-month scheduled sacrifice. Female mice had much

1 greater survival with only 30, 34, 19, and 34 unscheduled deaths prior to the 24-month sacrifice.
2 The authors did not note the effects of RB in mice, although the RD₅₀ for a 10-minute exposure
3 for male B6C3F1 mice has been reported at 4.9 ppm and 4.4 ppm (Steinhagen and Barrow, 1984;
4 Chang et al., 1981).

5 The first examination of the nasal cavities was conducted at the 12-month interim
6 sacrifice. Inflammation in the nasal turbinates was evident in mice in the 2 and 6 ppm treatment
7 groups (14/20 and 18/20, respectively), including adenitis of the nasal lacrimal duct, lacrimal
8 duct, and vomeronasal gland. Inflammation was not present in mice exposed at 15 ppm,
9 although serous rhinitis was seen in 4 of 20 animals. At 18 months, mice exposed at 2 and
10 6 ppm no longer exhibited adenitis in the nasoturbinates. Epithelial dysplasia was evident in 4 of
11 20 mice at 6 ppm exposure. Mice in the high-exposure group had significantly greater nasal
12 pathology; epithelial dysplasia and squamous metaplasia were reported in 18/19 and 17/19
13 female mice, respectively, exposed to 15 ppm. After 24 months, squamous epithelial hyperplasia
14 of the nasolacrimal duct (29/45) and atrophy of the olfactory epithelium (18/45) were also noted
15 in animals from the high-exposure group (male and female) (Battelle Columbus Laboratories,
16 1981). Similar pathology was reported in only a small fraction of mice exposed at 2 and 6 ppm
17 (5/48 and 11/60, respectively).

18 Three months after cessation of exposure, only nine female mice were available for
19 sacrifice, but within this small sample the data suggested recovery of nasal lesions: epithelial
20 dysplasia (4/9), squamous metaplasia (2/9), atrophy of the olfactory epithelium (1/9), and
21 squamous epithelial hyperplasia of the nasolacrimal duct (1/9) (Battelle Columbus Laboratories,
22 1981).

23 Of the 17 male mice that survived to 24 months in the 14.3 ppm exposure group, 2 had
24 SCC in the nasal cavity ($p < 0.05$). Of the two tumor-bearing mice, one exhibited significant
25 epithelial pathology, including rhinitis, dysplasia, squamous metaplasia, and hyperplasia.
26 Squamous metaplasia of the nasolacrimal duct was the only related pathology reported for the
27 second mouse. No SCCs were found in female mice, although 48 mice survived to 24 months.
28 The authors reported no other formaldehyde-related tumors. However, comparisons were based
29 on summary tables by organ. Although lymphomas were analyzed by organ and site (e.g.,
30 increase in salivary gland lymphoma considered separately from mandibular lymphoma), later
31 reanalysis of lymphoma in female mice, based on tumor-bearing animals (TBAs), does indicate
32 an association with formaldehyde exposure.

33

1 **4.2.2.5.2. *Rats.***

2 Holmström et al. (1989a) evaluated coexposure of inhaled formaldehyde with wood dust
 3 in 16 female Sprague-Dawley rats/group. Rats were exposed in whole-body chambers for 6
 4 hours/day, 5 days/week for 104 weeks to formaldehyde alone at 12.4 ± 1.1 ppm (15.21 ± 1.35
 5 mg/m^3), wood dust alone ($25 \text{ mg}/\text{m}^3$), or both wood dust ($25 \text{ mg}/\text{m}^3$) and formaldehyde ($12.7 \pm$
 6 1.0 ppm) or to room air as the control. The wood dust was generated from grinding of beech.
 7 Microscopic measurements of the wood particles indicated that approximately 70% had a
 8 geometric diameter of about $10 \mu\text{m}$, while 10–20% were about $5 \mu\text{m}$ or less. Animals were
 9 sacrificed at 104 weeks and histopathology was performed on five transverse sections of the
 10 nasal cavity (see Figure 4-6) and the lungs (not otherwise specified).

11 There were no differences in mortality among the groups at any time during the study
 12 period. Rats exposed to formaldehyde were reported to have exhibited yellow discoloration of
 13 the fur, and many displayed eye irritation. Formaldehyde exposure, with and without wood dust,
 14 induced squamous metaplasia, keratinization, and dysplasia of the nasal epithelium (see
 15 Table 4-37).
 16

17 **Table 4-37. Histopathologic findings and severity scores in the naso- and**
 18 **maxilloturbinates of female Sprague-Dawley rats exposed to inhaled**
 19 **formaldehyde and wood dust for 104 weeks**
 20

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 \pm 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

21 ^a $p < 0.01$.
 22 ^b $p < 0.05$.

23 Source: Holmström et al. (1989a).
 24
 25
 26
 27

1 Among the five levels of the nasal cavity that were examined, Holmström et al. (1989a)
2 presented findings for the naso- and maxilloturbinates since formaldehyde-induced tumors had
3 been associated with this level (Morgan et al., 1986a, b). The data also suggested an effect of
4 wood dust on formaldehyde-induced nasal pathology, with a slightly higher histologic score and
5 greater incidence of dysplasia than formaldehyde exposure alone. One SCC (1/16) occurred in
6 the group exposed to formaldehyde only but not in the group exposed to formaldehyde and wood
7 dust. Microscopic examination of the lungs revealed that emphysema (diagnostic criteria not
8 specified) was more prevalent in both groups exposed to wood dust compared with the control
9 group ($p < 0.05$). There was no significant difference in pulmonary epithelial histopathology
10 among the groups.

11 Tobe et al. (1985) also evaluated F344 rats (32/group) exposed to inhaled formaldehyde
12 for 28 months. Exposures were for 6 hours/day, 5 days/week to formaldehyde concentrations of
13 0, 0.3, 2, and 14 ppm (0, 0.37, 2.45, and 17.2 mg/m³). Fourteen of 32 rats (44%) in the high
14 concentration group developed nasal SCCs, compared with none in the other exposed groups and
15 the control group. Tobe et al. (1985) reported increased rhinitis, hyperplasia, and squamous
16 metaplasia of the nasal respiratory epithelium, including in the low-exposure group (0.3 ppm.)
17 However, some level of rhinitis, hyperplasia, and metaplasia were also present in controls.
18 Without a more complete report, it is unknown whether or not the pathology reported at 0.3 ppm
19 was a formaldehyde-related effect.

20 Kamata et al. (1997) evaluated the effects of inhaled formaldehyde in male F344
21 (F344/DuCrj) rats (32/group) exposed for 28 months. Formaldehyde exposure was generated by
22 metering 37% formalin (containing 10% methanol) into a sprayer in a glass bottle and diluting
23 with room air. Concentration in the chamber was monitored twice daily by the acetyl acetone
24 method. Exposures were for 6 hours/day, 5 days/week at nominal formaldehyde concentrations
25 of 0, 0.3, 2.0, and 15 ppm (0, 0.37, 2.45, and 18.4 mg/m³). Actual levels were 0, 0.3 ± 0.07, 2.17
26 ± 0.32, and 14.85 ± 2.22 ppm (mean ± SD). Rats in the 0 ppm group were given methanol to
27 inhale at the same concentration (4.2 ppm) as the 15 ppm group. A room control no-exposure
28 group was also included in the study. All animals were observed for clinical signs once a day
29 during the study. Body weights and food consumption were recorded weekly. Five animals per
30 group, randomly selected at the end of 12, 18, and 24 months, and all surviving animals at
31 28 months were sacrificed for hematological measurements (Hb, RBCs, PCV, MCV, mean
32 corpuscular hemoglobin [MCH], MCHC, and WBCs), biochemical determinations (TP, ALB,
33 BUN, ALP, AST, ALT, glucose, albumin/globulin ratio, phospholipids, triglycerides, and total
34 cholesterol), and pathological examinations. Wet weights were taken on brain, heart, lungs,
35 liver, kidneys, spleen, testes, and adrenal gland of each rat. Histopathology was performed on all

1 moribund or dead animals and those at specified sacrifices on all gross lesions and the following
2 tissues: pituitary, thyroid, nasal cavity, trachea, esophagus, stomach, small and large intestines,
3 prostate gland, urinary bladder, muscle, femur, sciatic nerve, spinal cord, and mesenteric lymph
4 nodes. Histopathologic sections of the nose were obtained from five anatomical levels, but these
5 did not correspond to the typical levels taken in other bioassays. Most notably, section level B
6 was anterior and not posterior to the incisor teeth. The incidence data for nasal histopathology
7 were not reported with respect to section level location, with the exception that the
8 nonproliferative lesions and tumors reported were described to occur predominantly at levels B
9 and C.

10 Yellow discoloration of the coats occurred in animals exposed at the 2 and 15 ppm levels.
11 Significant decreases in body weight and food consumption were observed in the high
12 concentration (15 ppm) group throughout the exposure period, and elevated mortality was noted
13 at 28 months (88.3 versus 31.8% in controls). The first death occurred after 6 versus 18 months
14 in the control group. Other effects noted in the 15 ppm exposure group include decreased
15 triglycerides, reduced liver weight (both relative and absolute), and increased relative adrenal
16 weights.

17 Treatment-related macroscopic and histopathologic findings were limited to the nasal
18 cavity. Squamous cell metaplasia was reported in all treatment groups: 16% (0.3 ppm), 37.5%
19 (2 ppm), and 91% (15 ppm) of exposed rats. Epithelial hyperplasia was similarly present in 12.5,
20 22, and 91% of the animals, respectively. Since a no-effect level could not be determined, the
21 authors reported benchmark doses (BMDs) of 0.25 and 0.24 ppm for squamous cell metaplasia
22 and epithelial hyperplasia (10% response.). Additional lesions only occurring in the 15 ppm
23 dose group were papillary hyperplasia (2/32), SCC (13/32), squamous cell papilloma (3/32), and
24 sarcoma (1/32). The majority of the tumors were located at levels B and C of the nasal cavity.

25 Albert et al. (1982) and Sellakumar et al. (1985) reported on a set of lifetime studies
26 performed in male Sprague-Dawley rats to evaluate the effects of inhaled formaldehyde alone
27 and in combination with hydrochloric acid (HCl). Rats were exposed 6 hours/day, 5 days/week
28 for life. In the first experiment (Albert et al., 1982), 8-week-old male inbred Sprague-Dawley
29 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)
30 formaldehyde, and there were two control groups: air-sham and untreated ($n = 50$).

31 Bis(chloromethyl)ether (BCME), a known animal carcinogen (Albert et al., 1975; Kuschner et
32 al., 1975; Figueroa et al., 1973; Laskin et al., 1971), is formed when formaldehyde and HCl are
33 mixed. BCME concentrations were estimated at about 1 ppb in the formaldehyde-HCl mixed
34 exposures, based on levels in the mixing chamber. Complete necropsies were conducted when
35 animals died naturally or were killed when moribund. Histologic sections were taken from the

1 nasal cavity, larynx, trachea, pulmonary lobes, liver, bladder, kidney, spleen, and other organs
2 with gross pathologic alterations.

3 Exposure to the mixed gases (formaldehyde-HCl-BCME) induced nasal lesions,
4 including epithelial hyperplasia (71%), squamous metaplasia (64%), squamous papilloma (3%),
5 and SCC (25%) (Albert et al., 1982). Although a few squamous metaplasias were noted in the
6 larynx, trachea, and bronchi, these lesions were also noted in controls. Mortality in exposed rats
7 was significantly increased over controls and was approximately 30% when the first carcinoma
8 was reported (233 days). Mortality in exposed rats rose quickly to approximately 60% after the
9 first year of exposure. Therefore, the authors used a life-table method to calculate a mortality-
10 corrected cumulative incidence, reporting a corrected cumulative incidence of 77% at 720 days
11 after first exposure.

12 In the second experiment performed in this laboratory (Sellakumar et al., 1985; Albert et
13 al., 1982), Sprague-Dawley rats were similarly exposed to HCl (10 ppm) alone, formaldehyde
14 alone (15 ppm), or a combination of both. The combination exposure was generated in two
15 different ways to better understand the influence of BCME formation on study results: premixed
16 at high concentrations and gases fed separately into the inlet air supply at the target
17 concentrations. BCME concentration measured by a gas chromatography/mass spectrometry
18 method in the premixed chamber varied between 0.1 and 0.4 ppb. Cage-side observations and
19 necropsy procedures were as described in Albert et al. (1982) with the exception of the histologic
20 preparation of the head. The head was cut transversely into four tissue blocks, and sections were
21 taken from the face of each.

22 Animals exposed to formaldehyde alone and formaldehyde-HCl (premixed or
23 nonpremixed) showed a marked decrease in body weight after 16 weeks. After 32 weeks rats
24 exposed to the premixed formaldehyde-HCl (with BCME) had higher mortality compared with
25 the other mixed gas exposures ($p < 0.05$). Nasal pathology was similar among rats exposed to
26 formaldehyde alone or the mixed gases (see Table 4-38). Desquamation of respiratory epithelial
27 cells was reported in the respiratory epithelium that covers the nasomaxillary turbinates and the
28 nasal septum (approximately section levels 2 and 3). Olfactory epithelium in the ET frequently
29 showed an inflammatory reaction with seropurulent exudate filling the lumen. Squamous
30 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

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1 that this singular tumor occurred in the absence of other changes in the ethmoid region or in the
2 lungs where BCME was also demonstrated to cause tumors. Furthermore, exposure was
3 approximately one-tenth the cumulative dose in the Kuschner et al. (1975) study that was
4 associated with a single similar tumor. Sellakumar et al. (1985) attributed the higher incidence
5 in the premixed-combination group to traces of other alkylating agents (not BCME) that could
6 have been formed. The results demonstrate that animals exposed to either a combination of
7 formaldehyde-HCl or to formaldehyde alone develop nasal tumors, principally SCCs, at about
8 the same frequency, indicating that HCl plays little or no role in the carcinogenicity of inhaled
9 formaldehyde.

10 In a companion study to the chronic mouse study described above (Kerns et al., 1983;
11 Swenberg et al., 1980), groups of F344 rats (approximately 120/sex/concentration) were exposed
12 to 0, 2.0, 5.6, or 14.3 ppm (0, 2.45, 6.87, or 17.5 mg/m³) formaldehyde 6 hours/day, 5 days/week
13 for 24 months. This exposure period was followed by up to 6 months of nonexposure to evaluate
14 recovery. Interim sacrifices were conducted at 6, 12, 18, 24, 27, and 30 months. Study
15 parameters and methods were as described above.

16 Formaldehyde exposure increased mortality of both male and female rats in all treatment
17 groups ($p < 0.05$ for 6 and 15 ppm groups). Severe treatment-related mortality was seen at the
18 highest exposure group beginning at 12 months with only 30% surviving to the 24 months.
19 There were no alterations in clinical chemistry, neurofunctional, or ophthalmological
20 measurements considered to be related to formaldehyde exposure. A concentration-dependent
21 increase in yellow discoloration of the hair coat was observed. This discoloration dissipated over
22 the 3-month postexposure period. Rats in the highest-concentration group were dyspneic
23 ($p < 0.01$) and emaciated ($p < 0.05$) and had many facial swellings that on closer examination
24 were revealed to be carcinomas protruding through the nasal cavity. Neoplastic lesions in the
25 URT were first observed clinically at day 358 in females and day 432 in males.
26 Macroscopically, these lesions originated in the anterior portion of the nasal cavity and, in a few
27 instances, extended into the ETs.

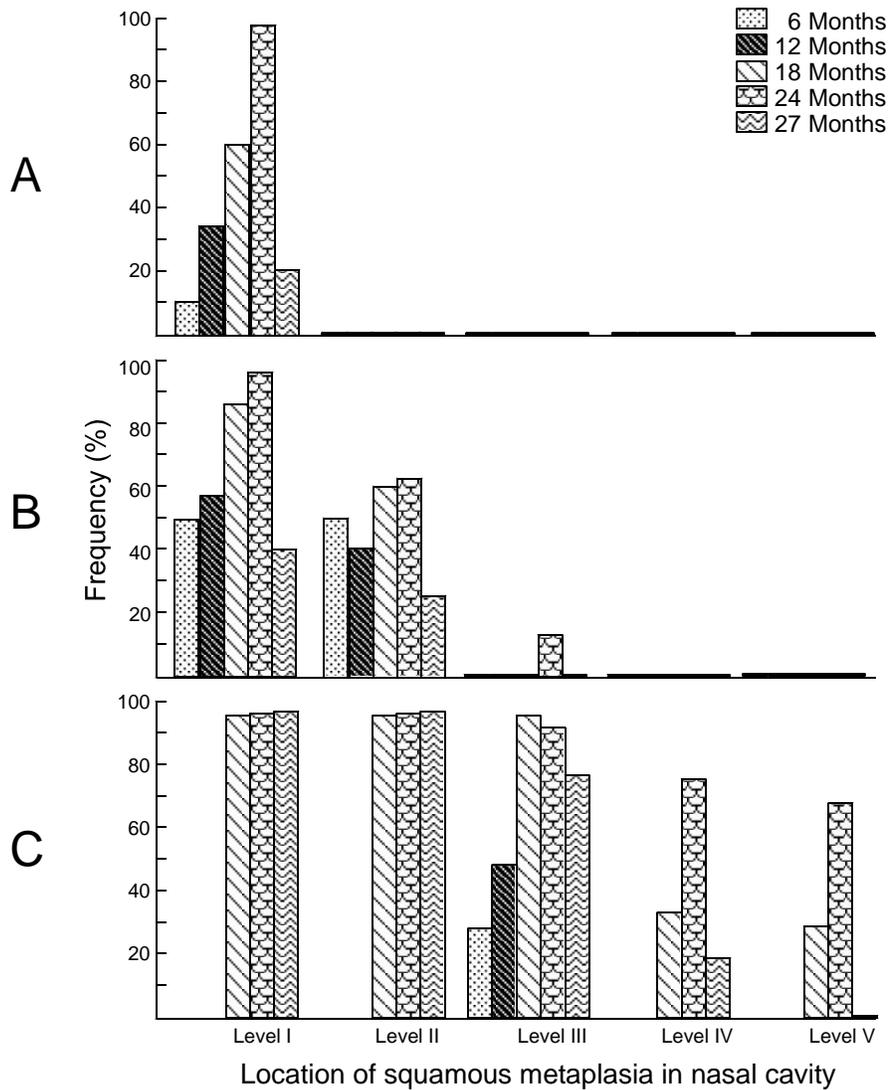
28 Figure 4-13 shows the frequency of squamous metaplasia by location in the noses of rats
29 sacrificed at various time points along the 2-year exposure period. Histopathologic lesions were
30 confined to the nasal cavity and proximal trachea in concentration-dependent fashion. The
31 morphologic diagnosis of squamous metaplasia was used to designate zones of altered
32 epithelium that were characterized by the presence of a well-differentiated germinal layer
33 (stratum germinativum) and superficial layers of epithelium (stratum spinosum and stratum
34 corneum). Keratin was produced only in areas of squamous metaplasia. Epithelial dysplasia was
35 detected earlier than squamous metaplasia and was characterized by a mucosa that had

1 undergone a transition from nonciliated simple cuboidal to one that was several cells thick and
2 squamoid with an organization and polarity of the individual cells that had changed from vertical
3 to horizontal with respect to the basement membrane. Similar histomorphologic changes have
4 also been called basal cell hyperplasia and epidermoid metaplasia (e.g., Albert et al. [1982]).
5 Figure 4-13 clearly illustrates that concentration is a dominant determinant of lesion distribution.
6 At low concentrations the lesions occur only in the most anterior region (cross-section level 1).
7 At 5.6 ppm, the squamous metaplasia in levels 1, 2, and 3 was also associated with purulent
8 rhinitis and epithelial dysplasia. At the highest concentration, the lesions progress to the more
9 distal URT, with lesions evident in level 5 and no difference in the incidence at level 1 or 2
10 across the various sacrifice times. Statistically significant ($p < 0.05$) regression of the lesion was
11 evident at most locations at the 27-month sacrifice (3 months postexposure) (e.g., level 1 in the
12 2 ppm group, all levels of the 5.6 ppm group, and levels 4 and 5 of the 14.3 ppm group).

13 Furthermore, progression of lesions distally to the lower respiratory tract (LRT) occurred
14 only in the high concentration group. Tracheal pathology observed at 18 months included
15 multifocal areas of minimal to mild epithelial hyperplasia, epithelial dysplasia, or squamous
16 metaplasia. There were no significant tracheal lesions present in the 0, 2.0, or 5.6 ppm exposure
17 groups, and tracheal lesions were not observed during the postexposure period in the 14.3 ppm
18 exposure group.

19 Table 4-39 provides the summary data of all neoplastic lesions in the nasal cavity of
20 exposed rats. The adjusted cumulative incidence rates of SCC in male and female rats from the
21 14.3 ppm exposure group at 24 months were 67 and 87%, respectively. In this group, the
22 formation of zones of squamous metaplasia with zones of squamous epithelial hyperplasia and
23 increased keratin production appeared to precede areas of squamous papillary hyperplasia with
24 foci of cellular atypia. More advanced lesions included carcinoma in situ and invasive SCC of
25 the nasal turbinates. The neoplasia were extremely osteolytic and were associated with excessive
26 keratin production and mild to severe purulent rhinitis. In many animals from the high-exposure
27 group (with or without carcinoma), the excessive accumulation of keratin and inflammatory
28 exudates within the lumen of the URT caused severe dyspnea and death. Polypoid adenomas
29 were also observed in eight rats (four/sex) from the low-exposure group, six male rats from the
30 intermediate-exposure group, and six rats (five males, one female) from the high-exposure group
31 in level 1, 2, or 3. One control male rat had a similar lesion. When adjusted and unadjusted data
32 were analyzed, no significant differences were observed in pair-wise analyses; however, a
33 significant adjusted trend ($p < 0.05$) was reported for male rats. There was no evidence of
34 progression from polypoid adenoma to SCC.

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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.

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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.

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Source: Redrawn from Kerns et al. (1983).

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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

^aOne 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

^aRounded to nearest whole number.

^bArea 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).

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1 In the 14.3 ppm exposure group, 98/103 rat noses had adequate numbers and quality of
2 slides for mapping the SCC distribution. Single neoplasia were present in 80 (40/sex), while
3 multiple neoplasia were present in 9 males (21 neoplasia) and 9 females (20 neoplasia). The
4 results were similar for cases with high or low accuracy. For example, more than half (57%) of
5 the SCCs occurred on the lateral side of the nasoturbinate and adjacent lateral wall at the front of
6 the nose (levels 1 and 2); approximately 25% were located on the midventral nasal septum
7 (levels 2 and 3); and about 10% were on the dorsal septum and roof of the dorsal meatus
8 (levels 1, 2, and 3). A small number (3%) were found on the maxilloturbinate (levels 2 and 3),
9 which only involved the medial aspect. All other regions of the nose where SCC was found were
10 considered to be involved as a result of invasion from one or more of the above sites. There
11 were two tumors in the 5.6 ppm group: one male had a single neoplasm on the ventral nasal
12 septum (level 3) while a female had an SCC from the lateral aspect of the maxilloturbinate to the
13 adjacent lateral wall (level 2).

14 On the basis of the morphology of 19 small neoplasia in this study and in additional work
15 described below (Morgan, 1997; Monticello et al., 1996), it was further concluded that the SCCs
16 arose from the epithelium lining the airway and not from the underlying glandular epithelium.
17 This mapping procedure and that of Monticello et al. (1996) described below were in good
18 concordance and showed a clear site specificity; most of the SCC arose in the anterior lateral
19 meatus (ALM) (57%), which is lined by transitional epithelium, and the midventral nasal septum
20 (26%), which is lined by respiratory epithelium (Morgan, 1997).

21 The CIIT performed a second bioassay on inhaled formaldehyde in 9-week-old male
22 F344 (CDF[F344]/CrIBr) rats (Monticello et al., 1996). The rats were exposed 6 hours/day, 5
23 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
24 18.4 mg/m³) formaldehyde. Study objectives were to repeat the Kerns et al. (1983) bioassay,
25 better defining the concentration response relationship and to seek a correlation between
26 localized data on tumor sites and concomitant cell proliferation assays. Histopathology was
27 performed on six cross-section levels of the nasal cavity on every animal of an unscheduled
28 death and all those of the terminal sacrifice after 24 months. The distribution of lesions for each
29 individual animal was recorded onto epithelial maps of the nasal cavity at 30 selected levels
30 designed to permit accurate localization (Mery et al., 1994). Cell proliferation was measured in a
31 subset of animals (five per treatment group) at 3, 6, 18, and 24 months of exposure in each of the
32 nasal regions to which tumors were mapped (see Table 4-41).

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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

^aTotal 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.

1 Regional analysis indicated that the SCCs arose in nasal regions lined with transitional or
2 respiratory epithelium and were most common in the lateral meatus and the midseptum (see
3 Table 4-41). Within the lateral meatus and mid-septum, there was clear evidence of a higher
4 tumor incidence rate in the anterior sample site ($p = 0.001$ and 0.02 , respectively). Smaller
5 numbers of SCCs were observed on the medial aspect of the maxilloturbinate and the dorsal
6 septum and on the posterior lateral wall and lining of the nasopharyngeal meatus (data not
7 shown). No SCCs were observed in the maxillary sinus, with the exception of one animal
8 exposed to 15 ppm that had a small tumor in the wall of the ostium of this sinus. Tumor rates
9 across the seven nasal epithelial sites are presented in Table 4-41. There was an increasing
10 tumor response between the 10 and 15 ppm exposure groups in all sites, except in the ALM. The
11 SCC rates at 10 and 15 ppm were virtually identical (13.3 and 11.6%, respectively), which is
12 probably attributable to the occurrence of many large neoplasia in the lateral meatus site that
13 were not suitable and not counted in the analysis.

14 The nonlinear tumor response is mirrored by a highly nonlinear response in cell
15 proliferation measured after 3, 6, 12, and 18 months of exposure. Significant treatment-induced
16 responses in cell proliferation indices at these time points were only observed at the two highest
17 exposure concentrations (10 and 15 ppm). Other treatment-induced lesions, predominantly
18 epithelial hypertrophy, hyperplasia, squamous metaplasia, and mixed inflammatory cell
19 infiltrate, were also most severe at these two exposure concentrations. Significant distortion and
20 destruction of the nasoturbinate architecture occurred in many animals exposed to 15 ppm.
21 Nasal turbinate adhesions and olfactory degeneration (usually confined to the walls of the
22 anterior dorsal medial meatus) also occurred in animals exposed to 10 and 15 ppm. Lesions in
23 the 6 ppm exposure group were limited to focal squamous metaplasia in the anterior regions.

24 As discussed briefly above, small numbers of polypoid adenomas were also induced by
25 formaldehyde exposure and were similar in acinar-like structure and location to those in the
26 Kerns et al. (1983) bioassay. No polypoid adenomas occurred in the control animals or in the
27 0.7, 2, or 6 ppm exposure groups. A clear concentration response was observed in the 10 and
28 15 ppm exposure groups. Five of 90 animals (5.6%) in the 10 ppm exposure group and 14 of
29 147 animals (9.5%) in the 15 ppm exposure group had a polypoid adenoma. Most of these
30 polypoid adenomas (79%) were located in or adjacent to the lateral meatus. The significance of
31 these tumors for risk assessment remains to be determined (Morgan, 1997).

32 Appelman et al. (1988) studied the effects of bilateral intranasal electrocoagulation
33 damage on susceptibility to inhaled formaldehyde in male SPF Wistar (Cpb: WU) rats. Rats
34 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,
35 1.23, or 12.3 mg/m³) formaldehyde. These concentrations were chosen because the various

1 short-term studies performed in the same laboratory (described in Section 4.2.1.2) showed that
2 formaldehyde was noncytotoxic to the nasal mucosa at levels of 0.3, 1.0, and 2.0 ppm, slightly
3 cytotoxic at 3 and 4 ppm, and strongly cytotoxic at 10 and 20 ppm (Zwart et al., 1988; Wilmer et
4 al., 1987; Woutersen et al., 1987). Furthermore, because nasal tumors have only been found at
5 exposure concentrations that also induced severe degenerative, hyperplastic, and metaplastic
6 changes in the nasal epithelium (Griesemer et al., 1985; Squire and Cameron, 1984), Feron et al.
7 (1984) and the investigators at the TNO-CIVO Toxicology and Nutrition Institute postulated that
8 formaldehyde at a subcytotoxic concentration was only a very weak initiator without promoting
9 activity. Appelman et al. (1988) used an electrocoagulation method in this study to evaluate if
10 damage to the mucosa followed by compensatory cell proliferation might render the epithelium
11 vulnerable to subcytotoxic levels of formaldehyde. One-half of the rats used in the study
12 (10/group) were damaged bilaterally and then subjected to the first 6-hour exposure to
13 formaldehyde approximately 20–26 hours after the electrocoagulation procedure. Ten
14 undamaged rats/group were also exposed at each concentration for either 13 or 52 weeks.
15 Histopathologic examination included six standard cross-section levels in the nose; livers of all
16 rats killed at 14 weeks and of all control and 10 ppm exposed rats killed in week 53; larynges,
17 tracheas, and lungs of all rats of the control and 10 ppm exposed rats killed in week 53; and
18 organs and tissues of control and 10 ppm exposed rats with an undamaged nasal mucosa killed in
19 week 53.

20 Yellow discoloration of the fur occurred in all animals of the two highest exposure
21 groups. Growth retardation was observed in the animals killed with or without damaged noses
22 after 2 weeks of exposure to 10 ppm formaldehyde. No toxicologically significant findings in
23 the body weights or organ weights of any animals in the other exposure groups were observed.
24 No relevant differences between groups were found in any of the hematological or urinary
25 parameters with the exception of frequent oliguria ($p < 0.05$) in the top exposure group without
26 nasal coagulation and killed in week 53. Three-way ANOVA revealed a significant increase in
27 TP content of the liver in rats with damaged noses as compared with rats with undamaged noses,
28 and there was a significant negative correlation between the formaldehyde exposure level and TP
29 in these same rats. Hepatic GSH was positively correlated with both nasal damage and age of
30 the animals. No treatment-related gross findings were observed in animals sacrificed at either 14
31 or 53 weeks except for yellow discoloration of the fur in rats exposed at the two highest
32 concentrations. No changes observed in the larynx, trachea, lungs, liver, or other tissues
33 evaluated were regarded as related to formaldehyde.

34 Few nasal lesions were noted in intact rats exposed at 0.1 or 1 ppm for either 13 or 52
35 weeks ($n = 10$ /group). Focal squamous metaplasia was noted in a single animal exposed at

1 1 ppm for 13 weeks. Rats exposed at 10 ppm formaldehyde demonstrated clear pathology in the
2 respiratory epithelium progressing from 13 to 52 weeks, including squamous metaplasia, basal
3 cell hyperplasia, and focal rhinitis. Additionally, focal nest-like infolds of the epithelium were
4 present in 4 of 10 rats at 52 weeks, and minor changes to the olfactory epithelium were noted
5 (thinning/disarrangement and focal basal cell hyperplasia.)

6 All rats with damaged nasal passages exhibited similar minor pathology of the respiratory
7 epithelium at 13 and 52 weeks (squamous metaplasia, focal basal cell hyperplasia, and focal
8 rhinitis). Formaldehyde-related effects were note at 52 weeks, where the squamous metaplasia
9 of the respiratory epithelium was no longer noted in controls (versus 13 weeks) but was clearly
10 present in all formaldehyde-treatment groups, including progression from focal to diffuse lesions
11 (at 1 and 10 ppm) and keratinization (3/10 and 4/10 at 0.1 ppm and 10 ppm, respectively). The
12 formaldehyde effects on the respiratory epithelium were much more severe in rats with damaged
13 nasal passages, with all animals demonstrating thinning and disarrangement of the olfactory
14 epithelium and 8 of 10 rats exhibiting “loosely arranged submucosal tissue.” Squamous
15 metaplasia and focal rhinitis of the olfactory epithelium were seen in less than half of the
16 formaldehyde-treated rats with damage. No changes in the olfactory epithelium due only to
17 electrocoagulation were encountered.

18 The most notable effects of nasal damage from electrocoagulation were the ones at the
19 highest formaldehyde exposure (10 ppm) on the olfactory epithelium. Damage to the respiratory
20 epithelium also occurred more posteriorly in rats with damaged noses. Since electrocoagulation
21 often induced damage that included partial or complete loss of turbinates and septal perforation,
22 a likely explanation for the posterior distribution of the damage is an abnormal airflow pattern.
23 This gross damage to the nasal structure may have also disrupted normal mucous production and
24 flow. Therefore, formaldehyde-induced pathology appearing deeper in the nasal passages,
25 including the respiratory epithelium, may be due to formaldehyde penetrating more deeply into
26 the nasal passages and resulting in greater tissue doses in these areas.

27 Woutersen et al. (1989) conducted a lifetime study in parallel to the 1-year study
28 described above for Appelman et al. (1988). Male Wistar rats with nasal damage induced by
29 electrocoagulation (60/group) or without nasal damage (30/group) were exposed 6 hours/day,
30 5 days/week to the same concentrations as in the previous study (0, 0.1, 1.0, and 10 ppm) for
31 28 months or for 3 months followed by a 25-month observation period. The general condition
32 and behavior of the animals were checked daily. Body weight, organ weight, and gross
33 pathology were evaluated as described for Appelman et al. (1988). Histopathologic examination
34 was conducted on all animals at the standard six cross sections (see Figure 4-6).

1 No remarkable findings on behavior were observed except for yellowing of the fur in
2 animals at the two highest concentrations. There were no relevant differences in mortality (data
3 not shown). Growth retardation was observed relative to controls in animals with or without
4 damaged noses exposed to 10 ppm from day 14 onward. Body weights were generally slightly
5 lower in formaldehyde-exposed animals with an intact nasal mucosa and slightly higher in
6 exposed animals with damaged noses than in the corresponding controls.

7 The effects of formaldehyde exposure on the respiratory and olfactory epithelium after
8 28 months of exposure were similar to those reported for 52 weeks exposure (Appelman et al.,
9 1988): rhinitis, squamous metaplasia with some keratinization of the respiratory epithelium, and
10 thinning/disarrangement and slight squamous metaplasia of the olfactory epithelium at the
11 10 ppm exposure. Effects attenuated from the anterior to posterior sections (I–II, III, IV, and
12 V–VI). A low incidence of olfactory epithelium replaced by respiratory epithelium (<10%) and
13 vacuolation and atrophy of olfactory cells (<10%) was reported, this in part may be due to the
14 larger study size (30 rats per group versus 10). Squamous metaplasia in levels I–II of the
15 respiratory epithelium at 10 ppm was the only treatment-related pathology remaining in rats
16 exposed for 3 months followed by a 25-month recovery period.

17 Similarly, as reported by Appelman et al. (1988), rats with noses damaged by
18 electrocoagulation did demonstrate increased pathology of the respiratory epithelium.
19 Formaldehyde exposure at 10 ppm exacerbated these changes, and effects were noted in more
20 posterior sections than in rats without nasal damage (levels III, IV, and V). Olfactory pathology
21 was also greater in formaldehyde-treated rats: basal cell hyperplasia, replacement of olfactory
22 epithelium by respiratory epithelium (10–20% at level III and <10% at level IV). Although the
23 incidences are low, there is some evidence that effects on the olfactory epithelium may be
24 increased at the lower formaldehyde exposures (0.1 and 1 ppm.) Analysis of the number of
25 animals with olfactory pathology would be helpful to better understand the potential of low-level
26 formaldehyde effects on these less frequent lesions. Interestingly, the recovery of the olfactory
27 and respiratory epithelium seen in rats with undamaged nasal cavities after a 25-month recovery
28 period was not evident in rats with damaged noses. Formaldehyde-exposure effects are only
29 present at the 10 ppm exposure for the respiratory epithelium (squamous metaplasia, basal cell
30 hyperplasia), and the formaldehyde-related effects on the olfactory epithelium
31 (thinning/disarrangement, basal cell hyperplasia, and replacement by respiratory epithelium) are
32 seen at 0.1 and 1.0 ppm as well.

33 A single SCC, 1 out of 30 rats, was found in each 28-month formaldehyde-treatment
34 group (1/26, 1/28, and 1/26, respectively) but not in any control animals ($n = 52$). SCCs were
35 also noted in rats with noses damaged by electrocoagulation (1/54, 1/58, 0/56, and 15/58 for

1 control rats and the formaldehyde-treatment groups, respectively). These data clearly indicate a
2 synergistic effect of high formaldehyde exposure and nasal damage on the formation of SCCs in
3 rats. One adenosquamous carcinoma and one adenocarcinoma were also reported as increasing
4 the frequency to 17/58 for all tumors. Additionally SCC was present in two rats in the 0.1 and
5 1 ppm 3-month exposure groups with damaged noses only, although only one SCC was reported
6 in the 10 ppm 3-month groups with and without damaged noses. Rats not surviving to
7 28 months are included in these results, as well as the histopathology reported above. Since no
8 mortality data are reported, it should be noted that the incidence of both nasal lesions and tumors
9 are not controlled for early deaths.

10 In total, 30 tumors were examined from this study. In general, the tumors (26/30 or 87%)
11 were SCCs, and 69% (18/26) of these clearly originated from the respiratory epithelium lining
12 the septum or nasal turbinates. The eight other SCCs, derived from the epithelium lining the
13 nasolacrimal duct, were seen in connection with severe odontodystrophy and periodontitis or
14 might have originated from the skin or salivary glands. Four remaining rats bearing a nasal
15 tumor developed a small polypoid adenoma located on the nasoturbinates, an adenocarcinoma
16 originating from the olfactory epithelium, an adenosquamous carcinoma of the respiratory
17 epithelium lining the septum or turbinates, or a carcinoma in situ of epithelium in the
18 nasolacrimal duct.

19 20 **4.2.2.5.3. Hamsters.**

21 Dalbey (1982) examined the effects of inhaled formaldehyde alone for a lifetime or
22 combined with diethylnitrosamine (DEN) in an initiation-promotion study design using male
23 Syrian golden hamsters. For the first experiment, hamsters were exposed at either 0 or 10 ppm
24 (0 or 12.3 mg/m³) formaldehyde in whole body chambers 5 hours/day, 5 days/week for a lifetime
25 (132 controls, 88 exposed). Histopathologic evaluations were carried out on two transverse
26 sections of the nasal turbinates (otherwise not specified), longitudinal sections of larynx and
27 trachea, and all lung lobes cut along the bronchus prior to embedding. In the formaldehyde-only
28 (10 ppm) experiment, mortality was increased relative to unexposed controls ($p < 0.05$). No
29 tumors and little evidence of toxicity to the nasal epithelium were observed. There was no
30 increase in rhinitis. Epithelial hyperplasia and metaplasia were increased in formaldehyde-
31 treated animals (5% incidence) versus none observed in controls.

32 The second set of experiments by Dalbey (1982) examined interaction of formaldehyde
33 exposure on tumor formation from DEN administered subcutaneously. The five treatment
34 groups included: (1) controls ($n = 50$); (2) formaldehyde only ($n = 50$); (3) DEN 0.5 mg, once
35 per week for 10 weeks ($n = 100$); (4) formaldehyde exposure for life with DEN injection for the

1 first 10 weeks given 48 hours after formaldehyde exposure ($n = 27$); and (5) DEN injection for
2 10 weeks, followed by formaldehyde exposure for life ($n = 23$). In all groups hamsters were
3 exposed at 30 ppm formaldehyde 5 hours/day, once a week. Histopathologic examinations were
4 conducted as above.

5 Although weekly exposures to formaldehyde alone (30 ppm once a week) did not
6 influence mortality, treatment with DEN alone significantly ($p < 0.05$) increased mortality above
7 that of untreated controls, and mortality was further elevated ($p < 0.05$) in the two groups
8 exposed to both DEN and formaldehyde compared with DEN alone. No respiratory tract tumors
9 were observed in untreated animals or those receiving only formaldehyde. DEN treatment alone
10 resulted in a high incidence (77%) of tumors (nasal, larynx, trachea, and lung). Formaldehyde
11 pre- or post-treatment did not further increase the number of TBAs-. All tumors observed were
12 classified as adenomas. Formaldehyde pretreatment nearly doubled the number of tumors per
13 animal in the trachea (but not lung or larynx) ($p < 0.05$). This increase in tumors initiated by
14 DEN given 48 hours after formaldehyde exposure suggests a role of formaldehyde- induced
15 changes in the respiratory tract in tumor promotion (e.g., cell proliferation and inflammation).
16

17 **4.2.2.5.4. Summary.**

18 Chronic rodent studies of inhalation exposure to formaldehyde provide a consistent
19 picture of the agent's toxicity—especially on the URT—on which most studies focus. All three
20 species tested—hamsters, mice, and rats—had some degree of hyperplastic and metaplastic
21 change in the nasal passages. The pathology defined in acute and subchronic exposures is
22 similarly described in chronic studies, where progression, severity, and presence in more
23 posterior sections of the nose increase with both the concentration and duration of exposure.

24 Pathology of the respiratory epithelium includes rhinitis, goblet cell hyperplasia,
25 pseudoepithelial cell hyperplasia, squamous metaplasia, and dysplasia (see Table 4-42). At
26 higher exposures and longer durations of exposure, similar effects are seen on the olfactory
27 epithelium, present further into the nasal passages. In addition to hyperplasia and squamous
28 metaplasia, thinning and disarrangement of the olfactory epithelium noted and, in a few cases,
29 cell damage and replacement of olfactory epithelium with respiratory epithelium appear,
30 including loss of sensory cells (Woutersen et al., 1989; Kerns et al., 1983; Battelle Columbus
31 Laboratories, 1981).
32

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
<i>Chronic bioassays</i>					
<i>Mice</i>					
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)
<i>Rats</i>					
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)
<u>Hamsters</u>					
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.	<p>Pathology: Increased mortality in conjunction with DEN—above DEN-only treated animals. Respiratory pathology not reported.</p> <p>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).</p>	<p>LOAEL = 30 ppm.</p> <p>Evidence for formaldehyde as a promoter</p>	Dalbey (1982)

1 Clear species differences in the severity of lesions are present. Although the bioassays in
2 mice, hamsters, and rats do represent similar exposure concentrations and duration of exposure,
3 hamsters exhibit little pathology and rats (three strains tested) exhibit gross toxicity and even
4 increased mortality. Mice similarly exposed exhibit a range of effects on the respiratory
5 epithelium but not near the severity seen in rats. Many factors may contribute to these observed
6 species differences. As Chang and Barrow (1983) reported, the increased RB of mice seems to
7 be protective of POE damage in comparison to that of rats. The reduced ventilation rate and
8 minute volume of rodents in the presence of a reactive gas can reduce the effective delivered
9 dose at the same exposure concentration (Chang and Barrow, 1983). Additionally, as illustrated
10 in the computational fluid dynamic (CFD) modeling (see Section 3.5), there are species
11 differences in nasal architecture that influence areas of formaldehyde absorption or flux into the
12 tissue. Localized differences in mucus flow and production as well as metabolic enzymes have
13 also been posited as having roles in differential toxicity of formaldehyde on the URT (see
14 Chapter 3).

15 Formaldehyde-induced tumors were present in exposed rats and mice and primarily
16 involved SCCs later in life (Kamata et al., 1997; Tobe et al., 1985; Kerns et al., 1983; Swenberg
17 et al., 1980). Although exposure of male Syrian hamsters to either 10 or 30 ppm did not result in
18 formaldehyde-induced nasal tumors, a classic initiation-promotion assay with DEN-induced
19 tumor formation did indicate that formaldehyde increased the tumor burden per animal, where
20 DEN induced tumors in 77% of the animals (Dalbey, 1982). This study suggests a role for
21 promotion in the observed carcinogenicity of formaldehyde. Less clear are the implications of
22 the synergistic effect of formaldehyde exposures and gross damage to the respiratory epithelium
23 by electrocoagulation on tumor formation (Woutersen et al., 1989).

24

25 **4.2.2.6. *Summary of Respiratory Pathology and Carcinogenic Potential***

26 The progressive pathology of the nasal passages from inhalation exposure to
27 formaldehyde is well documented, especially in rodents (rats and mice) (see Tables 4-12, 4-23,
28 4-36, 4-42). Although there are species differences in tissue dose (see Section 3.4) due to
29 variations in nasal architecture and breathing patterns, the nature and progression of the
30 pathology is fairly well conserved across species, including nonhuman primates. The observed
31 formaldehyde-induced pathology includes disruption of the mucociliary apparatus, rhinitis
32 (serous and purulent), hyperplasia (cell proliferation), metaplasia (transition of cell type),
33 dysplasia (disarrangement of cells), nest-like infolds and invaginations of the epithelium,
34 thinning of the epithelial layer and focal to diffuse lesions, atrophy of the olfactory epithelium,

1 thickening and keratinization (usually of squamous metaplasia), tumors (adenoma, sarcoma,
2 carcinoma) (see Section 4.2.2).

3 Progression of lesions can be viewed as progression from the anterior to posterior
4 sections of the nasal cavity or as a progression in severity of lesions at a particular location (e.g.,
5 level or region) of the nasal passages. In both cases, progression is evident with increasing
6 exposure concentration and with increasing duration of exposure (Kamata et al., 1997;
7 Monticello et al., 1996; Morgan et al., 1986b; Takahashi et al., 1986; Sellakumar et al., 1985;
8 Kerns et al., 1983; Albert et al., 1982). The data suggest that concentration and duration of
9 exposure do not act in a simply cumulative manner (e.g., $C \times t$). Additionally the influence of
10 concentration, duration, and repeated exposure may be different for various effects. For
11 example, some lesions may be transient (e.g., low-exposure cell proliferation), others may have a
12 threshold and vary little after that (e.g., rhinitis). Additionally, as the nasal epithelium responds
13 with both adaptive and adverse epithelial changes, the absorption of formaldehyde into the tissue
14 at that location may be reduced. As respiratory epithelium transitions to squamous metaplasia,
15 the effective tissue dose of formaldehyde increases posterior to these lesions. As barriers to
16 formaldehyde flux into the tissue develop (e.g., squamous metaplasia, keratinization),
17 formaldehyde penetrates more deeply into the nasal passages (Kimbell et al., 2006). Therefore,
18 although both concentration and duration of exposure do affect the adverse effect, the
19 relationship is difficult to define and in fact may be different for various adverse effects.

20 Respiratory histopathology has been commonly reported in response to exposure to
21 formaldehyde in rats and mice (Lino dos Santos Franco et al., 2006; Javden and Taher, 2000;
22 Kamata et al., 1996a, b; Cassee and Feron, 1994; Bhalla et al., 1991; Monteiro-Riviere and Popp,
23 1986; Buckley et al., 1984; Chang et al., 1983), rabbits (Ionescu et al., 1978), hamsters
24 (Schreibner et al., 1979), and rhesus monkeys (Monticello et al., 1989). The histopathologic
25 lesions ranged from inflammation to ulceration, necrosis, and metaplasia that occurred in nasal
26 turbinates, maxilloturbinates, and goblet and microvillus cells (Bhalla et al., 1991). These effects
27 were observed at a variety of doses (e.g., 10 ppm for 4 hours, 3.13 ppm for 6 hours for 1, 2, or
28 4 days, 6 or 15 ppm). Wilmer et al. (1989, 1987) assessed whether a dose and time-dependent
29 interaction ($C \times t$) is associated with histopathologic lesions. Results indicated that
30 concentration, rather than duration or cumulative exposure, correlates best with severity of
31 lesions (Wilmer et al., 1989, 1987).

32 Histopathologic lesions and changes to biochemistry have been reported in the lung as
33 well, though these effects were observed following a high dose of formaldehyde. In addition,
34 changes in clinical chemistry, P450 expression and activity in lung tissue, and gene expression
35 that is phenotypically anchored to the observed respiratory pathology have been reported.

1 Extrapulmonary effects have also been noted, including changes in liver chemistry, relative brain
2 weight, and focal, chronic inflammation in the heart and kidney. Most of these changes occurred
3 at exposures of 20 ppm, and those that occurred at lower formaldehyde exposures (3.7 ppm)
4 could not be strictly correlated with formaldehyde exposure.

5 Some researchers have reported formaldehyde-induced effects in the pulmonary region in
6 rats, mice, and rabbits. Kamata et al. (1996a) observed reduced lipid content of pulmonary
7 surfactant in rats exposed to 128.4 or 294.5 ppm formaldehyde. Kamata et al. (1996b) reported
8 biochemical changes in lung homogenates and altered lipid content of BAL at 145.6 ppm
9 formaldehyde. Lino dos Santos Franco et al. (2006) observed increased leukocytes (and
10 neutrophils) and degranulated mast cells recovered in BAL fluid (concentration of 1% formalin
11 not provided). In rabbits, Ionescu et al. (1978) observed frank necrosis of lung parenchyma after
12 aerosol inhalation of 3% formalin for 3 hours/day for 50 days (concentration of formaldehyde
13 not provided). These pulmonary effects may be due to frank toxicity resulting from the high
14 dose of formaldehyde used in these studies.

15 Several recent toxicogenomics studies have assessed gene expression changes in nasal
16 and lung tissue in animals and in humans by using in vivo and in vitro approaches. Hester et al.
17 (2005, 2003) documented changes in gene expression associated with DNA repair and apoptosis
18 in nasal tissue from male rats after a single instillation of formaldehyde. Other gene expression
19 changes were observed in those genes related to xenobiotic metabolism and in cell cycle and
20 repair. These preliminary results provide an initial basis for forming a phenotypically anchored
21 set of gene expression changes associated with exposure to formaldehyde and may assist in
22 determining the underlying MOA, as will be discussed in Section 4.5. Sul et al. (2007)
23 investigated gene expression genes in lung tissue from formaldehyde-exposed rats. Yang et al.
24 (2005) performed a proteomics analysis by using lung tissue extracted from formaldehyde-
25 exposed rats. Two studies used human tracheal cell lines to investigate formaldehyde-induced
26 gene expression changes in vitro (Lee et al., 2008, 2007). However, the relevance of these
27 findings to actual exposures remains unknown. In total, toxicogenomics studies hold promise,
28 but they must be interpreted with caution until results can be replicated and phenotypically
29 linked to observable changes.

30 Thus, formaldehyde-induced respiratory pathology has been commonly described in the
31 nasal passages and includes cellular proliferation, mucociliary function, and histopathologic
32 lesions. Pulmonary effects have been documented as well but at high doses. The nasal
33 pathology may occur as a result of both concentration and duration components of exposure.
34

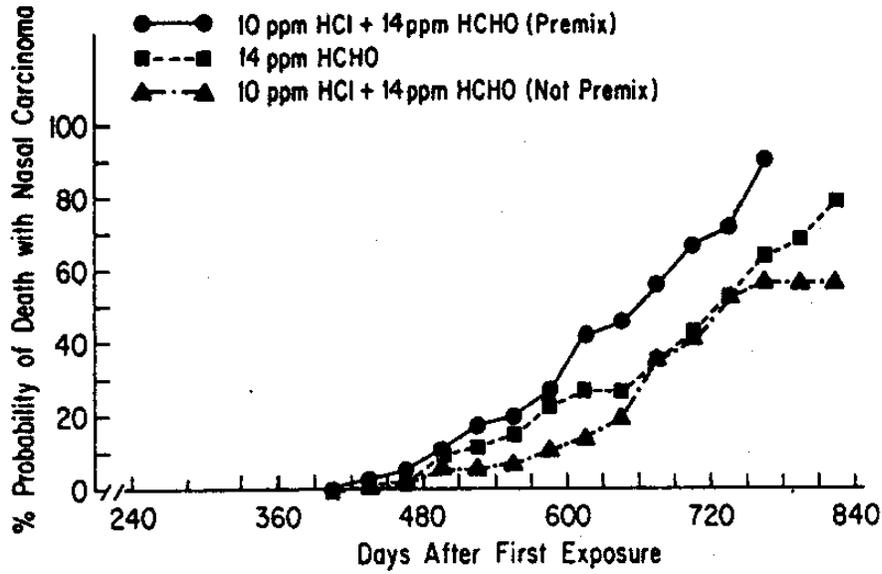
1 **4.2.2.6.1. Carcinogenic potential.**

2 In the respiratory tract, only nasal tumors are considered formaldehyde induced in rodent
3 studies. The majority of studies were conducted using rats (F344, Wistar, or Sprague-Dawley),
4 and all studies of 18 months or greater in mice and rats show evidence of formaldehyde-induced
5 nasal carcinogenicity. The nasal tumors are primarily SCCs, although papillomas, polypoid
6 adenoma, adenocarcinoma, fibrosarcoma, and esthesioneuroepithelioma have been reported
7 (Kamata et al., 1997; Monticello et al., 1996; Morgan et al., 1986a, b; Takahashi et al., 1986;
8 Sellakumar et al., 1985; Kerns et al., 1983; Albert et al., 1982). Although hyperplasia, dysplasia,
9 and squamous metaplasia of the respiratory epithelium have been observed beyond the nasal
10 cavity, other respiratory tract tumors have not been significantly increased by formaldehyde
11 exposure alone.

12 Increased tumor incidence and decreased latency are correlated with increasing
13 formaldehyde exposure concentration. Reviewing data from the only lifelong inhalation study
14 with multiple exposure groups, SCC is first noted at 8 and 9 months for high exposed (15 ppm)
15 female and male F344 rats autopsied as “early deaths” prior to the 12 month sacrifice, with an
16 incidence of 43% over the course of the study (unadjusted for mortality) (Kerns et al., 1983). In
17 contrast only two SCCs were found in male and female rats sacrificed after 24 months of
18 exposure (incidence of SCC 2.5% at 24 months) (Kerns et al., 1983). In a follow-up study by
19 Monticello et al. (1996), the incidence of SCC in rats exposed at 15 ppm was 47% with the first
20 tumor noted at 12 months. The incidence of SCC in male rats exposed at 10 ppm was 22% with
21 the first SCC noted at 18 months. Moreover, of 90 rats exposed at 6 ppm for 20 months only one
22 SCC was noted. No SCCs were detected in rats exposed at 0.7 or 2 ppm formaldehyde. These
23 incidence rates are not mortality adjusted and include animals from each scheduled sacrifice (3,
24 6, 12, and 18 months). In a lifelong study of male Sprague-Dawley rats exposed at 15 ppm
25 formaldehyde, the cumulative nasal tumor incidence was calculated as a function of time of
26 exposure (see Figure 4-14) (Sellakumar et al., 1985). After 2 years of exposure, the probability
27 of nasal carcinoma was greater than 60%.

28 There is some evidence that less-than-lifetime exposure to formaldehyde can induce nasal
29 tumors over an extended observation period. Two studies, both in male Wistar rats, report nasal
30 tumors in response to less-than-lifetime exposures (Woutersen et al., 1989; Feron et al., 1988).
31 A 13-week exposure at 20 ppm resulted in four nasal tumors (three SCCs), a cystic SCC of the
32 nasolacrimal duct, and an epithelial tumor on the mandible, for a total of six tumors observed
33 over 30 months of observation (Feron et al., 1988). No tumors were noted in 13-week controls.
34 A limited number of formaldehyde-related tumors were noted due to 4 or 8 weeks of exposure
35 followed by 30 months of observation. Although the tumor incidence of these less-than-lifetime

1 exposures is low, this is consistent with the 2-year bioassays in Wistar rats. Wistar rats are more
2 resilient to formaldehyde-induced nasal toxicity than F344 or SD rats (see Section 4.2.1), and
3 only 1 of 26 (4%) Wistar rats exposed at 10 ppm for 28 months developed SCC (Woutersen et
4 al., 1989) versus 22% in F344 rats (Monticello et al., 1996).



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7
8 **Figure 4-14. Mortality corrected cumulative incidences of nasal carcinomas**
9 **in the indicated exposure groups.**

10
11 Source: Sellakumar et al. (1985).

12
13
14 Woutersen et al. (1989) also examined the effect of severe nasal damage from
15 electrocoagulation on formaldehyde-induced SCC in Wistar rats. Nasal tumors were noted in
16 formaldehyde-exposed rats without damaged noses (exposed for only 3 months and observed for
17 25 months). However, the low incidence of tumors in each treatment group (1/26, 2/60, 2/60,
18 1/58) indicates these data should be considered suggestive even though no SCCs were noted in
19 control rats with or without damaged noses ($n = 83$). The studies by Woutersen et al. (1989) did
20 demonstrate a synergistic effect of nasal damage from electrocoagulation and 10 ppm
21 formaldehyde exposure (3 months), where 15/58 rats had SCC versus 1/26 with undamaged
22 noses. The study was originally designed to examine the effect of formaldehyde on the damaged
23 tissue on cancer promotion. However, it is unclear if the synergistic effect of formaldehyde
24 exposure on damaged nasal tissue is an effect of formaldehyde on the damaged cells and joint
25 effects of a mutagen with regenerative proliferation from the nasal damage. It is also possible

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1 the damaged nasal passages may alter airflow in the nasal passages, resulting in significantly
2 different flux of formaldehyde into the tissue.

3 There is a single inhalation study (Dalbey, 1982) that investigates the role of promotion
4 in formaldehyde-induced cancer. Although hamsters exhibit little to no effects of formaldehyde
5 on the nasal mucosa or other respiratory tract tissues (Rusch et al., 1983a, b; Dalbey, 1982),
6 DEN-induced respiratory adenomas were increased with formaldehyde exposure (10 ppm)
7 48 hours prior to DEN injection (but not by formaldehyde alone or formaldehyde exposure after
8 DEN injection). The number of tracheal tumors per TBA was doubled by formaldehyde
9 exposure. The study authors note that adenomas should be considered independent tumors and
10 that the increase in tracheal tumors is of biological significance even given the incidence of
11 TBAs (77%, DEN alone), was not further increased by formaldehyde exposure. It is of
12 particular interest that a promotion study in hamsters is positive, since so little nasal pathology
13 occurs with formaldehyde exposure. The absence of significant hyperplasia and tissue damage
14 in these animals suggests that formaldehyde may induce subtle changes in the respiratory tract
15 mucosa that permit formaldehyde to act as a tumor promoter.

16 17 4.2.3. Gastrointestinal Tract Pathology

18 As with inhalation, the POE is thought to be the principal target tissue in response to oral
19 exposure. A concentration-dependent pattern of toxicity longitudinally down the GI tract has
20 been observed upon oral exposure. Some evidence (Til et al., 1989, 1988) suggests that, with
21 regard to oral exposure, duration in addition to concentration is important in the development of
22 toxicity.

23 Formalin and paraformaldehyde were used to dose animals in oral toxicity studies.
24 Formalin contains 12–15% methanol as a preservative to inhibit the polymerization of
25 formaldehyde and subsequent precipitation as paraformaldehyde (Kiernan, 2000). The presence
26 of methanol in formalin may confound the results of a formaldehyde study. Methanol has been
27 shown to be a developmental and neurologic toxin (e.g., Degitz et al. [2004a, b]; Rogers et al.
28 [2004, 2002]; Weiss et al. [1996]; Sharpe et al. [1982]). Oral dosing with paraformaldehyde is
29 preferred because it allows for the preparation of methanol-free formaldehyde in the laboratory
30 by dissolving paraformaldehyde in slightly basic water.

31 32 4.2.3.1. *Short-Term and Subchronic Ingestion Studies*

33 Til et al. (1988) evaluated the oral toxicity of formaldehyde and acetaldehyde in a
34 subacute study in Wistar (Cpb:WU; Wistar random) rats. Groups of rats (10/sex/dose) were
35 exposed to paraformaldehyde dissolved in drinking water at 0, 5, 25, and 125 mg/kg-day for 4

1 weeks. The control group was comprised of 20 rats of each sex. To account for potential effects
2 of decreased water consumption in treated animals, an additional control group of 10 male and
3 10 female rats was given drinking water in an amount equal to the amount of liquid consumed by
4 the group given the highest dose. Examination of the GI tract was performed in all dose groups
5 and included the tongue, esophagus, and stomach. Histopathology for the other tissues was
6 performed on high-dose and control animals.

7 The rats appeared to be healthy throughout the study, and no effects on growth occurred
8 despite significant decreases in food and water intake that occurred at the high dose (125
9 mg/kg-day). Yellow discoloration of the fur occurred in the rats on the high dosage from week 3
10 onward. There were no significant changes in hematology among the exposed groups except for
11 slight (not statistically different) increases in PCVs in the water-restricted group and in high-dose
12 males. The high-dose groups of the formaldehyde exposed and in the water-restricted controls
13 had slightly increased urine density, but again this was not statistically significant. Plasma TP
14 and ALB levels were decreased in the males of the highest dose group. No changes in organ
15 weights occurred except for relative kidney weights that were slightly increased in the females of
16 the high-dose group. Gross pathological findings were restricted to the GI tract and revealed a
17 thickening of the limiting ridge of the forestomach in all animals exposed at the highest dose that
18 was accompanied by a yellowish discoloration of the mucosa. These latter changes were not
19 observed in the acetaldehyde-exposed animals. Treatment-related histopathologic changes were
20 seen in the GI tract only. Slight (8/20) or moderate (12/20) focal hyperkeratosis of the
21 forestomach and slight focal atrophic gastritis occurred in animals of the high-dose groups only
22 (see Table 4-43). One female had moderate focal papillomatous hyperplasia. No
23 histopathologic changes were observed in any animals of the lower-dose groups. The study
24 established a LOAEL and NOAEL for epithelial changes in the GI tract of male and female
25 Wistar rats exposed to formaldehyde in drinking water at 125 mg/kg-day and 25 mg/kg-day,
26 respectively.

27 Johannsen et al. (1986) performed a subchronic study by using rats exposed to
28 paraformaldehyde dissolved in drinking water and dogs exposed to paraformaldehyde in the diet.
29 Groups of albino Sprague-Dawley rats (15/sex) were administered the equivalent of 0, 50, 100,
30 or 150 mg/kg-day in their drinking water for 91 consecutive days. Pure-bred beagle dogs
31 (four/sex/group) were fed a diet with added aqueous formaldehyde to approximate 0, 50, 75, or
32 100 mg/kg-day. Dogs were observed daily and rats at frequent intervals for behavioral reactions.
33 Body weights and food and water intake were recorded on a weekly basis in both species.
34 Hematology (HCT, Hb, total and differential leukocyte counts), clinical chemistry (blood sugar,
35 BUN, ALP, AST and ALT in dogs only), and urine analyses (color, appearance, pH, specific

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2
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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

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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.

1 No deaths or abnormal reactions were observed in either species. Significant reductions
2 in weight gain were observed in dogs of both sexes at 100 mg/kg-day, in rats of both sexes at
3 150 mg/kg-day, and in male rats at 100 mg/kg-day of formaldehyde. There was a dose-related
4 decrease in liquid consumption of both sexes in rats given formaldehyde, but there was no
5 overall difference in mean food intake or feed efficiency, so the reductions in body weight gain
6 were considered to be systemic effects. Dogs administered formaldehyde had reduced food
7 consumption and feed efficiency at all doses tested. No significant effects on hematology,
8 clinical chemistry, or urine analyses were observed in either species. No effects in either species
9 were reported on organ weights. The GI mucosa in both species was reported to appear normal
10 with no indication of irritation. This study suggests a NOAEL of 150 mg/kg-day in Sprague-
11 Dawley rats and of 100 mg/kg-day in beagle dogs for formaldehyde in drinking water.
12 Differences in the results for the rats with those reported in other studies (Til et al., 1989, 1988;
13 Tobe et al., 1989) may be due to strain differences or duration of the exposure. The dog may be
14 a more sensitive species than the rat based on these results and on those of 2-week pilot studies.
15

16 **4.2.3.2. Chronic Ingestion Studies**

17 The same laboratory that tested formaldehyde and acetaldehyde in a 4-week study (Til et
18 al., 1988) performed a chronic bioassay with formaldehyde in drinking water. Til et al. (1989)
19 administered paraformaldehyde dissolved in drinking water to Wistar rats (Cpb: WU; Wistar
20 random) (70/sex/dose). Interim sacrifices (10/sex/dose) were performed at 12 and 18 months.
21 Formaldehyde was administered in drinking water to provide target doses of 0, 5, 25, and 125
22 mg/kg-day. The mean formaldehyde doses administered were 0, 1.2, 15, or 82 mg/kg-day for
23 males and 0, 1.8, 21, or 109 mg/kg-day for females. Concentrations were adjusted weekly for
24 the first 12 weeks based on dose estimates derived from body weight and liquid consumption
25 data. Such adjustments were made every 4 weeks from weeks 12 to 52 and kept constant. Fresh
26 solutions of the test concentrations were prepared weekly and stored at 15°C.

27 Endpoints examined included daily observations for condition and behavior, body weight
28 at weekly intervals for the first 12 weeks and then every 4 weeks thereafter, liquid intake weekly,
29 and food intake weekly for the first 12 weeks and then every 2 weeks for the remainder of the
30 study. Samples of blood were taken for hematological and clinical chemistry analyses on weeks
31 26 and 103. Analysis of blood glucose and urine pH, density, and volume was performed on
32 samples at weeks 27, 52, 78, and 104. Pooled urine samples were also evaluated for glucose,
33 occult blood, ketones, urobilinogen, and bilirubin in samples at weeks 27 and 104. Weights of
34 all major organs were recorded at interim sacrifices and at term. Gross and histopathologic
35 examinations were carried out on all major tissues of the rats in the high-dose and control

1 groups. The livers, lungs, stomach, and noses were examined in all rats. Additionally, the
2 adrenals, kidneys, spleens, testes, thyroids, ovaries, pituitaries, and mammary glands (for
3 females) were examined in all sacrificed animals at weeks 53 and 79 and at term.

4 The general health and behavior of the rats were not affected in any of the formaldehyde-
5 exposed groups. Slight yellowing of the fur did occur in the animals exposed at the mid and high
6 doses from week 3 onward. The mean body weights were decreased in the males from week 1
7 and in the females from week 24 onward. At the high dose, liquid consumption was significantly
8 decreased in both sexes, and food intake was significantly decreased in the males. There were no
9 toxicologically significant effects on hematological, urinary, or clinical chemistry parameters.
10 Decreases in absolute heart, liver, and testis (males) weights were attributed to lower body
11 weights. Relative kidney weights were increased in females of the high-dose group, and relative
12 brain weights were increased in both sexes of the high-dose group. Relative testis weight was
13 increased in males. Treatment-related changes in gross pathology were restricted to the
14 forestomach. Histopathologic examinations at the two interim sacrifices and final sacrifice
15 revealed GI tract changes. Renal changes were observed in the high-dose group at final
16 sacrifice. There was no indication of treatment-related effects in other tissues.

17 As shown in Table 4-44, significant histopathology in the GI tract was limited to the
18 forestomach and stomach of rats in the high-dose groups. Some progression with duration of
19 exposure may have occurred by week 105 because GI lesions were observed in the lower dose
20 groups at this time point, whereas none were observed in these groups at interim sacrifices. The
21 histopathologic changes included papillary epithelial hyperplasia in the forestomach that was
22 frequently accompanied by hyperkeratosis on the limiting ridge or its vicinity. The mucosa
23 showed an irregular layer of hyperplastic basal cells, but no atypical nuclei or other subcellular
24 structures were observed. Chronic atrophic gastritis occurred to varying degrees in the stomachs
25 of all high-dose rats. In some cases the inflammatory process involved the entire mucosa and
26 was seen to extend to the whole muscularis mucosae and met the criteria for ulceration.

27 Histologic examination also showed that the incidence and degree of renal papillary
28 necrosis was increased in animals of the high-dose groups at the terminal sacrifice. This change
29 was located at the tip of the papilla and was characterized by patchy necrosis of interstitial cells,
30 capillaries, and loops of Henle. There was no evidence of a dose-related response in chronic
31 nephropathy. The incidence of chronic nephropathy was lower in the males of the high-dose
32 group than in controls. In females, the incidence was slightly higher in the test groups than in
33 controls but only achieved statistical significance at the lowest dose. It is likely that the decrease
34 in liquid intake incurred in the high-dose groups contributed to the increased incidence and
35

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

^aIncidence in rats that died or were killed when moribund during the experiment or were killed at week 53, 79, or 105.

^bA few rats were lost because of advanced autolysis.

^cThe values differ significantly (Fisher's exact test) from the control value ($p < 0.001$).

Source: Til et al. (1989).

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1 degree of renal papillary necrosis observed in the high-dose animals because dehydration has
2 been shown to enhance its production by various analgesics.

3 The results of this chronic bioassay indicated that formaldehyde is cytotoxic to the
4 epithelial mucosa of the nonglandular (forestomach) and glandular stomach with a LOAEL of 82
5 and 109 mg/kg-day and a NOAEL of 15 and 21 mg/kg-day in males and females, respectively.
6 The findings provided no evidence of carcinogenicity in either the GI tract or systemic sites for
7 formaldehyde administered in drinking water to Wistar rats at doses as high as 82 mg/kg-day.

8 Tobe et al. (1989) performed a chronic toxicity study of Wistar rats (Slc:Wistar) exposed
9 to paraformaldehyde dissolved in drinking water. Groups of 20 male and 20 female rats were
10 given formaldehyde solution in their drinking water at concentrations of 0, 0.02, 0.10, and 0.50%
11 for 24 months. Interim sacrifices of six randomly chosen rats from each group were performed
12 after 12 and 18 months. Based on the estimated average amount of water intake and body
13 weight, the actual doses of formaldehyde in either sex were reported to be 0, 10, 50, and
14 300 mg/kg-day. Fresh test solutions were prepared twice each week. The rats were observed
15 daily for the entire study. Body weights and water and diet intake were measured once weekly
16 or biweekly. Hematology (RBC, WBC, and Hb) and serum clinical chemistry (TP, ALB, BUN,
17 uric acid, total cholesterol, inorganic phosphorous, ALP, AST, and ALT) were made at each
18 necropsy. Organ weights were measured for the brain, heart, lung, liver, kidney, spleen, adrenal,
19 testis or ovary, pituitary, and thyroid. These organs and the stomach, small and large intestine,
20 pancreas, uterus, lymph nodes, and all tumors were examined histopathologically.

21 The general condition of animals in the high-dose group was poor with significantly
22 reduced body weight gain as well as intake of water and diet. An increase in mortality was also
23 observed in this group. Some clinical chemistry parameters were altered in this group. No
24 significant changes in absolute or relative organ weights were observed. Mortality was 100% in
25 the high-dose group by 24 months. At the 12-month sacrifice, hyperplasia of the squamous
26 epithelium with or without hyperkeratosis was observed in the forestomach of all high-dose
27 animals (12/12). Basal cell hyperplasia with growth into the submucosa was also observed in
28 most cases (10/12). Erosions and/or ulcers with submucosal inflammatory cell infiltrates were
29 observed in the glandular stomach of most rats (10/12). Regenerative changes of the glandular
30 epithelium (glandular hyperplasia) were noticed in most cases (10/12) along the limiting ridge of
31 the fundic mucosa. No lesions were observed in the glandular stomach at the 50 mg/kg-day
32 dose, and forestomach hyperplasia was observed in only one of six males and in one of eight
33 females at 18 and 24 months. No lesions in either the forestomach or glandular stomach were
34 observed in rats treated at 10 mg/kg-day.

1 This study corroborates the Til et al. (1989) study and shows that the main targets for
2 formaldehyde toxicity administered by drinking water to rats are the forestomach and glandular
3 stomach. Although the lesions observed at the 50 mg/kg-day were minimal in this study, Tobe et
4 al. (1989) designated the NOAEL at 10 mg/kg-day, further supporting the NOAEL of 15
5 mg/kg-day from the Til et al. (1989) study.

6 Takahashi et al. (1986) studied the effects of formaldehyde in an initiation-promotion
7 model of stomach carcinogenesis in male outbred Wistar rats (Shizuoka Laboratory Center,
8 Shizuoka). Rats ($n = 17$) were given 100 mg/L of *N*-methyl-*N*¹-nitro-*N*-nitrosoguanidine
9 (MNNG) in drinking water and a diet supplemented with 10% sodium chloride (NaCl) for the
10 first 8 weeks as an initiation phase. This was followed by 0.5% formalin (which contains
11 12–15% methanol) in drinking water for 32 weeks as the promotion phase of the protocol. A
12 comparison group ($n = 10$) was given stock water and diet without any supplementation for the
13 first 8 weeks followed by 0.5% formalin in drinking water for 32 weeks. Animals were observed
14 daily and weighed once every 4 weeks. Small pieces of the stomach and other tissues in the
15 peritoneal cavity were fixed for histopathologic examination.

16 Body weight gain was reduced by exposure to MNNG with sodium chloride, and
17 formaldehyde exposure during the promotion phase exacerbated this effect. Histopathologic
18 investigations were restricted to the GI tract. Formaldehyde was shown to statistically increase
19 the incidence of lesions in the forestomach and stomach in the animals initiated with MNNG
20 with NaCl as compared with controls receiving no initiation (see Table 4-45). Increases in
21 papilloma in the forestomach, adenomatous hyperplasia in the fundus, and adenocarcinoma in
22 the pylorus were observed. Histopathology in the animals receiving formaldehyde alone during
23 weeks 9 through 32 showed an increase in forestomach papillomas but with no lesions in the
24 glandular stomach (see Table 4-45). The adenomatous hyperplasia were defined as proliferative,
25 noninvasive mucosal lesions, and the adenocarcinomas were defined as well differentiated and
26 composed of typical glandular structures, demonstrating a tubular pattern and cellular or
27 structural atypism without metastasis. No definition of criteria for papilloma diagnosis was
28 provided. The findings in this study are inconsistent with those of Til et al. (1989), who found
29 no evidence of carcinogenicity in a 2-year bioassay at comparable concentrations (assuming 37%
30 formaldehyde in formalin results in 0.19% formaldehyde in this study). As discussed above, the
31 differences may be due to differences in the strains of rat or in the diagnostic criteria. The lack
32 of more than one test concentration precludes dose-response analysis of this study and provides
33 only a stand-alone LOAEL of 0.2% formaldehyde in drinking water. The lack of consumption
34 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)							
	Gastroduodenal carcinoma	Forestomach papillomas	Glandular stomach tumors				
			Fundus		Pylorus		Duodenum
			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)							
	Gastroduodenal carcinoma	Forestomach papillomas	Glandular stomach tumors				
			Fundus		Pylorus		Duodenum
			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%

^aSignificantly different from control animals with MNNG initiation, $p < 0.01$.

^bSignificantly different from control animals with MNNG initiation, $p < 0.05$.

Source: Takahashi et al. (1986).

1 4.2.3.3. *Summary of Gastrointestinal Effects and Evaluation of Carcinogenic Potential*

2 Short-term and subchronic exposures to formaldehyde via drinking water for 4 weeks
3 yielded slight to moderate histopathologic lesions (focal hyperkeratosis) at 125 mg/kg-day in
4 male and female Wistar rats, as well as slight focal gastritis and submucosal infiltrate in one to
5 two animals of both sexes (Til et al., 1988). No histopathologic lesions were noted in albino
6 Sprague-Dawley rats or beagle dogs that received oral doses of formaldehyde in drinking water
7 for 91 days (Johannsen et al., 1986). In both studies, decreases in weight gain were noted in
8 exposed animals compared with controls.

9 As with the respiratory tract, the proximal portion of the GI tract exhibits formaldehyde-
10 induced lesions in the forestomach and glandular stomach (Til et al., 1989; Tobe et al., 1989;
11 Takahashi et al., 1986). In a chronic drinking water study, Til et al. (1989) reported that
12 formaldehyde is cytotoxic to the epithelial mucosa of the nonglandular (forestomach) and
13 glandular stomach with a LOAEL of 82 and 109 mg/kg-day and a NOAEL of 15 and 21
14 mg/kg-day in males and female Wistar rats, respectively. The findings provided no evidence of
15 carcinogenicity in either the GI tract or systemic sites for formaldehyde administered in drinking
16 water to Wistar rats at doses as high as 82 mg/kg-day. The incidence and degree of renal
17 papillary necrosis was increased in animals of the high-dose groups at the terminal sacrifice (Til
18 et al., 1989). Findings by Tobe et al. (1989) corroborate the Til et al. (1989) study and show that
19 the main targets for formaldehyde toxicity administered by drinking water to rats are the
20 forestomach and glandular stomach.

21 There is evidence that formaldehyde may act as a tumor promoter by the oral route as
22 well as the inhalation route (discussed above). Takahashi et al. (1986) studied the effects of
23 formaldehyde in an initiation-promotion model of stomach carcinogenesis in male outbred
24 Wistar rats (Shizuoka Laboratory Center, Shizuoka, Japan Takahashi et al. (1986) reported an
25 increase in MNNG-initiated GI cancers with formaldehyde exposure (29.4 versus 13.3% TBA in
26 controls); the greatest difference in tumor-containing versus nontumorigenic mice was associated
27 with adenocarcinoma in the glandular stomach (23.5 versus 3.3% in controls). Additionally,
28 forestomach papillomas and preneoplastic hyperplasia in the glandular stomach were increased
29 with formaldehyde exposure alone.

30 31 4.2.4. Immune Function

32 Leach et al. (1983) documented potential immunomodulatory effects of formaldehyde
33 inhalation exposure. F344 rats were exposed nose only to formaldehyde 6 hours/day,
34 5 days/week for up to 30 days. The target concentrations for exposure were 0, 3, 16, 61, and
35 99 ppm formaldehyde (0, 3.7, 19.7, 75.0, and 122 mg/m³). Body weight and food consumption
36 were recorded, and blood samples for standard hematology and immune assays were collected

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1 (details not given). Immune measures referenced include in vitro lymphocyte transformation,
2 hemagglutination assays, and enumeration of B cells, WBCs, and RBCs. No effects were seen at
3 3 ppm formaldehyde. However, dose-dependent responses were reported for weight loss,
4 decreased food consumption, increased WBCs, increased segmented neutrophils and nucleated
5 RBCs, and decreased ability to produce antibodies to sheep RBCs. The results of the
6 lymphocyte transformation assay were inconsistent, with a 25–30% reduction in stimulation after
7 exposure to 99 ppm but an initial stimulation seen after 16 and 61 ppm exposures. Further
8 details were not available, making it difficult to determine if these reported immunomodulatory
9 effects may have been, in part or in full, secondary to effects on the URT. Subchronic exposures
10 at 61 and 99 ppm formaldehyde would be expected to result in frank toxic effects in mice (see
11 Section 4.2.1). However, these findings suggest possible immunomodulatory effects due to
12 formaldehyde exposure and require further exploration.

13 Dean et al. (1984) investigated the effects of formaldehyde exposure on a range of
14 indicators of immune function. Female B6C3F1 mice were exposed to 15 ppm formaldehyde
15 (18.4 mg/m^3) 6 hours/day, 5 days/week for 3 weeks. Three trials were run with a total of 255
16 formaldehyde-treated mice. Body and organ weights were recorded at sacrifice for control and
17 formaldehyde-exposed mice (10 per group). Measures of host susceptibility, cell-mediated
18 immunity MP function, and antibody reactions were conducted 2 to 6 days after the end of
19 exposure (see Table 4-46). Lymphocyte subsets, spleen cellularity, bone marrow cellularity, and
20 progenitor cell subsets were enumerated. Host susceptibility and delayed type hypersensitivity
21 were measured in vivo. Lymphocyte proliferation, natural killer cell activity, phagocytosis,
22 hydrogen peroxide production, and IgM plaque-forming cells (PFCs) were measured ex vivo
23 after in vivo stimulation in some cases (see Table 4-46).

24 Body weight, organ weights and cellularity, progenitor cell populations, blood cell
25 counts, and differentials were unchanged in formaldehyde-treated mice (Dean et al., 1984).
26 Circulating blood monocytes were decreased in treated mice, which may be a reflection of the
27 local inflammatory response expected in the nasal epithelium (Dean et al., 1984). However,
28 there was no corresponding decrease in peritoneal MPs. There was a trend, but no statistical
29 significance, for decreased spleen weight, cellularity, and B cell precursors (87, 83, and 78% of
30 controls, respectively). The mean body weight of formaldehyde-treated mice was 21.1 versus
31 20.9 g in control mice, and thymus and spleen weights were not normalized by body weight.

32 All indicators of natural killer cell function, cell-mediated immunity, and humoral
33 immunity in formaldehyde-treated mice were unchanged from controls (Dean et al., 1984).
34 Phagocytic capacity of both resident and elicited peritoneal MPs was unchanged by formaldehyde
35 treatment. However, hydrogen peroxide production in elicited peritoneal MPs was significantly

1 increased in formaldehyde-treated mice, 78 versus 42 nmol/mg protein ($p < 0.05$) (Dean et al.,
 2 1984).

3
 4
 5
 6
 7

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	<i>Listeria monocytogenes</i>	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 ₂ O 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

8 ^aT-cell mitogen, phytohemagglutinin (PHA-P).
 9 ^bB-cell mitogen, lipopolysaccharide (*Escherichia coli*).
 10 Source: Dean et al. (1984).
 11
 12

13 As shown in Table 4-47, several indicators of host resistance in the female B6C3F1 mice
 14 were increased after formaldehyde exposure (Dean et al., 1984). Tumor mass and pulmonary
 15 foci after B16F10 melanoma cell challenge were significantly reduced in formaldehyde-treated

1 mice, indicating improved tumor immunity ($p < 0.05$). However, following PYB6 sarcoma cell
 2 challenge, formaldehyde-treated mice had a 7.1% tumor incidence versus 11.1% in controls,
 3 which was not statistically different. Mortality due to *Listeria monocytogenes* (LM) was
 4 decreased from 70 to 30% ($p < 0.05$). Because resistance to LM is primarily MP dependent, the
 5 authors speculated that this enhanced resistance might be due in part to increased bactericidal
 6 activity as was also suggested by increased hydrogen peroxide production ex vivo in elicited
 7 peritoneal MPs from female mice (Dean et al., 1984).

8
 9 **Table 4-47. Summary of the effects of formaldehyde inhalation on the**
 10 **mononuclear phagocyte system (MPS) in female B6C3F1 mice after a**
 11 **3-week, 15 ppm formaldehyde exposure (6 hours/day, 5 days/week)**
 12

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 ₂ O 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

13 ^aDean et al. (1984).

14 ^bAdams et al. (1987).

15 ^cPhorbol 12-myristate 13-acetate (PMA).

16 ^dPeritoneal MPs were elicited with the pyran copolymer Murray Valley encephalitis virus (MVE-2).

17 Sources: Adams et al. (1987); Dean et al. (1984).

1 Overall, the observations of increased hydrogen peroxide production and increased host
2 resistance in peritoneal MPs distant from the POE suggest that formaldehyde has an effect on the
3 mononuclear phagocyte system (MPS). The authors postulated that this effect may be indirect,
4 due in part to the tissue inflammatory response in the URT or a direct systemic effect on the
5 MPS by formaldehyde exposure (Dean et al., 1984). Subsequent studies by the same researchers
6 explored the possibility of systemic effects of formaldehyde exposure on MPS function and
7 maturation stage (Adams et al., 1987). Female B6C3F1 mice were exposed to 15 ppm
8 (18.4 mg/m³) formaldehyde 6 hours/day, 5 days/week for 3 weeks, as before (Adams et al.,
9 1987). Both resident and Murray Valley encephalitis virus (MVE-2)-elicited peritoneal MPs
10 were examined for hydrogen peroxide production, enzymatic activity, phagocytic ability,
11 binding, and lysis of tumor cells (Adams et al., 1987).

12 Similar to the findings of Dean et al. (1984), formaldehyde treatment increased hydrogen
13 peroxide production almost twofold in MVE-2 elicited peritoneal MPs (Adams et al., 1987). As
14 summarized in Table 4-47, no treatment differences were seen in phagocytic ability in either
15 resident or elicited MPs (Adams et al., 1987). Resident peritoneal MPs from formaldehyde-
16 treated mice were not different in their ability to bind or lyse tumor cells. Although
17 formaldehyde treatment did not increase the ability of elicited MPs to bind tumor cells, lysis of
18 the target cells (P815 tumor cells) was increased from 28 to 37% by formaldehyde treatment but
19 only at the midrange target-to-effector-cell ratio tested in the assay ($p < 0.05$) (Adams et al.,
20 1987). Although this is statistically significant, the authors questioned the biological
21 significance of this result since it was not observed at all three target cell ratios tested. However,
22 an increase in tumor cell lysis in vitro would be consistent with the in vivo increased tumor
23 resistance previously reported (Dean et al., 1984). The in vitro lysis response curve suggests that
24 assay conditions may result in a maximum cytolysis near 40%. If so, any treatment effects on
25 lysis would be difficult to discern at higher effector cell ratios.

26 Jakab (1992) investigated the effect of formaldehyde exposure on the alveolar MPs and
27 resistance to respiratory infections. The first set of experiments assessed bactericidal activity by
28 directly quantifying the pulmonary bacterial loading after exposure to *Staphylococcus aureus*.
29 White female Swiss mice were exposed to formaldehyde after bacterial infection (regimens A
30 and C), before bacterial infection (regimen B), or before and after infection (regimen D) (see
31 Table 4-48).

32

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

^a0, 1.2, 6.2, 12.3, or 18.5 mg/m³ formaldehyde.

^b0, 0.62, or 1.2 mg/m³ formaldehyde.

Source: Jakab (1992).

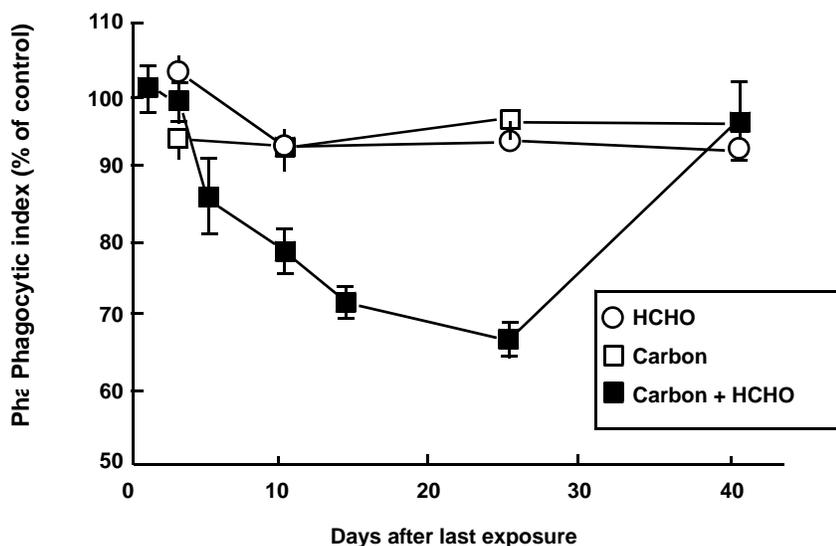
For regimen A, mice were exposed to 0, 1, 5, 10, or 15 ppm (0, 1.2, 6.2, 12.3, or 18.5 mg/m³) formaldehyde. For regimens B–D, mice were exposed to 0, 0.5, or 1 ppm (0, 6.2, or 1.2 mg/m³) formaldehyde. A 30-minute exposure to an infectious aerosol of *S. aureus* deposited 2×10^5 staphylococci in the lungs. 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

1 difficult to distinguish the potential for formaldehyde effects directly on the mucociliary
2 apparatus as a barrier to infection.

3 A second set of experiments in the same report (Jakab, 1992) examined the effects of
4 coexposure to formaldehyde and carbon black on pulmonary infection with *S. aureus*. The
5 particle size distribution of the carbon black aerosol was less than a 5 µm aerodynamic diameter
6 and, therefore, 98% respirable. Female Swiss mice were exposed nose only to formaldehyde and
7 carbon black. Experiments were run at two target concentrations: (1) 2.5 ppm (3.1 mg/m³)
8 formaldehyde and 3.5 mg/m³ carbon black or (2) 5 ppm (6.2 mg/m³) formaldehyde and
9 10 mg/m³ carbon black. Coexposure was given either for 4 hours after a 30-minute *S. aureus*
10 infection or 4 hours/day for 4 days as a pretreatment prior to *S. aureus* infection. Bacterial
11 loading was determined 0 and 4 hours after the *S. aureus* infection to assess bacterial survival.
12 Formaldehyde-carbon black coexposure did not alter bacterial survival either as a pretreatment or
13 post-treatment to bacterial exposure. However, this exposure regimen was not run for
14 formaldehyde or carbon black separately, and the 4 hours/day for 4 days pretreatment was not
15 included in the formaldehyde alone experiments (see Table 4-48).

16 Jakab (1992) also assessed the phagocytic activity of alveolar MPs collected by lavage at
17 various time points after formaldehyde, carbon black, or coexposure. Female Swiss mice were
18 coexposed to 5 ppm (6.2 mg/m³) formaldehyde and 10 mg/m³ carbon black 4 hours/day for
19 4 days. Mice were sacrificed and alveolar MPs harvested 1, 3, 5, 25, and 40 days after exposure.
20 Mice exposed only to formaldehyde or carbon black were sacrificed 3, 10, 25, and 40 days after
21 exposure. Fc-receptor-mediated phagocytosis was assessed ex vivo by using sensitized sheep
22 RBCs. The phagocytic index (PI) was reported as the total number of RBCs in 100 MPs.
23 Neither formaldehyde nor carbon black exposure alone significantly changed the PI (Jakab,
24 1992). These findings are consistent with the first coexposure experiment, since no changes in
25 PI were seen immediately after exposure. However, coexposure did decrease the PI of alveolar
26 MPs in a time-dependent manner, with maximal decrease to less than 70% of controls by 25 days
27 after exposure (see Figure 4-15). Decreases in the PI reflect changes in both the percentage of
28 phagocytic MPs and the number of RBCs phagocytized (Jakab, 1992). The PI recovered to
29 control levels by 40 days postexposure.

30



2
3
4 **Figure 4-15. Alveolar MP Fc-mediated phagocytosis from mice exposed to**
5 **5 ppm formaldehyde, 10 mg/m³ carbon black, or both.**

6
7 Note: Exposure was 4 hours/day for 4 days. Each value represents the mean \pm
8 SEM of five determinations.

9
10 Source: Redrawn from Jakab (1992).

11
12
13 Holmström et al. (1989b) evaluated the effects of long-term formaldehyde exposure on
14 antibody production. Female Sprague-Dawley rats were exposed to 12.6 ppm formaldehyde
15 (15.5 mg/m³) 6 hours/day, 5 days/week for 22 months. Body weight, tumor incidence, and
16 pathology were reported elsewhere (Holmström et al., 1989b). Rats were given a subcutaneous
17 injection of pneumococcal polysaccharide antigens or tetanus toxoid 21 to 25 days prior to
18 sacrifice. The two vaccines chosen represent T-cell-dependent and T-cell-independent antigens,
19 respectively. Antibody titers (IgG and IgM) were determined prior to vaccination and at
20 sacrifice. Formaldehyde treatment had no effect on antibody titers either before or after
21 vaccination (Holmström et al., 1989b).

22 23 **Summary of Formaldehyde Effects on Immune Function**

24 Although there were initial reports of systemic immunomodulation attributed to
25 formaldehyde exposure (Leach et al., 1983), formaldehyde effects on measures of humoral and
26 cell-mediated immunity were not confirmed by Dean et al. (1984). The authors did report
27 increased host resistance to both tumor and bacterial tumor challenges after a 3-week exposure to

1 15 ppm formaldehyde. An increased resistance to these challenges, presented distal to the site of
2 formaldehyde exposure (administered subcutaneously or intravenously), suggests a systemic
3 effect of formaldehyde exposure. In addition, increased host resistance and hydrogen peroxide
4 release from peritoneal MPs were reported and confirmed (Adams et al., 1987; Dean et al.,
5 1984). Chronic inflammation and tissue damage to the respiratory mucosa expected with
6 formaldehyde exposure may result in an up regulation of the MPS and therefore increase host
7 immunity. It is unclear if this response would be specific to formaldehyde or similar to
8 enhancement of immune function seen with chronic inflammation.

9 Jakab (1992) demonstrated decreased pulmonary resistance to bacterial infection where
10 animals were exposed to 15 ppm formaldehyde immediately after bacterial loading or when they
11 were given an 18-hour pre-exposure to formaldehyde followed by 1 ppm formaldehyde exposure
12 after bacterial loading. The authors speculated that formaldehyde may directly act on pulmonary
13 MPs, reducing their effectiveness. However, Jakab (1992) showed that there was no change in
14 Fc-mediated phagocytosis of alveolar MPs immediately after formaldehyde exposures.
15 Degradation of the protective mucus layer and possible epithelial cell damage may contribute to
16 more effective bacterial infection in the presence of formaldehyde without a direct action on MP
17 function. As mentioned above, degradation of the mucus layer may result in a more potent
18 inoculation and therefore higher bacterial loading.

19 Although neither formaldehyde nor carbon black alone impacted Fc-mediated
20 phagocytosis of alveolar MPs, Jakab (1992) demonstrated that there was decreased Fc-mediated
21 phagocytosis after formaldehyde and carbon black coexposure. Carbon black may have acted as
22 a carrier for formaldehyde, allowing higher levels of formaldehyde to be delivered more deeply
23 into the lungs than would be seen with formaldehyde alone.

24 Formaldehyde is known to break down the mucus layer protecting the respiratory tract,
25 allowing exposure of the underlying epithelium (Morgan et al., 1986a, c, d). Additionally,
26 formaldehyde can directly induce tissue inflammation through sensory irritation via substance P
27 from the trigeminal nerve (Fujimaki et al., 2004a). These actions together could contribute to
28 some of the observed effects on immune response attributed to formaldehyde exposures.
29 Degradation of the protective mucus layer would make antigens more available to the immune
30 system. It has been shown that direct application of an antigen to the nasal associated lymph
31 tissue, bypassing the mucus layer, is a more effective delivery of antigen (Hou et al., 2002).
32 Therefore, increased availability of these antigens to the immune system may in part explain
33 observed increased antibody production seen against ovalbumin (OVA) or common dust mite
34 allergen (Der f) during formaldehyde exposure (Sadakane et al., 2002; Riedel et al., 1996;
35 Tarkowski and Gorski, 1995). Neurogenic inflammation may also contribute to more efficient

1 antigen processing and presentation by activation of resident MPs. These factors are consistent
2 with the observation that formaldehyde exposures do not affect antibody production to antigens
3 administered outside of the respiratory tract, even after chronic exposures (Holmström et al.,
4 1989b).

5 This effect was initially observed several days after exposure was ended with maximal
6 suppression seen 25 days after a 4-day formaldehyde exposure. The delayed onset of this
7 response, however, suggests an effect beyond the POE effects observed at the time of exposure.
8 Table 4-49 presents a summary overview of the effects of formaldehyde on immune function in
9 laboratory animals.

10 11 4.2.5. Hypersensitivity and Atopic Reactions

12 Adverse reactions in humans exposed to formaldehyde in the workplace and homes have
13 been reported, which are consistent with an allergic response or a chemical sensitivity (see
14 Section 4.1.1 for details). Rashes and skin reactions are reported in some individuals after
15 dermal exposures, and in some cases exacerbation of asthma is reported after inhalation of
16 formaldehyde. However, the reports of human reactions do not allow a clear determination of
17 whether this sensitization is immunogenic or neurogenic in origin. Formaldehyde-induced
18 sensitization may have both neurogenic and immunologic components. Numerous animal
19 studies have been conducted in order to understand the potential for sensitization to
20 formaldehyde. Although hypersensitivity and allergic sensitization are often considered solely
21 immunologic in origin, neurogenic mechanisms may result in bronchial hypersensitivity and
22 increased immunologic sensitization. Therefore, the animal studies regarding formaldehyde-
23 induced sensitization are evaluated discretely in order to examine these etiologic possibilities.

24 Classically, hypersensitivity is characterized as an immune response to an antigen,
25 resulting in an inflammatory reaction that itself damages the tissues or is otherwise harmful
26 (Kuby, 1991). These reactions may be localized, as in topical dermatitis, or systemic, as in
27 anaphylactic shock from an allergen. Hypersensitivity can be mediated by a humoral immune
28 response or by a cell-mediated immune response. Four classes of hypersensitivity are generally
29 recognized that differ in their immune system components and functions. Although a single
30 agent (e.g., penicillin) may induce all four types of hypersensitivity, it is more usual for an agent
31 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 ₂ O 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 ₂ O 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 (<i>S. aureus</i>)	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.

1 Chemical sensitivity generally implies a neurogenically induced sensitization (Meggs,
2 1995). A chemical may directly interact with sensory nerves, releasing mediators or
3 neuropeptides such as substance P (a tachykinin) that trigger inflammation, hence called
4 neurogenic inflammation,. Repeated exposure to the same chemical is hypothesized to potentiate
5 neurogenic inflammation (Meggs, 1995). The resulting signs of tissue inflammation may be
6 similar to immunogenic inflammation (occurs by binding of antigen to antibody or leukocyte
7 receptor), but there would be no requirement that the immune system recognize the chemical as
8 an antigen for this type of response. Therefore, a chemical may induce one or more clinical
9 signs of atopic asthma without IgE-mediated type 1 hypersensitivity response. One form of
10 sensitivity directly affects sensory nerve endings, resulting in neurogenic inflammation and is a
11 well-known health effect attributed to formaldehyde. Neurogenic responses may result from the
12 direct and acute interaction of the chemical with sensory nerve ending receptors of the trigeminal
13 nerve that may lead to persistent rhinitis and an asthma-like reactive airway dysfunction
14 syndrome that may develop after short-term human exposures (Brooks et al., 1985). Thus, there
15 is evidence to suggest that neurogenic inflammation may contribute to observed increases in
16 formaldehyde-induced airway hyperresponsiveness and atopic responses. The available animal
17 studies that have investigated formaldehyde-induced airway hyperresponsiveness and atopic
18 responses are summarized below.

20 **4.2.5.1. *Inhalation Studies in Experimental Animals***

21 This section summarizes animal studies informing the role of formaldehyde-induced
22 chemical sensitization. The symptoms of sensitization (atopy, airway hyperresponsiveness) are
23 frequently associated with immunologic markers (cytokine production, leukocyte infiltration
24 histamine release, and antibody production) but may be mediated by neurogenic sensory
25 irritation, principally by activation of the trigeminal nerve (see Section 4.1.1.1 for a discussion of
26 sensory irritation). The animal studies that illuminate these neurogenic and immunologic
27 responses are discussed outside of the classic neurotoxicology and immunotoxicology study
28 summary sections to allow synthesis of these data.

29 Sensitization to chemical exposure by inhalation often manifests as an allergic or
30 asthmatic response as characterized by BC or BHR. This sensitization may be a result of
31 immune involvement, as in the case of hypersensitivity, or a neurogenic sensitization, where a
32 chemical may directly stimulate inflammation. Asthma is a specific manifestation of IgE-
33 mediated hypersensitivity, characterized by BHR and airway inflammation, resulting in lower
34 airway obstruction (Fireman, 2003; Kuby, 1991). In asthma, an allergen capable of cross-linking
35 membrane-bound IgE on mast cells initiates immunogenic inflammation resulting in an influx of

1 eosinophils, neutrophils, and lymphocytes. Mediators of BC, including histamine, eicosanoids,
2 and bradykinin (Kuby, 1991), are released during this process. Prior exposure to the allergen can
3 increase allergen-specific IgE, potentiating the allergic reaction; this is immunogenic
4 sensitization.

5 Biagini et al. (1989) evaluated the effect of a single pulmonary exposure of formaldehyde
6 on pulmonary mechanics, including BC. The researchers chose cynomolgus monkeys known to
7 be hyperreactive to methacholine (acetyl- β -methacholine chloride), which is a direct-acting
8 stimulant of BC (Cain, 2001). Measures of pulmonary mechanics included pulmonary flow
9 resistance; dynamic compliance; PEFr; FVC; FEV; FEF_{25-75%}, and 50% of VC; and FEFs
10 normalized for VC. Nine cynomolgus monkeys were exposed to increasing levels of
11 methacholine for 1 minute at 10-minute intervals (0, 0.125, 0.5, 2, and 8 mg/mL) as an aerosol
12 (0.065 mL/minute with a mean aerodynamic diameter of 1.0–1.5 μ m). Pulmonary mechanics
13 were measured to establish each monkey's response to methacholine. Methacholine challenge,
14 as the positive control, increased pulmonary flow resistance at increasing levels of methacholine
15 (0.125, 0.5, 2, and 8 mg/mL) to 196 ± 16 , 285 ± 57 , 317 ± 64 , and $461 \pm 120\%$ of baseline levels,
16 respectively. After a 2-week recovery period, each methacholine-sensitized monkey was
17 exposed to 2.5 ppm formaldehyde (generated from formalin, 15% methanol) for 10 minutes.
18 Measures of pulmonary function were performed at 2, 5, and 10 minutes after exposure.

19 Formaldehyde exposure increased pulmonary flow resistance from 11.3 ± 1.4 cm H₂O
20 prior to formaldehyde exposure to 16.1 ± 2.1 , 16.9 ± 2.8 , and 20.0 ± 3.4 cm H₂O at 2, 5, and
21 10 minutes after 2.5 ppm formaldehyde exposure (with 142, 150, and 177% change,
22 respectively). All other measures of formaldehyde-induced pulmonary mechanics were not
23 significantly different from controls. Increased pulmonary flow resistance, a measure of
24 increased BC, was induced by formaldehyde challenge in previously sensitized mice. However,
25 the differences between methacholine challenge and formaldehyde challenge were not
26 statistically significant. Although both formaldehyde challenge and methacholine challenge
27 increased pulmonary flow resistance, there was no correlation between individual methacholine
28 responsiveness and the magnitude of effect after formaldehyde exposure ($p > 0.1$). Therefore,
29 although formaldehyde exposure stimulated BC similarly to a known direct stimulating agent,
30 formaldehyde may not work through the same site of action as methacholine.

31 Swicichowski et al. (1993) assessed pulmonary resistance and airway reactivity due to
32 formaldehyde exposure alone and in response to increasing doses of acetylcholine chloride (a
33 direct-acting BC agent) after formaldehyde exposure in vivo. Male Hartley guinea pigs (eight
34 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/m³)
35 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/m³)

1 formaldehyde for 8 hours. Total pulmonary resistance increased after 2 hours formaldehyde
2 exposure at 9.4 and 31.1 ppm and reached similar peak resistance at the end of the exposure
3 period. This effect was rapidly reversible, with values returning to baseline within 30 minutes
4 after exposure. Although 2-hour exposures at 3 and 1 ppm did not increase pulmonary
5 resistance, 8-hour exposures at 0.3 and 1 ppm did increase pulmonary resistance to similar levels
6 as the 2-hour exposure at 30 ppm. The results indicate that both concentration and exposure time
7 impacted the measured increase in pulmonary resistance. However, a simple multiplicative
8 model (e.g., $C \times t$) does not adequately represent the effects observed. It is noted that an 8-hour
9 exposure at 1 ppm (8 ppm-hours), reached approximately the same pulmonary resistance as 2
10 hours at 9.4 ppm (19 ppm-hours). This may in part be due to a maximum practical increase in
11 pulmonary resistance in the animals. Conversely, there was no effect at 3 ppm for 2 hours
12 (6 ppm-hours), although significant increase in pulmonary resistance was recorded after an
13 8-hour exposure at 0.3 ppm (2.4 ppm-hours). Formaldehyde does not appear to exert its effects
14 via a classic $C \times t$ paradigm. Exposure concentration, however, did seem to impact recovery
15 time.

16 In addition, specific pulmonary resistance and airway reactivity to increasing doses of
17 intravenous acetylcholine chloride, a direct respiratory stimulant, were measured immediately
18 after formaldehyde exposure for up to 60 minutes. Formaldehyde-induced airway
19 hyperreactivity was defined as a decrease in the level of acetylcholine chloride needed to
20 produce twice the basal specific resistance (effective dose [ED]₂₀₀). The dose of acetylcholine
21 chloride required to double the specific pulmonary resistance (ED₂₀₀) and airway reactivity was
22 decreased in animals exposed for 2 hours to formaldehyde. When the duration was extended to 8
23 hours of formaldehyde exposure, the effective dose of formaldehyde required to elicit a doubled
24 pulmonary resistance (ED₂₀₀) in the presence of acetylcholine chloride was decreased to
25 1.07 ppm. Lower ED₂₀₀s were recorded in formaldehyde-treated animals. This indicates that
26 less acetylcholine was needed to produce BC when formaldehyde was present. Thus,
27 formaldehyde can exacerbate BHR. Additionally the formaldehyde-induced effect increased
28 with duration of exposure, indicating that time as well as exposure concentration are factors in
29 the magnitude of the response. Directly induced increases in airway hyperreactivity peaked 1
30 hour after exposure and persisted 6 hours after exposure.

31 In a second set of experiments, male Hartley guinea pigs were treated for 8 hours at
32 3.4 ppm (4.2 mg/m³) in order to measure airway hyperreactivity ex vivo (Swiecichowski et al.,
33 1993). After formaldehyde exposure, tracheae were excised and mounted in tissue baths, where
34 tracheal contraction was measured in response to direct application of acetylcholine and then
35 carbachol. Tracheae from similarly exposed guinea pigs were fixed and sectioned for histologic

1 examination and were assessed for signs of inflammation. Formaldehyde exposure did not
2 increase ex vivo tracheal constriction and suggests that changes in airway reactivity were
3 produced due to both local humoral changes and neural reflexes. However, no changes in
4 epithelial cell morphology or influx of inflammatory cells were recorded even up to 4 days after
5 formaldehyde exposure ended. The authors speculated on possible MOAs for BHR, such as the
6 role of an irritant receptor or altered epithelial cell biochemistry. It may be that the window of
7 acute inflammation occurred early in the exposure protocol and was resolved by the time of first
8 measurement, after 8 hours of exposure. The absence of inflammatory markers may argue
9 against a classic type 1 sensitivity.

10 The binding of an allergen to receptor bound IgE triggers degranulation of mast cells and
11 basophils, releasing mediators of type 1 hypersensitivity, including the histamine responsible for
12 BC. Brown Norway (BN) rats are known for their high capacity for IgE production and airway
13 hyperresponsiveness in response to allergens or other chemicals; they have often been used as a
14 model of allergic respiratory disease. Ohtsuka et al. (1997) compared the effects of
15 formaldehyde exposure on the nasal epithelium of F344 and BN rats. If the formaldehyde-
16 induced inflammatory response in the nasal epithelium is IgE mediated, BN rats would be
17 expected to display more severe effects of formaldehyde exposure than F344 rats. Both strains
18 of age- and sex-matched rats were exposed to formaldehyde aerosol for 3 hours/day, 5
19 days/week for 2 weeks. The aerosol was generated from a 1% formaldehyde solution by a two-
20 fluid atomizer, and formaldehyde level was maintained at 2 mg (1% sol.)/L (approximately
21 16 ppm or 20 mg/m³), by adjusting the flow rate for formaldehyde solution to the atomizer.
22 During the course of exposure, the following clinical signs were monitored: abnormal
23 respiration, stridor wheezing, nasal discharge, and sneezing. Rats were weighed weekly. Two
24 days postexposure, rats were sacrificed and tissues from the head, trachea, and lungs were fixed
25 and sectioned. Transverse sections were taken at the following palatal landmarks from three
26 animals: level 1 (lateral edge of incisor teeth), level 2 (between incisive papilla and the first
27 palatal ridge), and level 3 (on the second upper molar). The nasal septa of the remaining two
28 animals were revealed for examination by electron microscopy.

29 Formaldehyde-treated F344 rats showed less body weight gain over the 2-week
30 treatment, resulting in lower body weight at week 1 and week 2 than F344 controls ($p < 0.05$ and
31 0.01). Body weights of formaldehyde-treated BN rats were unchanged from BN controls. The
32 authors observed fewer clinical signs of respiratory irritation in the formaldehyde-exposed BN
33 rats compared with formaldehyde-exposed F344 rats, such as abnormal respiration (three versus
34 five) and nasal discharge (three versus five). Histologic analysis of lung and trachea tissues
35 revealed no distinct signs of inflammation in either strain. Formaldehyde exposure induced cell

1 damage in URT tissues. Epithelial cell damage was milder and impacted a smaller portion of the
2 URT in BN rats compared with F344 rats. Squamous metaplasia were present in the respiratory
3 epithelium (levels 1 and 2) in both strains in formaldehyde-treated rats. However, a distinct
4 keratinized layer was noted in level 1 epithelium of F344 rats, and the extent of lesions in level 2
5 respiratory epithelium was much greater than that seen in BN rats. Additionally, the olfactory
6 epithelium (level 2) in formaldehyde-exposed F344 rats exhibited degeneration, necrosis, and
7 desquamation not seen in BN rats. Mild squamous metaplasia was noted in level 3 of the
8 respiratory epithelium in the treated F344 rats but not the treated BN rats. No pulmonary
9 function measurements were taken, and, thus, no direct comparison in BHR or BC between BN
10 and F344 rats in response to formaldehyde can be made. It appears that BN rats are more
11 resistant to formaldehyde-induced cell damage than are F344 rats, despite the fact that BN rats
12 are known to be IgE responders. These results suggest that IgE responsiveness may be
13 protective of formaldehyde-induced cell damage, or IgE may not play a role at all. The authors
14 note that their earlier research indicated the BN rats have well-developed submucosal glands and
15 speculate that greater mucus flow may be partly responsible for the greater resistance of BN rats
16 to the histologic signs of formaldehyde toxicity.

17 In a subsequent study in the same laboratory, Ohtsuka et al. (2003) compared histology
18 and cytokine profiles in the nasal mucosa of formaldehyde-treated F344 and BN rats.
19 Formaldehyde aerosol was generated as above and rats (nine per group) were exposed
20 3 hours/day for 5 days to approximately 16 ppm of formaldehyde (20 mg/m³). Clinical signs
21 were recorded daily, and monitored respiratory parameters included abnormal respiration, stridor
22 wheezing, nasal discharge, and sneezing. Tissue sections of the nose (five rats per group) were
23 prepared for light microscopy as above: transverse sections at levels 1, 2, and 3. Th-1 cytokines
24 (IFN- γ , IL-2) and Th2 cytokines (IL-4 and IL-5) were determined from the whole nasal mucosa
25 in four rats of each treatment group.

26 As expected, lesions and neutrophilic infiltration were more severe in F344
27 formaldehyde-exposed rats compared with treated BN rats. In addition, lesions were observed in
28 all three levels of epithelium examined in F344 rats and impacted both respiratory and olfactory
29 epithelium. Mucosal lesions in formaldehyde-treated BN rats impacted the respiratory
30 epithelium of levels 1 and 2 only. Changes in formaldehyde-induced cytokine mRNA
31 expression were modest in both strains. Th-1-related cytokines (IFN- γ , IL-2) in formaldehyde-
32 treated BN rats were significantly decreased compared with control BN rats. A similar, although
33 not statistically significant, decrease in Th-2 cytokines (IL-4, IL-5) was observed in
34 formaldehyde-treated BN rats compared with unexposed BN rats. There were no treatment
35 differences in either Th-1 or Th-2 cytokine expression in formaldehyde-treated F344 rats

1 compared with unexposed F344 rats. The modest changes in cytokine profile reported in
 2 formaldehyde-treated BN rats were not consistent with type 1 hypersensitivity since type 1
 3 hypersensitivity reactions generally result in increased Th-2 cytokines. The mRNA expression
 4 results were not corroborated with protein levels and may not have been captured at their peak
 5 expression levels.

6 Lee et al. (1984) evaluated the potential for formaldehyde to act as a sensitizing agent
 7 through different routes of exposure in guinea pigs. The inhalation studies will be highlighted
 8 here. Dermal exposure and associated contact sensitivity results will be discussed in the dermal
 9 exposure section (see Section 4.2.5.2). Three groups of male English smooth-haired guinea pigs
 10 (four/group) were exposed via inhalation to either 6 or 10 ppm (7.4 or 12.3 mg/m³)
 11 formaldehyde 6 hours/day for 5 consecutive days. Depending on the group, animals were then
 12 subjected to bronchial provocation challenge with 2 or 4 ppm formaldehyde on day 7 or days 7,
 13 22, and 29 after exposure (see Table 4-50 for clarification).
 14

15 **Table 4-50. Study design for guinea pigs exposed to formaldehyde through**
 16 **different routes of exposure: inhalation, dermal, and injection**
 17

	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

18 Source: Lee et al. (1984).
 19
 20
 21

22 Dermal and injection groups are shown for comparison. Pulmonary hypersensitivity was
 23 assessed by measuring respiratory rate and tidal volume in response to exposure to 2 ppm
 24 formaldehyde challenge for 1 hour, 2 days postexposure for all three groups, and additional
 25 measurements were taken 22 and 29 days postexposure for group III. Blood was drawn to
 26 characterize IgE antibodies to formaldehyde in a passive cutaneous anaphylaxis (PCA) assay.

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1 Respiratory rate was measured following initial formaldehyde exposure and again after bronchial
2 challenge with formaldehyde.

3 Respiratory rate (exhibited as a pause during expiration) was depressed by 45% following
4 exposure to 10 ppm formaldehyde during the first hour of exposure. During the first hour of
5 exposure, decreased respiratory rate was accompanied by a pause during expiration that has been
6 categorized as RB and indicated sensory irritation. The decreased respiratory rate is consistent
7 with URT sensory irritation and induction of the trigeminal (neurogenic) reflex (Lee et al.,
8 1984). After the first hour of exposure, decreased respiratory rate was characterized by a pause
9 between breaths, which is similar to the breathing pattern seen in mice exposed to formaldehyde
10 via tracheal cannula (Alarie, 1981). This suggests a separate effect of formaldehyde on the LRT
11 after deep penetration of formaldehyde and suggests pulmonary irritation (Lee et al., 1984).

12 However, subsequent bronchial provocation challenge with either 2 or 4 ppm
13 formaldehyde for either 1 or 4 hours failed to elicit immediate or delayed-onset respiratory
14 sensitization (see Table 4-51). Respiratory rates were reported as being within $\pm 20\%$ of
15 prechallenge levels (data not shown) and did not reflect statistical significance (Lee et al., 1984).
16 Moreover, increased respiratory sensitivity was not observed in animals that had received an
17 emulsification of formaldehyde and Freund's complete adjuvant by injection. Only two to four
18 animals given formaldehyde injections in the presence of Freund's complete adjuvant developed
19 a low titer of antibodies to formaldehyde (Lee et al., 1984).

20
21 **Table 4-51. Sensitization response of guinea pigs exposed to formaldehyde**
22 **through inhalation, topical application, or footpad injection**
23

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

24
25 Source: Lee et al. (1984).
26
27

28 Thus, inhalation exposure to 6 or 10 ppm formaldehyde (8 hours/day for 5 days) followed
29 by bronchial challenge with 2 or 4 ppm formaldehyde failed to result in respiratory sensitivity
30 defined as greater than 20% change in respiratory rate. Second, for animals that received an
31 injection of formaldehyde with Freud's adjuvant, it was not effective in inducing pulmonary

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1 sensitivity. While neither inhaled formaldehyde challenge nor injected formaldehyde and
2 Freud's adjuvant emulsion were effective in producing pulmonary sensitivity, this study relied
3 on increased respiratory rate as an indication of hyperresponsiveness and may not be an accurate
4 measure of hyperresponsiveness. Thus, overall, conclusions are uninformative due to study
5 design flaws.

6 Riedel et al. (1996) tested the effects of formaldehyde inhalation on the development of
7 sensitization to a known allergen. Female Perlbright-white Dunkin-Hartley guinea pigs (12 per
8 group) were exposed to 0, 0.13, or 0.25 ppm formaldehyde 8 hours/day for 5 consecutive days.
9 On day 5, the animals were sensitized to the common model allergen, OVA, in a 3-minute, head-
10 only exposure to an aerosol of a 5% OVA solution. A booster sensitization with OVA occurred
11 on day 19. A compressor nebulizer with an output rate of 0.75 mL/minute generated the aerosol.
12 Particle size ranged from 0.5 to 5.0 μm . On day 26, bronchial provocation testing was conducted
13 with 1% OVA challenge (aerosol). Blood samples were taken and anti-OVA IgG antibodies
14 were quantified by ELISA. Significant airway obstruction was defined as an increase in
15 compressed air in the lung that cannot be expired. Three guinea pigs were exposed to
16 formaldehyde (0.20 ppm) or clean air for 5 days. Immediately after exposure, lung and tracheal
17 tissues were fixed for histologic and morphometric evaluation. Wall thickness of bronchial and
18 alveolar septa was measured systematically with a microscope-digitizing-table set.

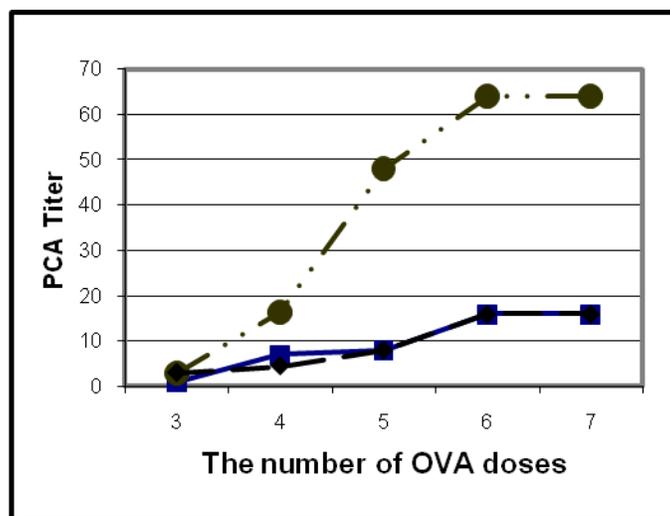
19 Significant airway obstruction as measured by compressed air was seen in 3 of 12
20 controls, 8 of 12 0.13 ppm-exposed, and 10 of 12 0.25 ppm-treated animals after OVA challenge.
21 The average airway obstruction was increased after 0.25 ppm (mean = 0.35 mL, $p < 0.01$) but not
22 after 0.13 ppm formaldehyde exposure. However, individual response to OVA sensitization was
23 highly varied, and animals exhibiting a 10-fold increase in obstruction (measured as compressed
24 air) were seen in both treatment groups (0.13 and 0.25 ppm). Even at the lower exposure (0.13
25 ppm), biologically significant responses were seen in individuals (see Figure 4-16).

26 Specific anti-OVA antibodies (IgG1 class) were not detected in animals prior to
27 sensitization or in control-treated animals after sensitization. Measurable anti-OVA antibodies
28 were elevated in 3 of 12 (at 0.13 ppm) and 6 of 12 (at 0.25 ppm) formaldehyde-treated guinea
29 pigs after sensitization (see Figure 4-17). The average anti-OVA titer for the high-dose group
30 was significantly higher than for controls ($p < 0.05$). The individual responses at the 0.13 ppm
31 exposure level indicate that, although the average group OVA titer may not have reached
32 statistical significance, there was a measureable biological response in three individuals. These
33 results indicate that formaldehyde exposure can sensitize previously naïve (nonsensitized)
34 animals to OVA.

1 The only significant, treatment-related histologic change was bronchial edema, with
2 thickening of the bronchial wall in formaldehyde-exposed animals compared with nontreated
3 animals subjected to OVA sensitization and subsequent OVA challenge. Bronchial walls were
4 measured as 40.9 ± 2.5 versus 28.2 ± 1.2 μm . No signs of inflammation in the bronchial mucosa
5 were seen with this edema.

6 Tarkowski and Gorski (1995) exposed female Balb/C mice to 0 or 6.63 ppm (0 or
7 2 mg/m^3) formaldehyde for either 6 hours/day for 10 days or 6 hours/day once a week for
8 7 weeks. All mice were sensitized intranasally to OVA for 10 days or once a week for 7 weeks.
9 IgE anti-OVA titers were determined from sera collected from four mice every 8 days (1 day
10 after OVA booster) by PCA. A parallel experiment to compare the role of the route of
11 administration was conducted with I.P. rather than intranasal sensitization ($1 \mu\text{g}$ OVA once every
12 7 days).

13 OVA titers increased similarly in control mice and mice exposed to formaldehyde once a
14 week (see Figure 4-18). In contrast, mice exposed to formaldehyde 6 hours/day for 10
15 consecutive days at the beginning of the experiment had increased anti-OVA beginning after the
16 fourth OVA sensitization, which continued to increase through seven doses of OVA to a peak of
17 70 PCA units ($p < 0.01$) (see Figure 4-18). Anti-OVA IgE titers were significantly different
18 between formaldehyde-treated and nonexposed mice.



20
21 **Figure 4-18. Anti-OVA titers in female Balb/C mice exposed to 6.63 ppm**
22 **formaldehyde for 10 consecutive days or once a week for 7 weeks.**

23
24 Note: ■ = control mice; ◆ = formaldehyde once a week \times 7; ● = formaldehyde
25 10 days.

26 Source: Redrawn from Tarkowski and Gorski (1995).

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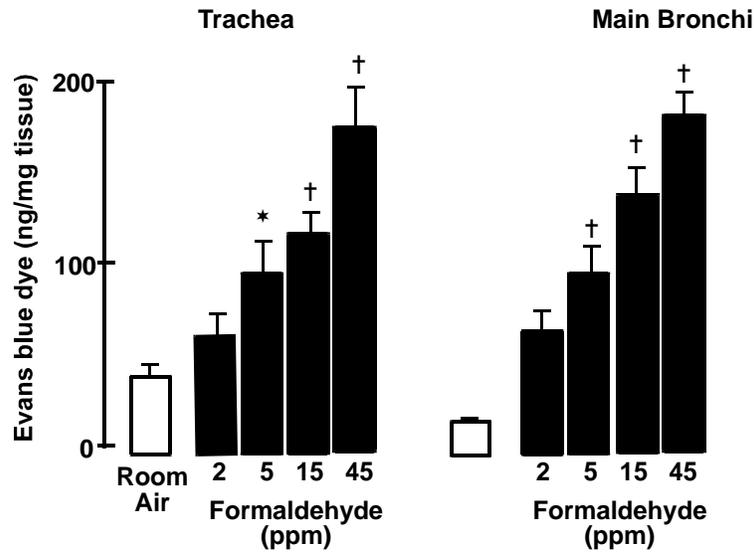
1 Intraperitoneal sensitization to OVA was much more effective than intranasal
2 sensitization, resulting in titers as high as 1,000 after 4 weeks. However, there were no
3 differences between controls and animals treated with formaldehyde via the I.P. route of
4 exposure. Thus, formaldehyde administered intranasally 6 hours/day for 10 days may facilitate
5 the sensitization to allergens. These changes were not observed when formaldehyde was
6 administered intranasally once a week for 10 weeks or via I.P. injection (Tarkowski and Gorski,
7 1995). The authors speculate that formaldehyde may increase permeability of respiratory
8 epithelium and destruction of immunologic barriers. Thus the respiratory tract may become
9 vulnerable to inhaled allergens after formaldehyde exposure (Tarkowski and Gorski, 1995).

10 Ito et al. (1996) conducted three experiments to examine the effects of acute
11 formaldehyde exposure on bronchoconstriction and the mediators of vascular permeability.
12 Male Wistar rats (five to eight per group) were exposed to 0, 2, 5, 15, or 45 ppm (0, 2.5, 6.2,
13 18.5, or 55.4 mg/m³) formaldehyde for 10 minutes. Baseline pulmonary insufflation and blood
14 pressure were determined prior to formaldehyde exposure and monitored throughout the
15 experiment. Vascular leakage was measured by injection of Evans blue dye prior to the
16 experiment and determining extravasation 5 minutes postexposure. Briefly, lungs were perfused
17 with 0.9% saline through an aortic cannula. The lower portion of the trachea and main bronchi
18 were removed, and the Evans blue dye remaining was determined and expressed as ng dye/g
19 tissue. A second experiment was conducted to determine if dye leakage continued to increase
20 after exposure. Seven rats were exposed to 15 ppm formaldehyde for 10 minutes, as above.
21 Evans blue dye was injected 5 minutes postexposure, and tissues were perfused and excised
22 15 minutes later. The final experiment was conducted to determine the effect of certain receptor
23 agonists on the formaldehyde-induced microvascular leakage. Ten groups of Wistar rats (four to
24 seven per group) were exposed to 15 ppm formaldehyde and injected with Evans blue dye, as
25 before. However, each receptor agonist under test or saline sham was injected 4–5 minutes prior
26 to the 10-minute formaldehyde exposure. Agonists tested included tachykinin NK₁ receptor
27 antagonist (CP-99,994) at 1, 3, or 6 mg/kg; a bradykinin B₂ receptor antagonist (HOE 140) at
28 0.65 mg/kg; and a histamine H₁ receptor antagonist (ketotifen) at 1 mg/kg.

29 Formaldehyde exposure did not change pulmonary insufflation pressure or blood
30 pressure. Formaldehyde increased vascular permeability in a concentration-dependent manner in
31 both the trachea and main bronchi for the first 5 minutes after exposure, as measured by Evans
32 blue dye extravasation (Ito et al., 1996) (see Figure 4-19). Vascular permeability was not
33 increased by formaldehyde exposure from 5 to 15 minutes postexposure (experiment 2).
34 Administration of a selective NK₁ receptor antagonist (CP-99,994) inhibited the formaldehyde-

1 induced vascular permeability, reducing Evans dye extravasation to control levels at the 3 and 6
2 mg/kg doses (see Figure 4-20).

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Figure 4-19. Vascular permeability in the trachea and bronchi of male Wistar rats after 10 minutes of formaldehyde inhalation.

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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 \pm SEM of five to seven animals. * $p < 0.05$ and † $p < 0.01$ versus room-air-exposed group (Williams' test).

Source: Redrawn from Ito et al. (1996).

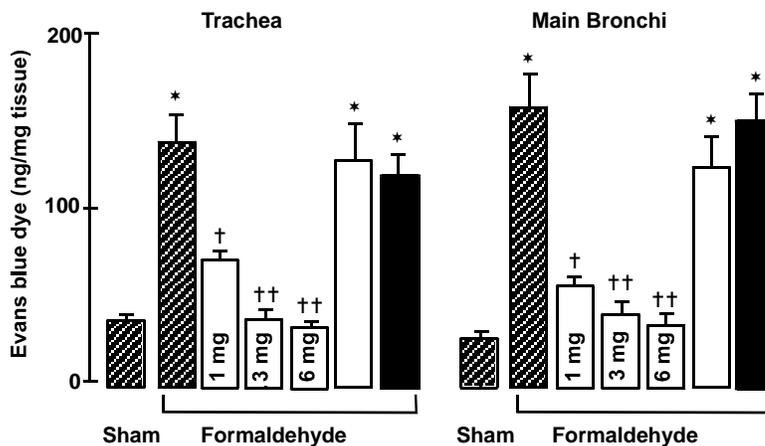


Figure 4-20. Effect of select receptor antagonists on formaldehyde-induced vascular permeability in the trachea and bronchi male of Wistar rats.

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 \pm 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 B₂ nor histamine H₁ 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 NK₁ receptor but does not seem to require the B₂ or H₁ 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

1 day prior to formaldehyde exposure (1.5 mg/animal). The same groups were challenged with
 2 intratracheal instillation of Der f (10 µg/animal) after 4 weeks. Three days after allergen
 3 challenge, mice were sacrificed and blood plasma and lung tissue were collected. Blood plasma
 4 was analyzed for Der f-specific immunoglobulins (IgG1 and IgE). Lungs from nine mice in each
 5 treatment group were homogenized, and Th1 cytokine IL-2, Th2 cytokines IL-4 and IL-5,
 6 granulocyte macrophage-colony-stimulating factor (GM-CSF), and the “chemokine regulated
 7 upon activation, normal T-cell expressed and secreted” (RANTES) protein levels were quantified
 8 in the supernatant via ELISA. Lungs from nine mice in each group were fixed, sectioned, and
 9 stained to evaluate eosinophil infiltration, lymphocyte infiltration, goblet cell proliferation, and
 10 localization of RANTES in the airway epithelium.

11 Der f-specific IgG1 was present in blood plasma of sensitized mice but was unchanged
 12 by formaldehyde exposure (Sadakane et al., 2002). IgE was too low to titer. IL-2 and GM-CSF
 13 were undetected in lung homogenate supernatant, and IL-4 was unchanged by sensitization or
 14 formaldehyde exposure. However, RANTES was increased by both formaldehyde exposure and
 15 allergen sensitization and challenge (see Table 4-52). These increases were more pronounced
 16 but less than additive for formaldehyde-exposed, allergen-sensitized mice. IL-5 was increased
 17 by allergen but unaffected by formaldehyde exposure only. However, formaldehyde exposure
 18 potentiated the IL-5 increase seen with allergen challenge.

19

20 **Table 4-52. Cytokine and chemokine levels in lung tissue homogenate**
 21 **supernatants in formaldehyde-exposed male ICR mice with and without**
 22 **Der f sensitization**
 23

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}

24

25 ^aNone detected.

26

^b*p* < 0.05 from control.

27

^c*p* < 0.001 from control.

28

^d*p* < 0.05 from Group 2.

29

^e*p* < 0.001 from Group 2.

30

^f*p* < 0.001 from Group 3.

31

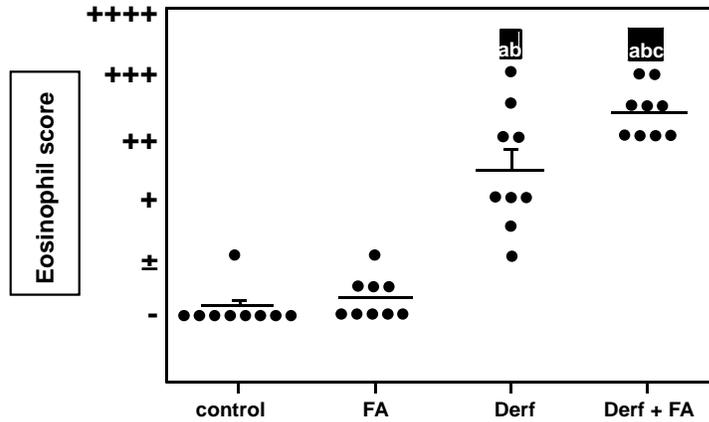
32 Source: Sadakane et al. (2002).

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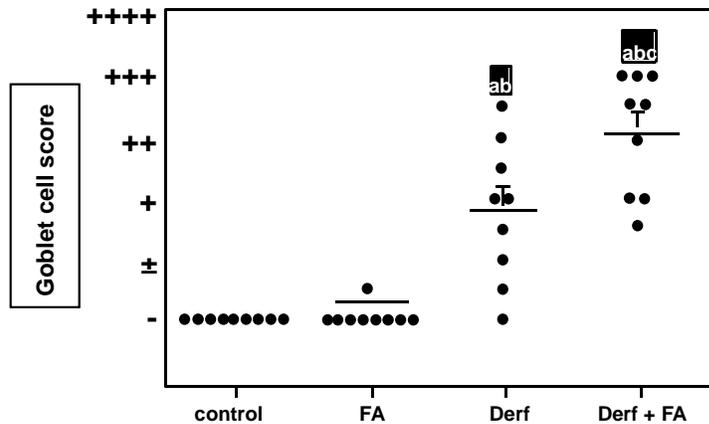
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1 Der f sensitization and challenge increased eosinophil infiltration into the interstitium
 2 around the bronchi and bronchioles as well as goblet cell proliferation in the bronchial
 3 epithelium (see Figure 4-21). Formaldehyde exposure exacerbated the eosinophilic and goblet
 4 cell responses to a challenge dose of Der f ($p < 0.05$) (Sadakane et al., 2002). Formaldehyde-
 5 induced eosinophilic infiltration in the absence of sensitization and challenge was not different
 6 from nontreated, nonsensitized mice.

8 *Panel A*



9 *Panel B*



10 **Figure 4-21. The effects of formaldehyde inhalation exposures on eosinophil**
 11 **infiltration (Panel A) and goblet cell proliferation (Panel B) after Der f**
 12 **challenge in the nasal mucosa of male ICR mice after sensitization and**
 13 **challenge.**

14 Note: ^a $p < 0.001$ compared with control group; ^b $p < 0.001$ compared with
 15 formaldehyde group; ^c $p < 0.05$ compared with Der f group.

16 Source: Redrawn from Sadakane et al. (2002).

1
2 These results suggest that formaldehyde exposure may aggravate eosinophilic infiltration and
3 goblet cell proliferation that accompanies allergic responses. This response is associated with an
4 increase in IL-5, an eosinophilic attractant, and an increase in RANTES, which recruits
5 eosinophils by chemotaxis in formaldehyde-exposed and Der f challenged animals, although the
6 effect was not statistically significantly elevated compared with Der f challenge-induced levels
7 of IL-5 and RANTES alone.

8 Fujimaki et al. (2004a) investigated the long-term effects of low-dose formaldehyde
9 exposure on immunologic and neurological inflammation. Female C3H/He mice were exposed
10 to 0, 0.082, 0.393, or 1.87 ppm (0, 0.1, 0.48, or 2.3 mg/m³) formaldehyde 16 hours/day,
11 5 days/week for 12 weeks. Six mice at each exposure level were given injections of OVA plus
12 adjuvant before the initial exposure and in weeks 3, 6, 9, and 11 of the experiment. Five mice at
13 each formaldehyde-exposure level did not receive OVA injections. One day after the last
14 exposure, mice were weighed and blood, BAL, spleen, and thymus were collected from each
15 animal. After weighing, spleens were disaggregated and spleen cells harvested for cell culture.
16 Immunophenotype of the spleen cells was determined by flow cytometry (CD4, CD8, CD3, and
17 CD19 positive cells). Lymphocyte proliferation in response to lipopolysaccharide (LPS),
18 phytohemagglutinin A (PHA), or OVA was determined after 72 hours in culture. Splenocytes
19 were cultured for 48 hours in the presence of LPS, PHA, and OVA (immunized mice only), and
20 supernatants were collected for cytokine analysis (IL-4, IL-5, and IFN- γ). Splenocytes were
21 cultured for 24 hours in the presence or absence of OVA to assess chemokine production (MCP-
22 1 and MIP1- α). Anti-OVA IgE, IgG₁, IgG₂, and IgG₃ were quantified in blood plasma.

23 Body and thymus weights were unchanged by formaldehyde exposure or OVA injection
24 (Fujimaki et al., 2004a), while, in nonimmunized mice, spleen weights were reduced by
25 formaldehyde exposure from 152 mg in controls to 128, 118, and 121 mg in mice exposed to
26 0.08, 0.4, and 1.8 ppm formaldehyde, respectively. Spleen weights tended to increase in groups
27 exposed to 400 and 2,000 ppb formaldehyde compared with controls in OVA-immunized mice
28 (control: 117.8 mg compared with 400 ppb: 168.6 mg and control: 121.0 mg compared with
29 2,000 ppb:153.2 mg, respectively) but were not statistically significant.

30 To gain insight on the overall pulmonary inflammatory response of mice exposed to
31 formaldehyde in both immunized and nonimmunized mice, the total number and differential
32 count of MPs, neutrophils, lymphocytes, and eosinophils in BAL were counted and were found
33 to be unchanged by formaldehyde in nonimmunized mice. By contrast, in immunized mice
34 exposed to 1.8 ppm formaldehyde, the total number of BAL cells, MPs, and eosinophils were
35 significantly increased compared with nonimmunized controls (9.65 versus 2.84, 7.22 versus
36 2.74, and 2.0 versus 0.02 $\times 10^4$ cells, respectively).

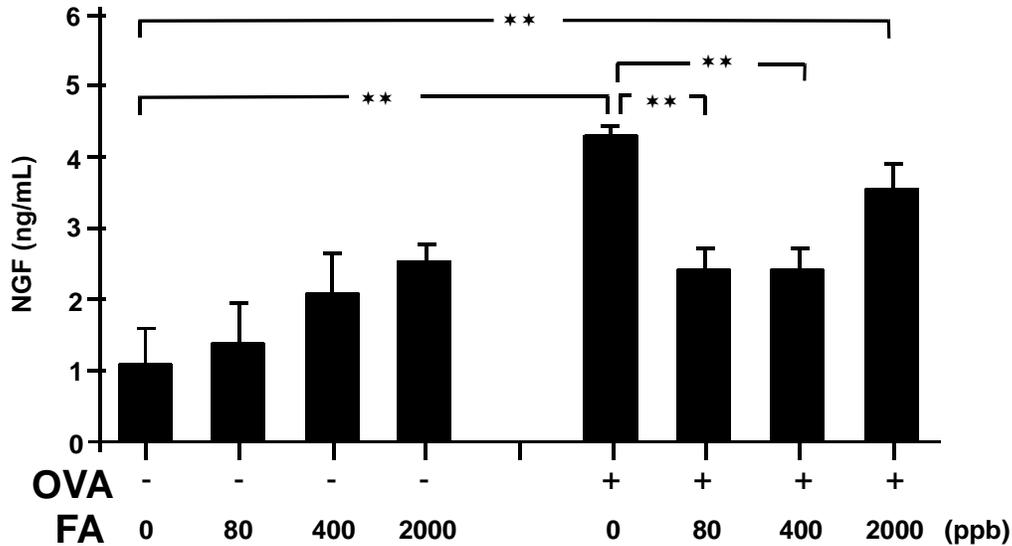
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1 To further assess the pulmonary inflammatory response, protein levels of inflammatory
2 cytokines were determined by ELISA in BAL fluid. Levels of IL-1 β in BAL of immunized mice
3 were decreased by formaldehyde exposure ($p < 0.05$ at 1.8 ppm formaldehyde), but IL-1 β levels
4 after formaldehyde exposure were not different from controls in nonimmunized mice (Fujimaki
5 et al., 2004a). All other cytokines or chemokines were either unchanged (TNF- α , IL-6, and GM-
6 CSF) or not detected (eotaxin, MIP-1 α , and MCP-1).

7 Various neuropeptides, such as brain-derived neurotrophic factor (BDNF), nerve growth
8 factor (NGF), and substance P are released from vagal nerve endings and mediate a neurogenic
9 inflammatory response. Levels of BDNF, NGF, and substance P were assessed in BAL fluid
10 and/or in plasma. BDNF was not detected in BAL or in plasma. NGF levels in immunized mice
11 were significantly higher than in nonimmunized mice in both BAL fluid and in plasma. NGF
12 levels in immunized mice were significantly attenuated by 0.08 and 0.4 ppm formaldehyde
13 exposure (see Figure 4-22) in both BAL fluid and in plasma. Plasma level of substance P (a
14 mediator of neurogenic inflammation) was increased by formaldehyde exposures in
15 nonimmunized mice (see Figure 4-23) in both BAL fluid and plasma. This increase appears to
16 be dose-dependent and reaches statistical significance at 2,000 ppb formaldehyde exposure in
17 nonimmunized mice compared with nonimmunized controls. Similar to NGF, levels of
18 substance P increased in OVA-immunized mice compared with nonimmunized mice in both
19 BAL fluid and plasma. Similar to NGF, levels of substance P in OVA-immunized mice were
20 attenuated by formaldehyde exposure at 80 ppb.

21 Fujimaki et al. (2004a) further investigated the effect of low-level formaldehyde exposure
22 from both immunized and nonimmunized mice on the systemic immune response. Spleens were
23 removed from formaldehyde-exposed mice and were cultured in the presence of LPS or PHA
24 (for nonimmunized samples) or OVA (for immunized samples). The secretory ability of
25 immunized and nonimmunized spleen cells was assessed by measuring IFN- γ release by ELISA.
26 Formaldehyde exposure (1.8 ppm) increased IFN- γ fourfold in LPS-stimulated cultured spleen
27 cells from nonimmunized mice. No other cytokine or chemokine was changed by formaldehyde
28 exposure in cultured spleen cells from nonimmunized mice. In OVA-immunized mice,
29 formaldehyde had no significant effect on cytokines from stimulated spleen cells. OVA in vitro
30 stimulation significantly increased the chemokines MIP-1 and MCP-1 for control and
31 formaldehyde-treated OVA-immunized mice. The OVA-stimulated release of MCP-1 in vitro
32 was enhanced by formaldehyde exposure in a concentration-dependent manner, increasing
33 threefold and fourfold at 0.40 and 1.8 ppm, respectively. Increases in MCP-1 correlate with
34 reported increases in the associated cytokine, RANTES, which recruits eosinophils by
35 chemotaxis (Sadakane et al., 2002). These formaldehyde-induced increases in cytokine levels

1 contribute to pulmonary inflammation. The inflammatory response is not mediated by
2 lymphocytes, since lymphocyte subsets and in vitro cell proliferation were unchanged by OVA
3 immunization or formaldehyde treatment (Fujimaki et al., 2004a).
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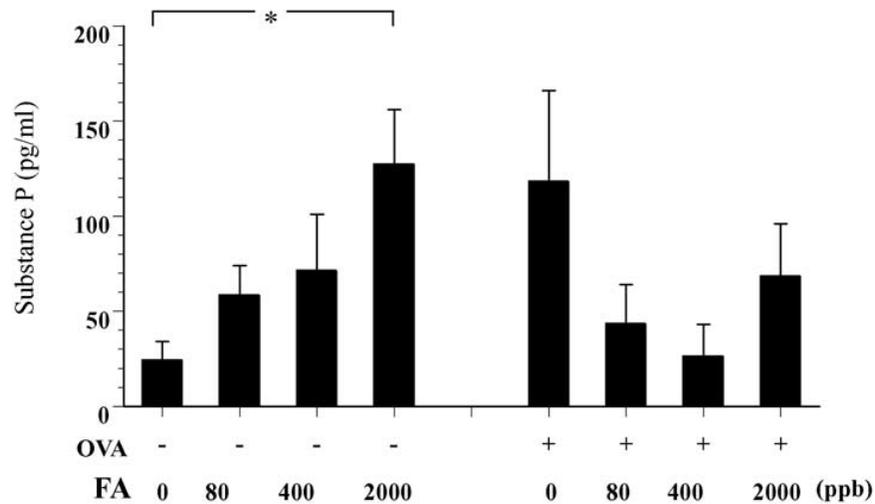


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Figure 4-22. NGF in BAL fluid from formaldehyde-exposed female C3H/He mice with and without OVA sensitization.

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 \pm SEM from five to six animals. $**p < 0.01$.

Source: Redrawn from Fujimaki et al. (2004a).



2
3
4 **Figure 4-23. Plasma substance P levels in formaldehyde-exposed female**
5 **C3H/He mice with and without OVA sensitization.**
6

7 Note: The day after the final formaldehyde inhalation, plasma samples were
8 collected from formaldehyde-exposed, nonimmunized, and formaldehyde-
9 exposed OVA-immunized mice, and the levels of substance P were determined by
10 ELISA. Data are mean \pm SEM from five to six animals. * $p < 0.05$. FA =
11 formaldehyde.
12

13 Source: Fujimaki et al. (2004a) as amended in Fujimaki et al. (2005; errata).
14
15

16 Anti-OVA (IgE and IgG_{2a}) levels in plasma were unchanged by formaldehyde exposure.
17 Anti-OVA IgG₁ was reduced in immunized mice exposed to 400 ppb formaldehyde compared
18 with nonexposed animals. However, this effect did not persist as dose increased. Anti-OVA
19 IgG₃ was depressed in immunized mice exposed to 0.08 and 0.4 ppm formaldehyde (Fujimaki et
20 al., 2004a). Formaldehyde exposure did not induce an inflammatory response in lung or tracheal
21 epithelium in sections viewed by light microscopy (Fujimaki et al., 2004a). Although there was
22 a mild infiltration of mast cells into the epithelium of OVA-immunized mice, there were no
23 effects of formaldehyde treatment on mast cell infiltration.

24 A recent study by Lino dos Santos Franco et al. (2009) exposed male Wistar rats for
25 3 days, 90-minutes/day, to 1% formaldehyde (by weight; exact doses not reported) by inhalation.
26 Of these, one group was sensitized I.P. to OVA (10 μ g), a common allergen, immediately
27 following formaldehyde exposure, and subsequently challenged with OVA 2 weeks later. Other
28 rats were sensitized and challenged but were not exposed to formaldehyde. PCA reaction as well
29 as BAL analysis and whole blood analysis were conducted. Immunohistochemical analysis of

1 platelet endothelial cell adhesion molecule-1 (PECAM-1) expression, an inflammatory mediator,
2 in lung tissue was also measured. When formaldehyde exposure was followed by OVA
3 sensitization and challenge, decreased lung inflammation was reported compared with the group
4 that was OVA-sensitized but had not been exposed to formaldehyde. Reduced lung mast cell
5 degranulation was also reported in the formaldehyde/OVA group compared with the nonexposed
6 OVA group. Total circulating leukocytes, total bone marrow cells, and lung protein expression
7 levels of PECAM-1 were also significantly decreased in formaldehyde/OVA rats compared with
8 non-formaldehyde exposed OVA rats. The reduction in inflammatory parameters in response to
9 formaldehyde may be attributed to different study designs, since in this study animals were
10 sensitized after exposure rather than prior to exposure. The results suggest that formaldehyde
11 may functionally alter the activity of certain cells, like mast cells, that may downgrade an
12 appropriate immune response to antigen and might serve to threaten lung homeostasis. Due to
13 the unique experimental design of this study, it cannot be directly compared with Sadakane et al.
14 (2002) or Fujimaki et al. (2004a). In addition, this study did not intend to measure whether
15 formaldehyde can exacerbate an asthmatic response but rather set out to identify whether
16 formaldehyde could affect immune homeostasis.

17 In summary, studies suggest that formaldehyde exposure may induce a predominantly
18 neurogenic inflammatory response via release of neuropeptide, such as NGF and substance P
19 from vagal nerve endings. Formaldehyde does not appear to potentiate a systemic immune
20 response. However, localized pulmonary inflammation can be potentiated by formaldehyde
21 exposure, as indicated by the increased presence of eosinophils and certain proinflammatory
22 cytokines (IFN- γ). This response does not appear to be mediated by classic immunogenic
23 mechanisms since studies have failed to report elevated levels of anti-formaldehyde-specific IgE.
24 Several studies have shown that exposure to formaldehyde can facilitate allergic sensitization in
25 previously naïve animals, and it is thought that this effect may occur due to formaldehyde's
26 ability to increase microvascular leakage in the nasal epithelium and by causing damage to the
27 nasal barrier (Ito et al., 1996). Sadakane et al. (2002) demonstrated that formaldehyde exposure
28 can also exacerbate allergic responses by enhancing the response to challenge allergen. Thus,
29 formaldehyde may exacerbate allergic responsiveness by aggravating the sensitization response
30 in previously naïve animals by altering the permeability of the mucosal barrier in nasal
31 compartments. Neurogenically derived inflammation, including stimulation of the trigeminal
32 nerve and release of bradykinin, suggests that the MOA for sensitization may ultimately have its
33 roots in neurogenic inflammation rather than an immunogenic response. In addition, using a
34 different protocol, Lino dos Santos Franco et al. (2009) suggest that formaldehyde exposure can
35 adversely affect lung homeostasis by reducing the activity of important inflammatory mediators

1 (mast cells, circulating leukocytes, PECAM-1 expression) when it occurs prior to sensitization,
2 thus downgrading an appropriate immune response.

3 4 **4.2.5.2. Dermal Sensitization**

5 Wahlberg (1993) used Hartley strain guinea pigs as test animals to determine the skin
6 irritancy of a suite of industrial chemicals, including formaldehyde. Aqueous solutions of the
7 compound in a 0.1 mL volume were applied to the shaved flanks of guinea pigs and gently
8 rubbed into the skin with a cotton-tipped applicator. Sites were left open and the treatments
9 repeated once daily for 10 days. A number of indices of acute skin irritation were monitored,
10 including erythema via visual scoring and edema and skin-fold thickness using Harpenden
11 calipers. Varying concentrations of formaldehyde (up to a 10% solution) induced a dose-
12 dependent increase in skin-fold thickness. Responses also showed shorter latencies at the higher
13 concentrations. For example, erythema was first observed on day 2 when 10% formaldehyde
14 was applied, day 5 (for 3%), and day 6 (for 1%).

15 Lee et al (1984) investigated the role of different routes of exposure in formaldehyde-
16 induced allergic sensitization. Two sets of four male English smooth-haired guinea pigs received
17 topical applications of 100 μ L 37% w/v formalin distributed over two shaved, depilated dorsal
18 sites two times over the course of 2 days at different sites. The total dose was calculated as
19 74 μ g/animal. In addition, eight animals received a single topical application onto a 15 mm area
20 of the dorsal surface. The applied dose of 25 μ L formaldehyde was dissolved in saline. Two
21 other groups of guinea pigs were exposed to either 6 ppm (6 hours/day for 5 days) or 10 ppm
22 (6 hours/day for 5 days) formaldehyde by inhalation. A third group of guinea pigs was exposed
23 to 10 ppm formaldehyde for 8 hours/day for 5 consecutive days by inhalation. All animals were
24 evaluated for contact sensitivity by topical application of 20 mL formaldehyde diluted with
25 saline and distributed in a 15 mm area on the backs of the shaved guinea pigs (Lee et al., 1984).
26 Sites were visually inspected for erythema at 1, 6, 24, and 48 hours following the topical
27 application, and reactions were scored. No erythema was observed in control animals. None of
28 the guinea pigs in the 6 hours/day inhalation groups (6 and 10 ppm formaldehyde) developed
29 skin sensitivity tested on day 9 (4 days after the initial exposure regimen ended). Two of four
30 guinea pigs exposed to 10 ppm formaldehyde for 8 hours/day for 5 consecutive days developed
31 mild skin sensitization tested on day 31. Contact sensitivity increased in a dose-dependent
32 fashion in groups of animals that had been sensitized via the dermal route. Thus, dermal
33 exposure resulted in contact sensitivity. Inhalation exposure did not consistently produce contact
34 sensitivity.

1 Arts et al. (1997) used a local lymph node assay (LLNA) and the induction of IgE to
2 monitor the sensitization of female Wistar rats (low IgE-responders) and BN rats (high IgE
3 responders). For the LLNA assay, animals were sensitized by the application of varying
4 concentrations of formaldehyde in raffinated olive oil on the dorsum of both ears on days 0, 1,
5 and 2. Control animals were treated with raffinated olive oil alone. Animals received an I.P.
6 injection of BrdU on day 5 and were subsequently sacrificed. Ear-draining lymph nodes were
7 collected, fixed, and sectioned, and the mitotic activity was monitored following successive
8 incubation of the sections in anti-BrdU, biotin-labeled rabbit anti-mouse antibody, peroxidase-
9 conjugated streptavidin, and 3,3-diaminobenzidine tetrahydrochloride. For serum IgE responses,
10 150 μ L of different concentrations of formaldehyde were applied to the shaved flanks of rats on
11 day 1, then 75 μ L of the same chemical at 50% of the initial concentration were applied to the
12 dorsum of each ear on day 7. The amount of IgE in the blood was measured using ELISA but
13 appeared to be little affected by formaldehyde treatment in either species of rat. However, the
14 ear-draining lymph nodes of both strains of rat showed a comparative increase in weight in
15 response to formaldehyde, and proliferation (BrdU positive) of paracortical cells was observed in
16 response to increasing doses of the compound. This response was most notable in BN rats
17 treated with 10% formaldehyde. Arts et al. (1997) concluded that the irritant and sensitizing
18 properties of formaldehyde may act through non-IgE-immune mechanisms.

19 Hilton et al. (1998) used the LLNA assay in female CBA/Ca (H-2^k haplotype) mice to
20 compare the skin sensitizing potencies of formaldehyde and glutaraldehyde. The comparison
21 was set on a quantitative basis by determining the concentration of each compound necessary to
22 induce a threefold increase in lymph node cell proliferative activity (effective concentration
23 [EC₃]). While both aldehydes induced a dose-dependent proliferative response, the
24 incorporation of [³H]-methylthymidine was far greater in animals exposed to glutaraldehyde
25 versus formaldehyde (with EC₃ values of 0.002–0.006 mol/L for glutaraldehyde versus
26 0.11–0.18 mol/L for formaldehyde). These data indicate the potential of both chemicals to
27 induce skin sensitization, although the potency of glutaraldehyde was far greater than that of
28 formaldehyde.

29 Xu et al. (2002) evaluated the extent to which the expression of some cytokines may
30 change as a result of cutaneous exposure to formaldehyde in mice. Female Balb/C mice were
31 skin painted with three topical applications of 100 μ L of 17.5% formaldehyde or distilled water
32 with a 1-day interval between each application. Spleen and draining lymph nodes were
33 harvested on days 3, 5, 7, 9, or 12 after the last skin painting. In some animals, contact
34 hypersensitivity was induced by applying 2% formaldehyde to both sides of mouse ears on day 3
35 following the last skin painting. For this endpoint, the percent increase in thickness of the ears

1 was monitored. For the cytokines, mRNA expression levels of IL-2, IL-4, IL-5, IL-10, IL-12,
 2 IL-13, IL-15, IL-18, and INF- γ were determined semiquantitatively by measuring the amount of
 3 individual mRNAs following amplification with the reverse transcriptase (RT)-PCR. The
 4 relative amounts of cytokine mRNAs were calculated as the ratio of cytokine mRNA to that of
 5 glyceraldehyde-3-phosphate dehydrogenase, as revealed in specific bands on an agarose gel.

6 Cutaneous formaldehyde treatment was associated with the long-lasting expression of
 7 IL-4 and IFN- γ mRNAs in mouse spleen and draining lymph nodes and with IL-15 mRNA only
 8 in mouse spleen. Only IL-13 mRNAs displayed a transient increase in expression in both spleen
 9 and draining lymph nodes. Levels of IL-2, IL-12, and IL-15 were increased in the mouse spleen
 10 but not the lymph nodes. The mouse ear swelling test gave positive correlations with enhanced
 11 expression of mRNA for IL-4 and IFN- γ (see Table 4-53).

12
 13 **Table 4-53. Correlation coefficients among ear swelling responses and skin**
 14 **mRNA levels in contact hypersensitivity to formaldehyde in mice**
 15

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

16
 17 ^aStatistically significant ($p < 0.05$).

18
 19 Source: Xu et al. (2002).

20
 21
 22 **4.2.5.3. Summary of Sensitization Studies**

23 Several animal studies report increased airway resistance and BC due to inhalation
 24 exposures to formaldehyde (Nielsen et al., 1999; Swiecichowski et al., 1993; Biagini et al., 1989;
 25 Amdur, 1960). Changes in pulmonary resistance were observed as early as 10 minutes after
 26 exposure (Biagini et al., 1989), and reported effect levels ranged from 0.3–13 ppm. Other
 27 pulmonary effects were reported in conjunction with BHR, such as increased tracheal reactivity
 28 and decreased pulmonary elasticity (Swiecichowski et al., 1993; Amdur, 1960). Although BHR
 29 is a common result of Type I hypersensitivity reaction to an allergen, the observation of BHR
 30 alone is not sufficient to demonstrate that an agent induces Type 1 hypersensitivity.

31 BHR may be directly induced both pharmacologically and neurogenically (Joos, 2003;
 32 Cain, 2001; Meggs, 1995). There is little evidence that formaldehyde itself is an allergen
 33 recognized by the immune system, especially via inhalation (Lee et al., 1984). Although

1 formaldehyde exposure has been reported to alter cytokine levels and immunoglobulins in some
2 experimental systems, these immunomodulatory effects do not support a type 1 hypersensitivity.
3 IgE was unchanged (Fujimaki et al., 2004a; Lee et al., 1984), and cytokine profiles were not
4 consistent with the Th-2 cytokines expected in IgE mediated hypersensitivity (Fujimaki et al.,
5 2004a; Ohtsuka et al., 2003).

6 Formaldehyde-induced dermal sensitization show parallel results. The physical signs of
7 irritation and sensitization are consistently shown (e.g., rashes, edema). Some involvement of
8 the immune response has been demonstrated with positive LLNA assays, indicating proliferation
9 of lymphocytes in lymph nodes draining the affected area (Hilton et al., 1998; Arts et al., 1997).
10 Increased expression of Th-2 cytokines in the lymph nodes of mice given dermal applications of
11 formaldehyde does indicate an immune component to the observed sensitization. However, the
12 response does not seem to be mediated by IgE (Arts et al., 1997; Lee et al., 1984).

13 Ito et al. (1996) reported that a tachykinin NK₁ receptor, but not the histamine H₁ or
14 bradykinin B₂ receptors, is involved in formaldehyde-induced vascular permeability.
15 Neuropeptides NGF and substance P were affected in BAL and stimulated splenocytes from
16 formaldehyde-exposed mice, with greater effects seen in OVA-immunized mice. Tachykinins
17 (e.g., substance P and neurokinin A) are produced by nerve cells and can directly stimulate
18 bronchoconstriction (Van Schoor et al., 2000). Substance P is also a mediator of neurogenic
19 inflammation. Therefore, although formaldehyde may induce some of the symptoms of type 1
20 hypersensitivity, these symptoms are more likely neurogenic than immunogenic in origin.

21 In contrast, formaldehyde enhances immunogenic hypersensitivity of known allergens
22 (Sadakane et al., 2002; Riedel et al., 1996; Tarkowski and Gorski, 1995). This potentiation
23 varied based on sensitization protocols (respiratory tract versus systemic, frequency and timing
24 of immunization, allergen, etc.) and formaldehyde exposure regimens (concentration, continuous
25 versus intermittent exposures). Taken as a whole, the results support the finding that
26 formaldehyde exposure can aggravate a type 1 hypersensitivity response (see Table 4-54).

27

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
<i>Inhalation studies</i>					
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
<i>Dermal sensitization</i>					
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.

1 4.2.6. Neurological and Neurobehavioral Function

2 **4.2.6.1. *Inhalation Exposure***

3 There are a number of published reports examining the effects of formaldehyde exposure
4 on nervous system structure and function. The reports evaluating behavioral effects fall into
5 three main categories: (1) behavioral responses evaluated during or immediately following
6 formaldehyde exposures, which may include effects due to the potential irritant properties of the
7 chemical, (2) acute or short-term exposures followed by behavioral assessments conducted 2–24
8 hours after termination of formaldehyde exposure, which reflect sustained effects of chemical
9 exposure independent of its irritant properties, and (3) repeated exposures to formaldehyde
10 followed by neurological assessments performed throughout the treatment period or several days
11 to weeks after termination of treatment. In addition to reports evaluating changes in behavior,
12 there are several reports evaluating neuropathological effects or changes in brain chemistry.

13

14 **4.2.6.1.1. *Behavioral response.***

15 **4.2.6.1.1.1. Clinical signs.**

16 Several studies that were focused on general toxicity or carcinogenicity of formaldehyde
17 also assessed clinical signs in exposed animals, which may be related to adverse effects on the
18 nervous system. Procedural details for the assessments, or specific data regarding findings, were
19 not provided. Signs recorded included uncoordinated locomotion and climbing of cage walls at
20 20 ppm formaldehyde in rats (Woutersen et al., 1987); restlessness at 15 ppm formaldehyde in
21 rats (Morgan et al., 1986a); dyspnea, listlessness, and hunched posture at 20 ppm and ataxia at
22 40 ppm in mice (Maronpot et al., 1986); and dyspnea in rats at 14.3 ppm formaldehyde (Kerns et
23 al., 1983). Given the lack of information regarding procedures used for these evaluations and the
24 limited reporting of results, the utility of these data is limited.

25

26 **4.2.6.1.1.2. Irritant threshold detection.**

27 Wood and Coleman (1995) evaluated the irritant properties of acute formaldehyde
28 exposure in mice. Adult male Swiss mice (eight/group) were initially trained to terminate a
29 60-second exposure to an irritant gas (ammonia, 1,000 ppm) by poking their noses into a conical
30 sensor five times to produce a 60-second facial shower of clean air. Each test session consisted
31 of 25 exposure trials. Following training, response to formaldehyde was evaluated, using the
32 same testing scenario. Each day mice had a morning exposure session to ammonia and an
33 afternoon session to formaldehyde. Formaldehyde concentrations tested were different each day,
34 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/m³) and
35 then stepping back again from 10 to 0 ppm. Half of the animals were tested in an ascending

1 order of formaldehyde concentrations, the other half in a descending order. The frequency of
2 terminating exposure, error rate, and the time lapse to termination were recorded. The
3 concentration at which 50% of the formaldehyde deliveries would be expected to be terminated
4 was estimated (AC_{50}) by simple linear regression or by analysis of covariance on the logit
5 transform of percentage terminated as a function of log concentration.

6 All mice were trained successfully to terminate 100% of ammonia exposures, but varied
7 responses were observed with formaldehyde exposure. In general, time taken to terminate
8 formaldehyde exposure decreased significantly with increasing formaldehyde concentration.
9 Mice terminated more exposures to 1 ppm formaldehyde than to air alone ($p < 0.0005$), and the
10 error rate, generally below 40%, did not significantly differ with formaldehyde concentration
11 tested. Each animal had two test sessions with each formaldehyde concentration (once during
12 the ascending sequence and again during the descending sequence); both the time to termination
13 ($p < 0.0012$) and AC_{50} were decreased in the second series of tests. One method of estimation by
14 the authors yielded an AC_{50} of 3.63 ppm for the first series of tests versus 1.88 ppm for the
15 second series. A two-way repeated measures ANOVA with replication and concentration as
16 within variables was highly significant ($p < 0.00005$). These studies indicate mice are sensitive
17 to the irritant properties of formaldehyde at exposure concentrations as low as 1 ppm, and
18 animals reacted more swiftly and with greater accuracy to terminate formaldehyde exposure as
19 the concentration increased. However, a wide variety of responses was noted on an individual
20 animal basis. Two of the eight mice terminated 90% of the trials during 1 ppm exposures and
21 80–100% of trials at all other tested formaldehyde concentrations. One mouse terminated fewer
22 than 10% of the formaldehyde exposure trials (1–10 ppm) during the testing regimen but had a
23 92% response rate to 20 ppm formaldehyde. The remaining five mice responded with increasing
24 termination frequency as formaldehyde concentration increased from 1 to 10 ppm, with an AC_{50}
25 of 2.72 ppm (3.34 mg/m³).

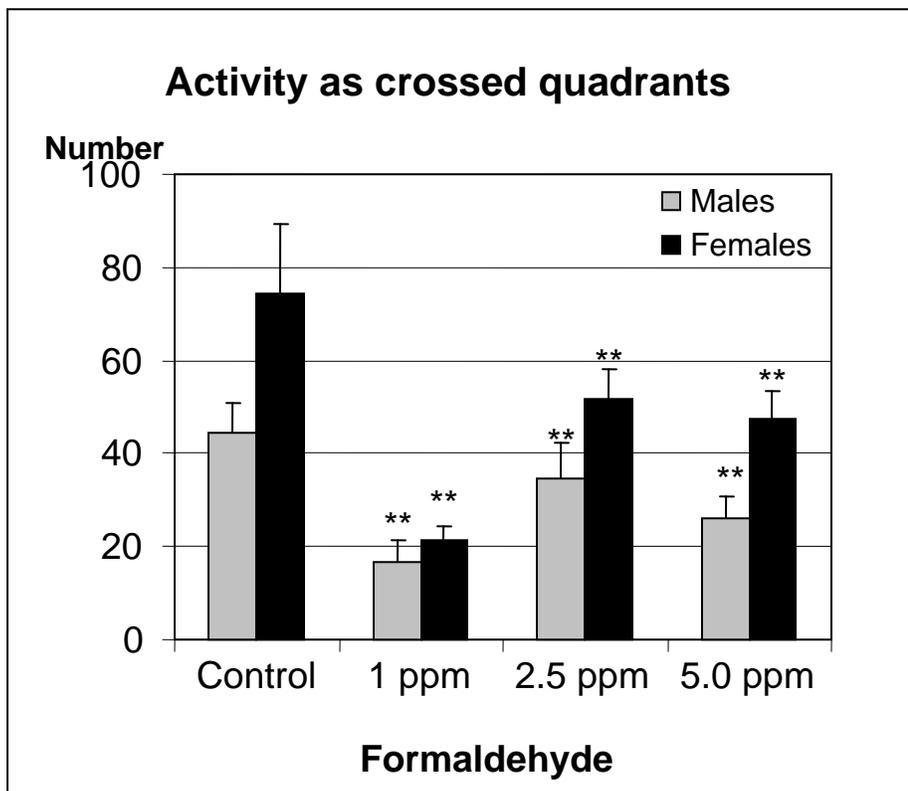
26 27 **4.2.6.1.2. Motor activity and habituation.**

28 Malek et al. (2003a) examined open field behavior of rats after acute formaldehyde
29 exposures. Male and female LEW.1K rats (15/sex/group) were exposed to 0, 1, 2.5, or 5 ppm (0,
30 1.23, 3.08, or 6.15 mg/m³) formaldehyde for 2 hours. Formaldehyde was vaporized from
31 aqueous solutions directly below the exposure chamber. Formaldehyde levels were checked 16
32 times throughout the 2-hour exposure periods. Mean formaldehyde levels of 1.01 ± 0.29 ppm,
33 2.51 ppm (SD is missing), and 5.0 ± 0.27 ppm were achieved. Locomotor activity was assessed
34 for 3 minutes in an open field 2 hours after termination of formaldehyde exposure and again 24
35 hours later, using an automated device to count the number of squares crossed. Other behaviors

1 were noted, including grooming (face cleaning, fur licking, and scratching), rearing, sniffing (air
2 and floor), wall climbing, and defecation.

3 The authors reported no signs of irritation or changes in activity or food or water intake
4 during exposure. In general, sniffing was increased after formaldehyde exposure and movement
5 was decreased (crossed quadrants and climbing) in both male and female rats ($p < 0.05$).
6 Significant reductions in horizontal movements (crossed quadrants) were observed at all dose
7 levels and were characterized by a U-shaped dose response (see Figure 4-24). The lowest dose
8 tested (1 ppm) demonstrated a higher level of activity suppression than the two higher doses, but
9 all groups were still suppressed relative to controls. Although female rats displayed a greater
10 level of activity overall, a similar U-shaped dose-response pattern was also observed.

11



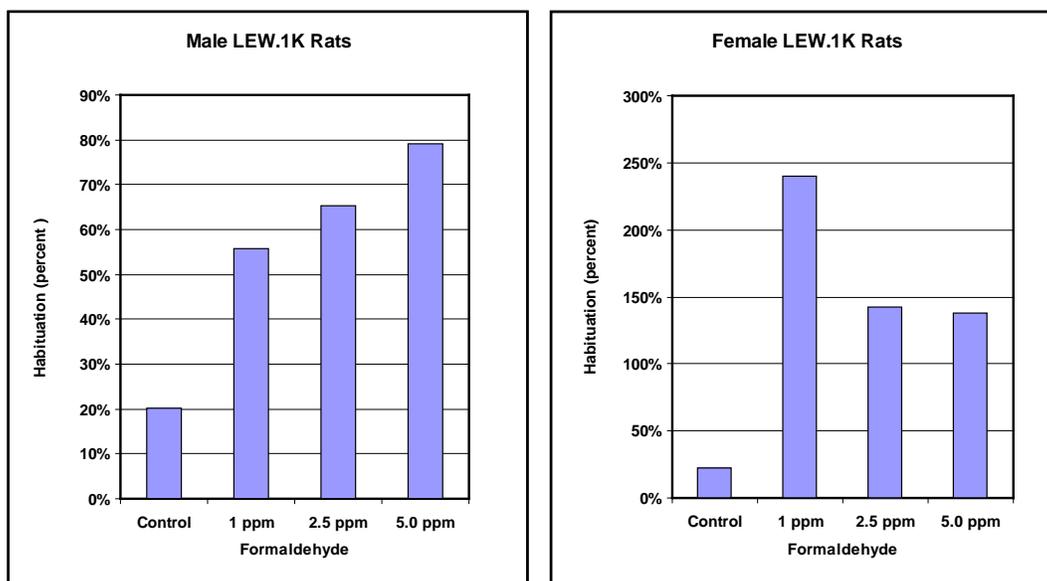
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Figure 4-24. Motor activity in male and female rats 2 hours after exposure to formaldehyde expressed as mean number of crossed quadrants \pm SEM.

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).

1 Activity in the same apparatus was reassessed 24 hours later. As expected, controls
2 demonstrated habituation to the test apparatus, exhibiting only 20% of the motor activity
3 observed on day 1 (see Figure 4-25). In contrast, formaldehyde-treated animals failed to
4 demonstrate the same degree of habituation. Activity levels for males observed on day 2 were
5 60–80% of the activity levels seen on day 1 ($p < 0.005$). Formaldehyde-treated females also
6 failed to habituate and actually demonstrated increases in activity on day 2 relative to day 1 at all
7 formaldehyde exposure levels ($p < 0.005$).



9
10
11 **Figure 4-25. Habituation of motor activity was observed in control rats**
12 **during the second observation period (day 2, 24 hours after formaldehyde**
13 **exposure).**

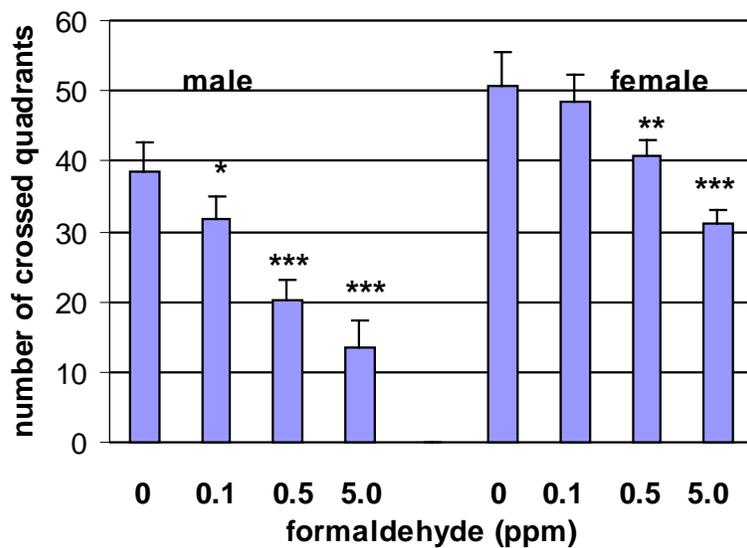
14
15 Note: Habituation is shown here as the percent decrease in number of crossings
16 between sessions from day 1 to day 2. The degree of habituation was reduced in
17 male rats exposed to formaldehyde (left panel) since their activity was closer to
18 100% of that seen on day 1. Females (right panel) had increased activity on day 2
19 (greater than 100% of activity on day 1), which is a sensitization rather than
20 habituation.

21
22 Source: Drawn from data reported by Malek et al. (2003a).

23
24
25 A follow-up study by Malek et al. (2003b) further expanded the dose-response analysis
26 for acute formaldehyde exposure. As described above, male and female LEW.1K rats (10 per
27 sex per group) were exposed at 0, 0.1, 0.5, or 5 ppm (0, 0.123, 0.615, or 6.15 mg/m³)
28 formaldehyde for 2 hours. Formaldehyde levels were checked nine times per hour during the

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1 exposure periods, and mean values were found to be 0.13 ± 0.04 , 0.48 ± 0.05 , and
 2 5.18 ± 0.66 ppm. Open field behavior was evaluated for each animal 2 hours after formaldehyde
 3 exposure. The number of crossed quadrants for both controls and a 5 ppm group were generally
 4 comparable with those observed in the first study, although female values were somewhat lower.
 5 Horizontal movement was decreased by formaldehyde exposure in a dose-dependent manner
 6 with significant reductions in motor activity as low as 0.1 ppm in males and 0.5 ppm in females
 7 (see Figure 4-26). The consistency of the findings across studies and between genders provides
 8 greater confidence in the effects of low-level formaldehyde exposure on this standard test of
 9 neurotoxicity.



10
 11 **Figure 4-26. Motor activity was reduced in male and female LEW.1K rats**
 12 **2 hours after termination of 10-minute formaldehyde exposure.**

13
 14 Note: Values are means \pm SDs. * = different from control, $p < 0.05$. ** = different
 15 from controls, $p < 0.01$. *** = different from controls, $p < 0.001$.

16
 17 Source: Drawn from data reported in Malek et al. (2003c).

18
 19
 20 Malek et al. (2004) also assessed the capacity of formaldehyde to induce persistent
 21 behavioral deficits in mice. Groups of 20 male AB mice received a single 2-hour exposure to 0,
 22 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
 23 after exposure for a series of behavioral responses, including ambulation (crossed squares),
 24 grooming, sniffing, rearing, wall climbing, and defecation. Even though there were no clinical
 25 signs of toxicity in any of the exposed groups, a number of behavioral anomalies were apparent

1 in response to formaldehyde exposure, some of which persisted for at least 24 hours, as indicated
 2 in Tables 4-55 and 4-56.

3
 4
 5
 6

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

7 ^aValues are means ± SDs.
 8 ^bStatistical significance of differences from controls ($p < 0.005$).
 9 ^cStatistical significance of differences from controls ($p < 0.05$).
 10 Source: Malek et al. (2004).

11
 12
 13
 14
 15

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

16 ^aValues are means ± SDs.
 17 ^bStatistical significance of differences from controls ($p < 0.005$).
 18 ^cStatistical significance of differences from controls ($p < 0.05$).
 19 Source: Malek et al. (2004).

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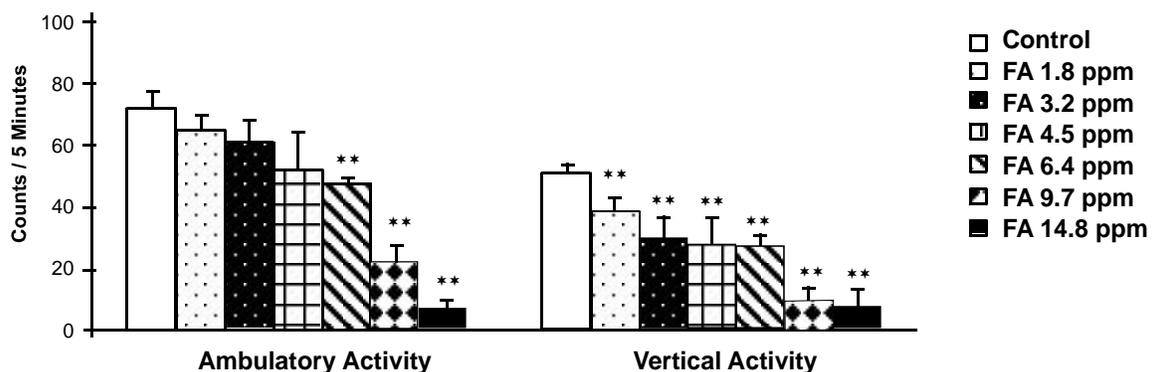
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1 Usanmaz et al. (2002) assessed spontaneous locomotor activity (SLMA) in Balb/c mice
 2 (4–14 per group, sex unspecified) after both acute and subchronic formaldehyde exposures.
 3 Prior to the acute exposure, mice were acclimated to the exposure chamber for 4 days but
 4 exposed only to clean air. On the fifth day, mice (six/group, sex unspecified) were exposed for
 5 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/m³)
 6 formaldehyde. Mice were removed from the exposure chamber, and SLMA behavior was
 7 evaluated by direct observation for 5 minutes. In addition to horizontal and vertical movement,
 8 wet dog shake (WDS) behavior was noted. In a separate trial, Balb/c mice (six/group, sex
 9 unspecified) were exposed to 8.2 ppm formaldehyde for 1 week, 2 ppm formaldehyde for
 10 2 weeks, or 3.3 ppm formaldehyde for 3 weeks (3 hours/day, 5 days/week) compared with
 11 controls exposed only to air. SLMA behavior was observed for 5 minutes after the last exposure.
 12 Mice exposed to 8.2 ppm formaldehyde for 1 week, 3.3 ppm formaldehyde for 2 weeks, and
 13 2 ppm formaldehyde for 3 weeks lost weight over the course of the treatment ($p < 0.05$). All
 14 other treatment groups had weight gain similar to control mice.

15 As shown in Figure 4-27, acute 3-hour formaldehyde exposures resulted in a dose-
 16 dependent decrease in SLMA. Decreases in horizontal activity were significant for the three
 17 highest dose groups (6.4, 9.7, and 14.8 ppm), and decreases in vertical activity were significant
 18 for all six formaldehyde treatment groups. SLMA was similarly decreased following subchronic
 19 exposures (data not shown here). Although the experimental protocol included longer exposures
 20 and a slightly longer observation period (5 versus 3 minutes) than in Malek et al. (2003a, b), the
 21 results are consistent, indicating decreased activity in formaldehyde-exposed animals several
 22 hours after exposure was ended.

23

SLMA after acute exposures



24

25

26 **Figure 4-27. The effects of the acute formaldehyde exposures on the**
 27 **ambulatory and vertical components of SLMA.**

28 Note: FA = formaldehyde exposure concentration. ** = $p < 0.01$ from controls.

29 Source: Usanmaz et al. (2001).

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1 Usanmaz et al. (2002) noted an increase in WDS, after the acute exposures, as a possible
2 preconvulsive effect. However, the mice were only observed for 5 minutes, and it is unclear how
3 the researchers distinguished between a WDS due to an irritating odor and a preconvulsive
4 movement. No other study has noted convulsive effects from formaldehyde exposure in any
5 species. A second set of trials was reported in the same paper that sought to evaluate
6 formaldehyde effects on CNS excitability. Balb/c mice (six/group, sex unspecified) were
7 exposed to 0, 1.8, 6.4, or 14.8 ppm (0, 2.2, 7.9, or 18.2 mg/m³) formaldehyde for 3 hours.
8 Subchronic exposures were at 2 ppm (2.5 mg/m³) formaldehyde for 3 weeks or 3.3 ppm
9 (4.1 mg/m³) formaldehyde for 2 weeks. Seizures were induced by I.P. injection of
10 pentilene tetrazole (PTZ), and the incidence, severity, and course of induced seizures were
11 recorded. The PTZ injection induced seizures in 83, 88, and 91% of controls, with 16, 38, and
12 67% mortality in controls in the three trials. Mortality was highly variable in treatment groups as
13 well. The authors report that PTZ-induced seizures were decreased in incidence by acute
14 formaldehyde exposure in a dose-dependent fashion with only 33% of mice exposed to 14.8 ppm
15 formaldehyde experiencing seizures versus 91% in control mice ($p < 0.05$ at the highest dose
16 only). However, the methodology for observing and scoring seizures is unclear. Additionally,
17 there was high mortality and high variability of results for the three similarly treated control
18 groups. Therefore, it is difficult to assess data quality and interpret these findings.

19 Boja et al. (1985) exposed male Sprague-Dawley rats to air or to formaldehyde at 5, 10,
20 or 20 ppm for 3 hours on 2 consecutive days. On the second day, half the rats received the same
21 exposure as the previous day, while half the rats were switched (e.g., half those rats receiving air
22 the first day received formaldehyde the second day, and half those receiving formaldehyde the
23 first day received air the second day), for a total of four possible exposure combinations. During
24 the exposure period, activity levels were monitored by observation, once per minute for the first
25 hour and once every 5 minutes for the second hour. At the end of the second exposure session,
26 rats were sacrificed and brains removed for neurochemical analysis (see Section 4.2.6.1.5).

27 Behavioral results were described in detail only for control and 5 ppm groups. During
28 the first exposure session, activity levels of formaldehyde-exposed animals were significantly
29 decreased (approximately 50% of control levels). On the second day of exposure, those animals
30 previously exposed to formaldehyde exhibited partial recovery, those experiencing their first
31 formaldehyde exposure behaved similarly to those initially exposed on the first day, and those
32 animals exposed to formaldehyde for a second time had a greater decrease in activity than during
33 the first exposure (to approximately 30% of control levels). The authors stated that a similar
34 effect was seen in animals exposed at 10 ppm but that results at 20 ppm were not interpretable

1 (data were not presented). Overall, the decreased activity seen in this study is consistent with
2 effects seen by other authors.

3 Senichenkova (1991) exposed pregnant female rats to 0 or 0.5 mg/m³ (0 or 403 ppb)
4 formaldehyde on gestation days (GDs) 1–19 for 4 hours/day. Reproductive aspects of this study
5 will be discussed in the reproduction section; however, results from behavioral assessments
6 conducted on the neonates are discussed here. The author stated that maturation of motor
7 reflexes (assessed as surface righting and pendular reflex), open field behavior, and maze
8 learning ability were assessed. Detailed descriptions of procedures and results were not provided
9 for all assessments, but it was stated that motor reflex development did not differ in treated and
10 control animals. Open field motor activity assessments in 40-day-old (juvenile) offspring
11 revealed an increase in squares visited and an increased frequency of rearing on the second and
12 third days of testing, indicating a lack of habituation in the offspring of formaldehyde-treated
13 dams; similar levels of activity by both measures were found on the first test day. Counts of
14 defecation and urination were increased on all 3 days of testing. Increased exploratory behavior,
15 described as increased impulses, was also noted in a learning task (not otherwise described), but
16 the author stated that learning rate and ability of the formaldehyde-treated group was not
17 different from controls (no data were provided).

18 Mobility and neuromuscular excitability (not otherwise described) in offspring of female
19 white rats were also evaluated by Sheveleva (1971). Dams were exposed to 0.005 or
20 0.0005 mg/L (approximately 4,000 or 400 ppb, respectively) formaldehyde on GDs 1–19.
21 Spontaneous mobility (over 15 minutes) and neuromuscular excitability were evaluated in
22 offspring at 1 or 2 months of age (other results from this study are discussed under
23 developmental toxicity, above). At 1 month, spontaneous mobility was reduced at the low dose
24 in males (52% of control levels; $p < 0.01$) but not at the high dose, and at both doses in females
25 (to 64 and 56% of control levels at the mid dose and high dose, respectively; $p < 0.02$). At two
26 months, there was a dose-related increase in activity for both sexes, statistically significant ($p <$
27 0.001) in high-dose females only (391% of control levels).

28 29 **4.2.6.1.3. Learning and memory.**

30 The effects of repeated formaldehyde exposures on learning were investigated by Malek
31 et al. (2003c), using a labyrinth swim maze. In this task, animals are required to make a series of
32 consecutive right or left turns to gain access to an escape platform (Malek et al., 2003c). Adult
33 male and female LEW.1K rats (15/sex/group) were exposed to 0, 0.1, 0.5, or 5.4 ppm (0, 0.123,
34 0.615, or 6.64 mg/m³) formaldehyde 2 hours/day for 10 consecutive days. Formaldehyde levels
35 were checked eight times throughout the 2-hour exposure periods. Mean formaldehyde levels of

1 0.1 ± 0.02, 0.5 ± 0.1, and 5.4 ± 0.65 ppm were achieved. Body weight was measured on days 1,
 2 5, and 10 of the experiment. Two days prior to beginning the formaldehyde exposures, all
 3 subjects were given an acclimation trial in which they were individually placed into the water-
 4 filled basin at the start position and allowed to navigate to the escape platform with manual
 5 assistance to learn the correct route. Thereafter, the water labyrinth test was run on each day of
 6 formaldehyde treatment, 2 hours after completion of each daily exposure. Time taken to
 7 complete the test and errors made were recorded for each rat (see Table 4-57). An error was
 8 defined as swimming toward the start position or circling in the same position without moving
 9 forward toward the escape platform. Rats were sacrificed at the end of the experiment, and
 10 tissues were taken from the lung, heart, thymus, kidney, liver, pancreas, skeletal muscle, and
 11 spleen. Tissues were fixed and prepared for histologic examination by light microscope. No
 12 differences were noted in food consumption or body weight gain for either male or female rats
 13 (Malek et al., 2003c). No treatment-related differences in organ pathology were reported (with
 14 the possible exception of focal microatelectasis (lung collapse at the microscopic level) seen in
 15 two to three animals in each formaldehyde-exposed group but not control animals).

16
 17 **Table 4-57. Effects of formaldehyde exposure on completion of the labyrinth**
 18 **test by male and female LEW.1K rats**
 19

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

20
 21 ^aRats were exposed to formaldehyde for 2 hours/day, for 10 consecutive days.

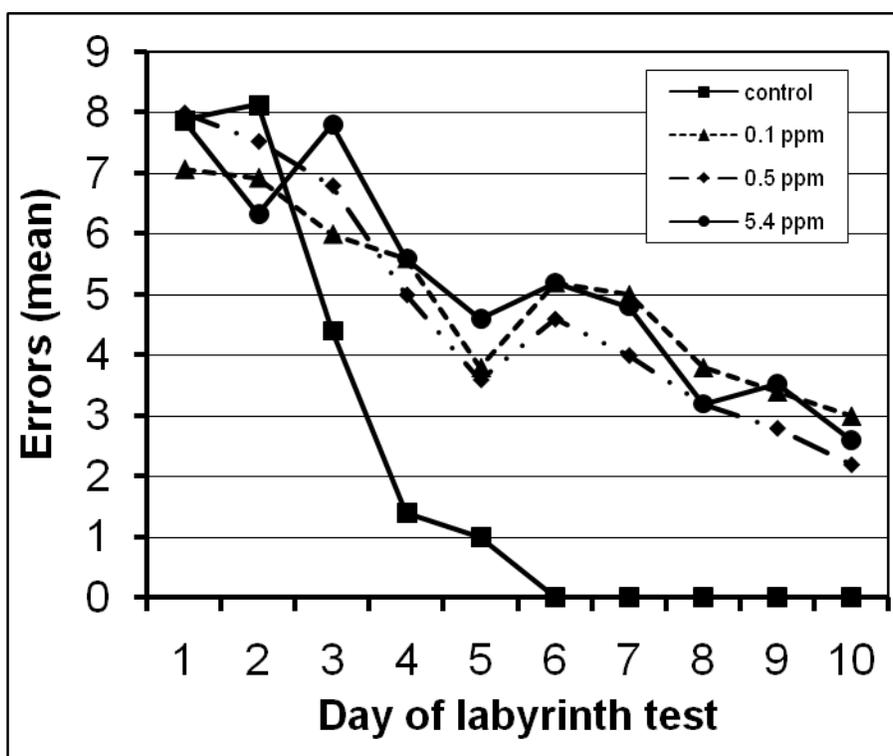
22 ^bDifferent from control, *p* < 0.05.

23 ^cDifferent from control, *p* < 0.005.

24
 25 Source: Malek et al. (2003c).
 26
 27

1 A clear learning curve was evident in control animals, with rats completing the task in
2 less time and with fewer errors over days (see Table 4-57). Although the number of errors
3 decreased with increasing experience in all groups, error rates in formaldehyde-exposed rats at
4 all doses were consistently higher than those observed in controls, starting on day 3 (see
5 Figure 4-28). All control animals performed without errors by day 6, whereas all treated animals
6 were still making two to three errors on day 10, the final day of testing. Time required (latency)
7 to complete the maze was also reduced over days. Although this measure of performance was
8 not as sensitive as error rate, formaldehyde-induced deficits were still evident in the 0.5 and 5.4
9 ppm exposure groups of both sexes.

10



11

12

13 **Figure 4-28. Effects of formaldehyde exposure on the error rate of female**
14 **LEW.1K rats performing the water labyrinth learning test.**

15

16 Source: Drawn from data reported in Malek et al. (2003a).

17

18

19 Impaired performance on formaldehyde-treated subjects cannot be attributed to
20 alterations in swimming ability, since latencies to complete the maze were identical for 0 and
21 0.1 ppm groups, yet acquisition of the task was still impaired in the 0.1 ppm group based on
22 number of errors committed (see Figure 4-28). This study reports an adverse effect level of

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1 0.1 ppm for increased error rate in the labyrinth water test, and all dose groups were equally
2 impaired across a broad range of exposures, 0.1–5.4 ppm. An independent estimate of
3 swimming speed was not included, so motor competency could not be directly evaluated.
4 However, comparable latency scores and error rates at the beginning of testing across all groups
5 and latency scores that track together over days suggest that impaired swimming ability does
6 account for the observed differences in latency, which are most likely reflective of the increased
7 number of errors in treated animals (errors usually increase the distance traveled and thus time
8 required for completion of the trial).

9 Pitten et al. (2000) evaluated the effects of very brief formaldehyde exposures
10 (10 minutes) but prolonged duration (90 days) on previously learned performance in a land
11 version of the labyrinth maze. Adult male and female Wistar rats (13/group) were trained on the
12 task for 14 days, two trials/day. Animals were required to make a series of five left or right turns
13 from the entrance of the maze to retrieve a piece of cheese placed in the goal box at the opposite
14 end. Animals were guided by the experimenter through the maze during this acclimation phase
15 until all subjects were able to retrieve the food without aid. After animals were trained (but prior
16 to formaldehyde exposure), performance was assessed once daily for 11 days, and the latency to
17 complete the maze as well as the number of errors committed when traversing from the entrance
18 to the goal box was recorded. Animals were then assigned to one of three dose groups (five to
19 eight/sex/group) such that task performance was equivalent across groups prior to
20 commencement of formaldehyde exposures. Animals were exposed to 0 ppm, 2.6 ppm (0.25%
21 formaldehyde solution to yield $3.06 \pm 0.77 \text{ mg/m}^3$), or 4.6 ppm (0.70% formaldehyde solution to
22 yield $5.55 \pm 1.27 \text{ mg/m}^3$) formaldehyde 10 minutes/day, 7 days/week for 90 days. Animals were
23 assessed for performance in the maze every seventh day, at least 22 hours after the exposure on
24 the previous day. At the end of the 90-day exposure period, monitoring of maze performance
25 continued once every 10 days for an additional 30 days. All rats were sacrificed at the end of the
26 postexposure trials and tissue sections were prepared for histologic examination by light
27 microscopy, including liver, trachea, lung, kidney, heart, spleen, pancreas, testicle, and brain.
28 No treatment-related changes in food or water consumption weight gain or in histologic samples
29 obtained at the termination of the experiment were observed.

30 Pitten et al. (2000) reported that no gender differences existed as a function of
31 formaldehyde treatment; therefore, data were presented by combining sexes. Control rats
32 showed no change in error rate but a slight decrease in running time through the maze during the
33 course of the experiment. The formaldehyde-exposed groups began with a similar performance
34 level and error rate as controls, but their performance degraded over the course of formaldehyde
35 exposure. By the fourth week of exposure, increased numbers of errors were evident in both

1 exposed groups relative to controls. This trend reached statistical significance at the 12-week
2 time point, with a greater than twofold increase in number of errors ($p < 0.05$). Formaldehyde-
3 treated rats also tended to have increased run times through the maze ($p = 0.04$), but no
4 difference was seen by formaldehyde concentration. By 4 weeks after termination of exposure,
5 no statistical differences among the three groups were evident, but the tendency for the two
6 exposed groups to make more errors and have longer latencies remained. Since Pitten et al.
7 (2000) tested animals after the task was acquired, these results indicate deficits in the retention of
8 a previously learned task.

9 Lu et al. (2008) evaluated the effects of formaldehyde on performance of mice in a
10 Morris water maze. Kunming mice (five males/group) were exposed to formaldehyde at 0.2, 1,
11 or 3 mg/m³ 6 hours/day for 7 days (measured concentrations: 0.2 ± 0.01 , 0.99 ± 0.04 , and
12 3.03 ± 0.16 mg/m³). Mice were trained to locate a hidden platform in a large, circular tank
13 (106 cm diameter, 31 cm deep). Each animal received four training trials per day, beginning
14 30 minutes after the end of exposure. During training, latency to locate the platform was
15 recorded for each trial, with a maximum of 60 seconds, after which the animal was guided to the
16 platform. After the last day of training, an additional trial was conducted with the platform
17 removed (the probe trial); time spent in each maze quadrant was measured to determine the time
18 the animal spent searching for the platform in the correct area of the maze. Performance in the
19 water maze, measured as mean escape latency across the seven training trials, was significantly
20 impaired in the 3 mg/m³ group. No significant difference was seen at 1 mg/m³, although there
21 appeared to be an increased latency during the second day of testing. During the probe trial,
22 control animals spent significantly more time in the correct quadrant, but neither formaldehyde-
23 exposed group did so. Results of this study indicate deficits in learning and retention of the
24 Morris water maze following formaldehyde exposure, with greater effects seen in the higher dose
25 group.

26 Apfelbach and Reibenspies (1991) published a brief report of formaldehyde effects on
27 olfactory learning. Ferrets were exposed to 0.25 ppm (0.31 mg/m³) formaldehyde gas
28 continuously for 6 months. A Y-shaped maze was used to test odor detection, discrimination
29 between odors, and odor threshold. Ferrets were conditioned to distinguish ethyl acetate (0.1 vol
30 %) from clean air. Untreated ferrets achieved 75% success after an average of 110 trials.
31 However, formaldehyde-treated ferrets required on average 320 trials to reach a 75% success
32 rate. A 90% success rate was achieved by untreated ferrets after 420 trials. However, this level
33 of success was not reached in formaldehyde-treated ferrets.

34 The same researchers also tested olfactory function in formaldehyde-treated ferrets, as
35 summarized in Section 4.2.7 (Apfelbach et al., 1992). A decrease in olfactory discrimination and

1 a reduction in the percentage of olfactory cells in the olfactory epithelium were reported after
2 3–12 months exposure to 0.25 or 0.5 ppm formaldehyde. Decreased olfactory sensitivity in rats
3 exposed to 0.25 or 0.5 ppm formaldehyde has also been reported by the same researchers (Weiler
4 and Apfelbach, 1992; Apfelbach and Weiler, 1991), and Weiler and Apfelbach (1992) reported
5 in an abstract that shifts in olfactory thresholds were greater when exposure was initiated at PND
6 30 than at adult ages. Given the documented changes in olfactory thresholds, observed changes
7 in olfactory learning would likely be confounded by the potential for decreased olfactory
8 function by formaldehyde exposures, and definitive conclusions regarding formaldehyde effects
9 specific to learning cannot be made based on these studies.

11 **4.2.6.1.4. Neurosensitization.**

12 Sorg et al. (1996) studied the potential for formaldehyde exposure to induce sensitization
13 in the CNS, possibly through the limbic pathways in the brain. The authors hypothesized that
14 multiple chemical sensitivity (MCS) has an onset and progression similar to CNS sensitization
15 and may, therefore, be a similar process. These experiments were conducted to test this
16 hypothesis and to determine whether formaldehyde exposure could be used as a model for MCS.
17 Behavioral sensitization can be initiated by psychostimulants (e.g., cocaine) and manifest as
18 increased locomotor activity upon subsequent challenge with the stimulant.

19 Sorg et al. (1996) evaluated cross-sensitization of cocaine-induced increases in activity
20 from an initial formaldehyde exposure. Female Sprague-Dawley rats (eight to nine) were
21 exposed to 0 or 11 ppm (0 or 13.5 mg/m³) formaldehyde 1 hour/day for 7 days. Locomotor
22 activity was measured (by photocell) after saline injection (1 day postexposure) and after cocaine
23 injection (2 days postexposure). A similar protocol was conducted on days 36 and 37
24 postexposure. Motor activity levels following saline injection were similar for controls and
25 formaldehyde-treated rats. However, formaldehyde exposure initiated sensitization to cocaine as
26 evidenced by a greater increase in locomotor activity in mice treated with formaldehyde
27 followed by cocaine ($p < 0.05$) with an average count of crossed grids greater than 40,000 (2
28 hours) in treated animals compared with 25,000 (2 hours) in controls. The cross-sensitization
29 was transient, with no treatment effects on cocaine-induced activity either 29 or 37 days
30 postexposure. When examining individual data, the authors suggested that the formaldehyde-
31 treated groups in both cases have a cluster of high responders (HRs), suggesting some animals
32 may have been more sensitive. A second group of similarly treated female rats was pretested for
33 locomotor activity and divided into subgroups of HRs or low responders (LRs). They were then
34 given a panel of neurobehavioral tests: anxiety (elevated plus maze, day 11); memory (passive
35 avoidance training, day 12; passive avoidance test, day 19); and nociceptive test (day 20). Trunk

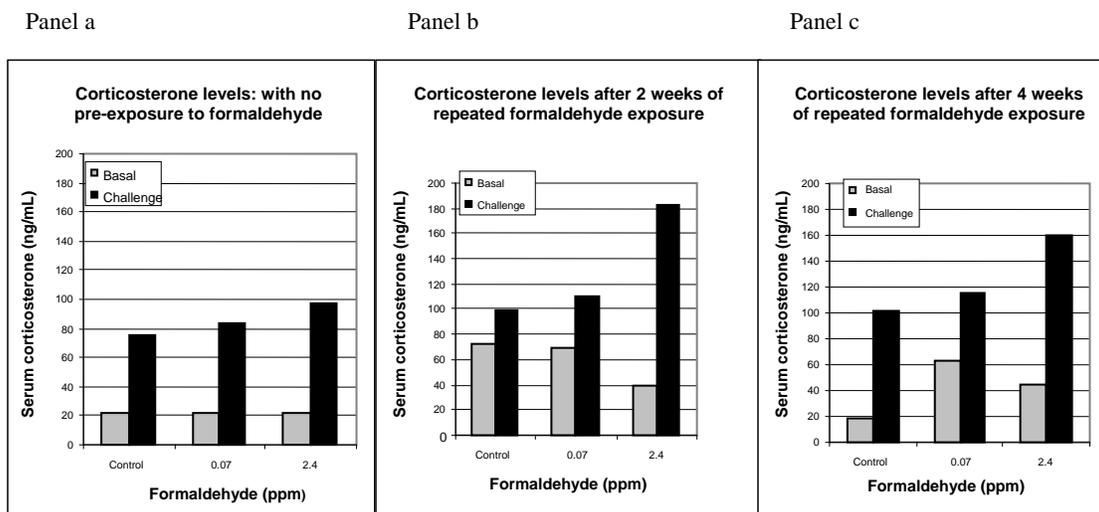
1 blood corticosterone levels were determined during stress on day 35 postexposure. No
2 significant treatment differences were found in the passive avoidance test, nociception, or
3 corticosterone levels (basal or stress induced). On the elevated plus maze, a two-way ANOVA
4 indicated no overall formaldehyde treatment effects, but the HR rats had higher open arm time
5 ratios (indicating greater anxiety) regardless of treatment. Within the treatment groups, the
6 difference in behavior between HR and LR subgroups was only significant for the formaldehyde-
7 treated rats ($p < 0.05$). Authors suggested that cross-sensitization to cocaine-induced locomotor
8 activity was caused by enhanced dopamine transmission within the mesolimbic system (ventral
9 tegmental area to nucleus accumbens projection) following repeated formaldehyde exposure. A
10 critical role of the hypothalamic-pituitary-adrenal (HPA) axis has also been implicated in cross-
11 sensitization.

12 Sorg et al. (1998) and Sorg and Hochstatter (1999) further explored formaldehyde-
13 induced behavioral sensitization using the cocaine model. In contrast to the results following
14 exposure to 11 ppm (Sorg et al., 1996), rats exposed to only 1 ppm for 7 days showed no cross-
15 sensitization to cocaine injection. However, animals exposed to 1 ppm formaldehyde for
16 4 weeks exhibited increased cocaine-induced vertical activity (with no difference in horizontal
17 activity) for 4–6 weeks after cessation of exposure. Activity levels of formaldehyde-exposed
18 rats were approximately threefold those of control rats 3–4 days postexposure and still 1.5-fold
19 control levels at 4–6 weeks postexposure ($p < 0.05$).

20 Sorg et al. (2001) examined changes in corticosterone levels in rats with and without
21 formaldehyde treatment. Basal corticosterone levels in trunk blood were established in naïve
22 male Sprague-Dawley rats taken directly from their home cage immediately prior to sacrifice. In
23 an acute trial, male rats were exposed to 0, 0.7, or 2.4 ppm (0, 0.86, or 2.96 mg/m³)
24 formaldehyde for either 20 or 60 minutes, and trunk blood was collected for corticosterone
25 analysis. Therefore, these rats were challenged with a new environment (the exposure chamber)
26 in the presence or absence of formaldehyde. In a separate trial, basal and challenged
27 corticosterone levels were measured after repeated exposure (1 hour/day, 5 days/week for 2 or
28 4 weeks). Basal corticosterone levels were measured in trunk blood immediately after removing
29 the animal from its home cage. Challenged corticosterone levels were measured after rats were
30 placed into the exposure chamber for a final 20-minute exposure. Body weight was measured at
31 the beginning of each week of exposure and was unchanged by formaldehyde treatment.

32 Corticosterone levels were increased over basal levels when rats were placed in the
33 exposure chamber for 20 minutes (see Figure 4-29, panel a) but returned to basal levels after
34 60 minutes in the exposure chamber (not shown). This response may reflect the stress of the new

1 environment and acclimatization after 60 minutes in the chamber. Corticosterone levels were the
2 same in the presence or absence of formaldehyde, indicating no treatment effect.



23 **Figure 4-29. Basal and stress-induced trunk blood corticosterone levels in**
24 **male LEW.1K rats after formaldehyde inhalation exposures.**

25
26 Note: Panel a: no pretreatment, corticosterone levels after 20-minute
27 formaldehyde exposure. Panels b and c show both basal and induced
28 corticosterone levels after a 2- or 4-week pretreatment to formaldehyde
29 1 hour/day. Challenge to induce corticosterone was a 20-minute reexposure at the
30 formaldehyde level tested.

31
32 Source: Sorg et al. (2001).

33
34
35 Control animals exhibited an increase in basal corticosterone after 2 weeks, which
36 returned to naïve levels after 4 weeks (see Figure 4-29, panels b and c). Formaldehyde-treated
37 rats demonstrated a comparable increase in basal corticosterone levels at 2 weeks, but these
38 levels did not return to naïve levels at 4 weeks as seen with controls. Control and 0.7 ppm
39 exposed rats showed a similar response to challenge (the final 20-minute exposure). However,
40 rats exposed to 2.4 ppm were hyperresponsive, with exaggerated corticosterone levels during this
41 final exposure. Differences in basal corticosterone levels after formaldehyde exposure and the
42 hyperresponsiveness seen in animals exposed at 2.4 ppm provide evidence of
43 formaldehyde-induced perturbations of the HPA axis. Authors suggested that elevated
44 corticosterone levels induced by repeated formaldehyde exposures may contribute to the cross-
45 sensitization to cocaine-induced motor activity.

1 Formaldehyde-induced changes in the HPA axis may contribute to behavioral effects of
2 formaldehyde exposure reported by Sorg et al. (2004) and Sorg and Hochstatter (1999). The
3 authors also reported an enhanced conditioning to odor in animals previously exposed to
4 repeated formaldehyde. Male and female Sprague-Dawley rats (60–80 days of age) were
5 exposed at 1 ppm (1.23 mg/m³) formaldehyde 1 hour/day, 5 days/week for 4 weeks (Sorg and
6 Hochstatter, 1999). Two weeks after exposure ended, rats were trained to the conditioned fear
7 task. Rats were conditioned to a fear response by either odor only or odor associated with
8 footpad shock. Orange-oil extract was used as the odor conditioned stimulus (CS). One day
9 after conditioning, rats were reintroduced into the environment without an odor cue, and time
10 spent motionless in the freezing posture (freezing) was observed. On day 2 after conditioning,
11 rats were placed in a novel environment, and time spent in the freezing posture was evaluated in
12 the absence and then the presence of odor. This was repeated on day 12 after conditioning to
13 measure the loss of the freezing response to the conditioned odor.

14 Both treated and exposed rats showed similar responses on reintroduction into the
15 conditioning environment in the absence of an odor cue on day 1 (Sorg and Hochstatter, 1999).
16 As expected, rats conditioned with a footpad shock demonstrated greater time motionless than
17 odor-trained only rats, and there was no difference between control and formaldehyde-treated
18 rats. However, in the presence of odor on days 2 and 12, formaldehyde-exposed rats who were
19 conditioned with odor associated with foot shock spent significantly more time freezing than
20 odor-only trained rats ($p < 0.05$); control animals on those days showed no difference in time
21 freezing in the presence and absence of odor. The authors concluded that the formaldehyde-
22 treated rats had more difficulty than controls in extinguishing the fear response to the
23 conditioned odor, and speculated that an enhancement of the fear-conditioned response by
24 formaldehyde pretreatment supports the hypothesis that sensitization may include effects through
25 the limbic system of the brain.

26 In a second experiment, adult male and female Sprague-Dawley rats were exposed at 0 or
27 2 ppm (2.45 mg/m³) formaldehyde 1 hour/day, 5 days/week for 4 weeks (Sorg et al., 2004). Two
28 to 3 weeks after exposure ended, rats were trained to the conditioned fear task. Rats were given
29 a foot shock either associated with an odor (paired group) or unassociated with an odor (unpaired
30 group). Orange-oil extract was used as the odor CS. After training, freezing behavior was
31 assessed (1) in the same context in the absence of odor (1 day), (2) in a new context in the
32 presence and absence of the CS (5 consecutive days), and (3) in another novel context in the
33 presence and absence of the CS.

34 Formaldehyde-exposed male rats demonstrated increased conditioned fear response to an
35 odor CS (orange oil) paired with foot shock with no change in the degree of conditioning to the

1 context. For female rats, formaldehyde exposure did not affect the percent of time spent
2 freezing, either in the conditioning context or the novel context in the absence of the conditioned
3 odor. In contrast, male rats spent an increased time freezing in a novel context in the presence of
4 odor, indicating a greater conditioned fear response to the olfactory cue ($p < 0.05$). This is in
5 agreement with the previous study where formaldehyde effects were seen in the presence of the
6 conditioning odor but not the environment (Sorg and Hochstatter, 1999). However, in this study
7 female rats did not exhibit a similar enhancement of fear conditioning to the olfactory CS.

8 The authors suggested that repeated exposure to low levels of formaldehyde acts as a
9 stressor in much the same way as inescapable foot shock, with resulting sensitized responses
10 within the olfactory/limbic pathways (Sorg et al., 2004). This interpretation is consistent with
11 work described above in which augmented basal corticosterone levels following repeated
12 formaldehyde exposures were demonstrated. However, while the fear conditioning in the present
13 study and cross-sensitization to cocaine described above (Sorg and Hochstatter, 1999) occurred
14 3–4 weeks after termination of exposure to formaldehyde, the duration of corticosterone
15 elevation induced by repeated exposure to formaldehyde has not been determined. It is possible
16 that augmentation of corticosterone levels following formaldehyde exposure results from direct
17 action of formaldehyde on the HPA axis. Experiments designed to compare HPA activation
18 following standard stressors (repeated inescapable foot shock or restraint stress), stress induced
19 by other irritants (chemicals with strong irritant odors but no CNS action), and repeated
20 formaldehyde exposures are necessary to dissociate primary from secondary action of
21 formaldehyde on CNS function in this paradigm. It is also possible that enhanced conditioning
22 to an odor stimulus results from formaldehyde-induced increases in airway irritation, rendering
23 the conditioned odor stimulus a more salient cue, producing a conditioned response that is not
24 extinguished as readily as in air-exposed controls. However, damage of the nasal mucosa and
25 lesions would be expected to be minimal at 1 ppm formaldehyde exposures and most likely
26 resolved 2 weeks after exposure was ended (see Section 4.2.1.2). Therefore, a more salient cue
27 for fear conditioning to odor due to physical irritation is not likely. Alternatively, formaldehyde
28 may act to up regulate olfactory activity, producing a stronger sense of odor during conditioning.

30 **4.2.6.1.5. Neurochemistry and neuropathology.**

31 Several studies that were focused on general toxicity or carcinogenicity of formaldehyde
32 also assessed histopathology in exposed animals, including pathological evaluation of the brain.
33 In all cases, details of the pathological evaluation were not provided. Reported results stated that
34 no significant lesions were seen on unspecified tissues (Appelman et al., 1988; Maronpot et al.,
35 1986; Kerns et al., 1983) or that an increase in relative brain weight (data not provided) was

1 considered of no toxicological significance (Woutersen et al., 1987). The absence of procedural
2 information, or specific reported results, limits the utility of this information.

3 Boja et al. (1985) measured changes in several neurotransmitters (norepinephrine,
4 dopamine, 5-hydroxytryptamine) and their major metabolites (3,4-dihydroxyphenylacetic acid
5 [DOPAC] and 5-hydroxyindoleacetic acid [5-HIAA]) following one or two 3-hour exposures to
6 formaldehyde at 0, 5, 10, or 20 ppm. Animals were sacrificed immediately following the second
7 exposure, and brains were immediately removed, frozen, and sectioned. Regions of interest were
8 analyzed by high-pressure liquid chromatography with electrochemical detection. Authors stated
9 that neurotransmitter concentrations were measured in multiple brain regions, but results were
10 reported only for the 5 ppm exposure and only for the hypothalamus. No change was seen in
11 concentrations of norepinephrine or 5-hydroxytryptamine for any exposure paradigm. For those
12 animals exposed twice to formaldehyde, there was a slight (statistically significant) increase in
13 dopamine and a larger (approximately fourfold) increase in 5-HIAA. DOPAC was increased
14 (approximately 30%) in animals receiving formaldehyde during the second exposure only.

15 Recent work by Hayashi et al. (2004) indicates that formaldehyde exposure increases the
16 activity of periglomerular (PG) cells in the main olfactory bulb. Tyrosine hydroxylase activity
17 was measured as a marker for activity of olfactory function. The authors surmised that
18 expression levels of this enzyme are useful markers since it has been reported that the protein is
19 up regulated after sensory stimulation and is down regulated by odor deprivation or when the
20 olfactory epithelium is removed (Cho et al., 1996; Stone et al., 1991; McLean and Shipley, 1988;
21 Baker et al., 1983). Eight-week-old female C3H/HeN mice were exposed at 0, 0.08, 0.4, or
22 2 ppm (0, 0.1, 0.49, or 2.45 mg/m³) formaldehyde 16 hours/day for 1 day or 12 weeks
23 (5 days/week). Formaldehyde exposure did not affect body weight. Mice were sacrificed
24 24 hours after exposure; the brains were removed, fixed, and prepared for sectioning. One side
25 of the olfactory bulb was sliced into 40 µm-thick serial frontal sections and immuno-stained for
26 tyrosine hydroxylase activity. The number of tyrosine hydroxylase-positive PG cells was
27 determined by examining digital photomicrographs of three tissue sections, averaging the counts
28 from 10–15 glomeruli per section.

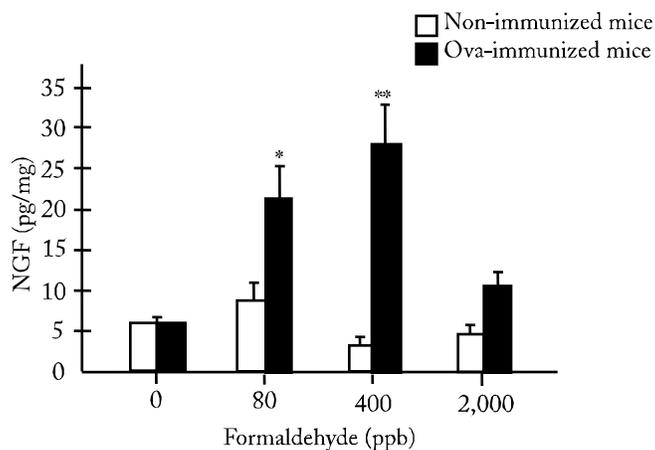
29 Neither the size of the olfactory bulb (rostrocaudal, dorsoventral, and mediolateral
30 lengths) nor the total number of PG cells was changed by formaldehyde exposure. The number
31 of tyrosine hydroxylase-positive PG cells per glomerulus was unchanged by a single
32 formaldehyde exposure but increased after 12 weeks of repeated exposures. The increases were
33 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
34 at 2 ppm or 196, 167, and 196% of controls, respectively. As an indicator of activity, it is not
35 unexpected that the enzyme was up regulated after repeated exposure to an odorous compound.

1 Hayashi et al. (2004) hypothesize that the increased tyrosine hydroxylase activity is an indication
2 of increased sensitivity and, therefore, may be a model for MCS. However, it is unknown if the
3 increase in enzyme activity after repeated exposures is transient or could result in sensitization.
4 Tyrosine hydroxylase is the first enzyme in the dopamine synthetic pathway, but the role of
5 dopamine in PG cells is not known. Further research would be needed to understand the
6 potential for formaldehyde to act as a sensitizing agent in this model.

7 In an abstract, Kakeyama et al. (2004) outline the results of experiments to address the
8 effects of subchronic exposure to low levels of formaldehyde on changes in neurotransmitter-
9 related mRNA expressions in mice forebrains. An unstated number of female C3H/He mice
10 were exposed 16 hours/day, 5 days/week to 400 ppb (0.49 mg/m³) formaldehyde for 12 weeks.
11 The authors used RT-PCR methodologies to quantify mRNA encoding for the glutamate receptor
12 subunits GluR1 and GluR2, the dopamine receptor D1, and the serotonin receptor 5-HT1A in the
13 neocortex, hippocampus, amygdala, and hypothalamus. Raised levels of mRNA expression were
14 observed for GluR1 in the neocortex and hippocampus; GluR1, GluR2, and the dopamine
15 receptor D1 in the amygdala; and the serotonin receptor 5-HT1A in the hypothalamus. Reduced
16 mRNA expression was observed for GluR2 in the hippocampus and neocortex. When other
17 mice were subjected to a radiofrequency-induced lesion of the hippocampus then exposed to
18 formaldehyde for 12 weeks as before, the altered expression of GluR1 and GluR2 in the
19 neocortex was abolished. However, the increment of mRNA expression of 5-HT1A in the
20 hypothalamus was further enhanced. In demonstrating that formaldehyde affects neocortical
21 GluR1 and GluR2 mRNA expressions through a hippocampal function, Kakeyama et al. (2004)
22 concluded that subchronic exposure to low concentrations of formaldehyde can affect neural
23 transmission in the forebrain.

24 Fujimaki et al. (2004b) examined the effects of formaldehyde on NGF in the brain and
25 hippocampus. Ten female C3H/HeN mice/group were exposed to 0, 80, 400, or 2,000 ppb (0,
26 0.1, 0.5, or 2.45 mg/m³) formaldehyde 16 hours/day, 5 days/week for 12 weeks. Some groups of
27 mice received the same treatment after I.P. injection of 10 µg of OVA and 2 mg alum prior to the
28 commencement of formaldehyde exposure. For this subgroup, booster injections of OVA were
29 administered on days 21, 42, 63, and 77 during the formaldehyde exposure regimen.
30 Quantitative measures of NGF and BDNF in homogenates of whole brain and hippocampus were
31 obtained by ELISA and mRNA determination. The amount of NGF protein in whole brains
32 remained unchanged in the nonimmunized mice. However, brain NGF levels were significantly
33 increased in OVA-immunized mice exposed to 80 and 400 ppb (but not 2,000 ppb)
34 formaldehyde (see Figure 4-30). This result was confirmed by parallel increases in the
35 concentrations of hippocampal NGF mRNA that were produced in immunized mice exposed to

1 formaldehyde at the same concentrations. However, there were no comparable increases in the
2 amounts of brain-derived neurotrophic factor in either immunized or nonimmunized mice. In
3 discussing the mechanisms potentially associated with their results, Fujimaki et al. (2004b)
4 considered it likely that low-level exposure to formaldehyde could enhance NGF production
5 through the stimulation of the HPA axis together with immunization.



8
9
10 **Figure 4-30. NGF production in the brains of formaldehyde-exposed mice.**

11
12 Note: Female C3H mice were exposed to formaldehyde 16 hours/day,
13 5 days/week for 12 weeks. NGF in homogenates of whole brain and
14 hippocampus were measured by ELISA. Values are means \pm SEM ($n = 5-6$).
15 * = $p < 0.05$ and ** = $p < 0.01$ versus control mice, as calculated by the authors.

16
17 Source: Redrawn from Fujimaki et al. (2004b).

18
19
20 The enhancement of NGF in the brains of immunized mice exposed to formaldehyde
21 gave rise to the suggestion that NGF may promote the survival of hippocampal neurons when
22 challenged with formaldehyde. To examine whether or not apoptosis plays a role in this process,
23 Tsukahara et al. (2006) measured the effects of formaldehyde on apoptotic mechanisms
24 regulating the survival and death of cells and on *N*-methyl-D-aspartate (NMDA) receptors.
25 Female C3H/HeN mice (13/group) were exposed to 0 or 400 (393 ± 34) ppb (0 or $490.8 \mu\text{g}/\text{m}^3$)
26 formaldehyde 16 hours/day, 5 days/week for 12 weeks. Seven control and formaldehyde-treated
27 mice were immunized with $10 \mu\text{g}$ OVA plus 2 mg aluminum hydroxide prior to exposure.
28 Subsequently, these mice received OVA via aerosol as a booster during weeks 3, 6, 9, and 11.
29 Hippocampi were dissected from all animals 1 day after the final exposure and homogenized in
30 hypotonic buffer. The 12,000 rpm supernatants were analyzed by Western blotting for the

1 presence of the proteins Bcl-2 (which inhibits apoptosis) and Bax (which opposes Bcl-2 action
2 and promotes apoptosis) and the NMDA receptor subtypes 2A and 2B (NR2A and NR2B).
3 Immunohistochemical analysis was also carried out for the presence of active caspase-3, an
4 apoptosis marker.

5 The levels of NR2A and NR2B were unaffected by exposure to formaldehyde in either
6 immunized or nonimmunized mice. Likewise, the number of caspase-3 immunoreactive cells
7 did not change as a result of formaldehyde exposure. However, when measured amounts of
8 Bcl-2 and Bax were normalized to the amount of β -tubulin, the ratio Bcl-2/Bax was significantly
9 increased in immunized mice exposed to formaldehyde. Nonimmunized mice did not show this
10 apparently compound-related response. Consistent with the concept that the proportions of Bcl-2
11 and Bax are critical for the regulation of cell survival and death, the authors interpreted their data
12 as an indication that changes to the ratio of Bcl-2/Bax expressions might be an important
13 adaptive response to the effects of formaldehyde, such that the antiapoptotic changes might
14 contribute to the protection of hippocampal neurons from the pernicious effects of formaldehyde
15 exposure itself.

16 The same research group used the immunized mouse model to determine whether
17 formaldehyde exposure affected mRNA expression of genes related to synaptic plasticity
18 (Ahmed et al., 2007). Ten female C3H/HeN mice were exposed to 0 or 400 ppb formaldehyde
19 16 hours/day, 5 days/week for 12 weeks. All mice were immunized with 10 μ g OVA plus 2 mg
20 aluminum hydroxide prior to initial formaldehyde exposure then treated in weeks 3, 6, 9, and 11
21 with aerosolized OVA as a booster. Five treated and control animals were I.P. injected with 1
22 mg/kg MK-801, a noncompetitive NMDA receptor agonist before the last formaldehyde
23 exposure. At term, hippocampi were dissected and frozen at -80°C until processing. At that
24 point, total mRNA was extracted and first strand cDNA was synthesized by using reverse
25 transcriptase. Expression levels of various proteins/receptors, including NMDA NR2A and
26 NR2B receptor subunits, dopamine D1 and D2 receptors, cyclic AMP responsive element-
27 binding proteins (CREB-1 and CREB-2), and the transcription factors FosB and Δ FosB were
28 determined by using the PCR. The expression level of each mRNA species was expressed
29 relative to the sample's content of 18S rRNA. The total protein lysate was also assayed for
30 pCREB by Western blotting.

31 In the first of a sequence of histograms, Ahmed et al. (2007) demonstrated a significant
32 increase in mRNA expression of NR2A as a result of formaldehyde exposure. However, this
33 effect was abolished in animals treated with MK-801. A similar trend in the mRNA expression
34 of NR2B in response to formaldehyde exposure did not achieve statistical significance. MK-801
35 treatment significantly reduced receptor in mRNA expression in the presence of formaldehyde.

1 The authors provided data showing an increased expression of dopamine D1 and D2 receptor
2 mRNA response to formaldehyde, in both cases abolished by treatment with MK-801. The
3 expression of CREB-1 mRNA also conformed to the pattern of being increased as a result of
4 formaldehyde exposure but abolished by MK-801. However, the expression of CREB-2 and
5 FosB/ Δ FosB was unaffected by formaldehyde. When normalized to the amount of β -tubulin,
6 there were no significant effects of formaldehyde exposure and MK-801 treatment on the protein
7 levels of pCREB. Finally, there was no significant difference in the expression of transient
8 receptor potential vanilloid receptor (TRPV1) between control and formaldehyde-exposed mice,
9 and MK-801 itself did not significantly alter the mRNA level of TRPV1. In seeking to explain
10 their results, the authors speculated that low-level exposure of immunized mice to formaldehyde
11 had an effect on hippocampal synaptic plasticity at the mRNA level, as evidenced by the
12 enhancement of mRNA for NR2A, the dopamine D1 and D2 receptors, and CREB1, with up
13 regulation compensating for the sustained levels of enhanced protein expression under low-level
14 formaldehyde exposure. The interpretation of these changes in NR2A mRNA, in the context of
15 the results of Tsukahara et al. (2006), showing no change in NR2A and NR2B protein
16 expression, was not discussed.

17

18 **4.2.6.1.6. Neurogenesis.**

19 Two papers have examined the effects of subacute exposure to formaldehyde on the
20 overall size (volume) of discrete cellular areas of the hippocampus in neonatal rats. The
21 researchers also used an optical fractionator counting method to derive a plausible estimate of
22 cell number. Aslan et al. (2006) studied the effects of formaldehyde exposure on the number and
23 volume of granular cells in the hippocampal dentate gyrus. Sarsilmaz et al. (2007) examined the
24 impact of postnatal formaldehyde exposure on brain hemisphere volume and on the size and cell
25 number of pyramidal cells in the cornu ammonis region of the hippocampus. The in-life phase
26 was the same in each study, featuring the exposure of 10 neonatal male Wistar rats/group to 0, 6,
27 and 12 ppm (0, 7.36, and 14.7 mg/m³) formaldehyde 6 hours/day, 5 days/week for 30 days. Five
28 rats/group were sacrificed at that point (PND 30), while the rest were maintained without further
29 treatment until PND 90.

30 For both pyramidal and granular areas, a much lower number of cells was seen on
31 PND 90 versus PND 30 ($p < 0.001$). This response was evident irrespective of the amount of
32 exposure to formaldehyde and is consistent with normal brain development. Compound-specific
33 effects of formaldehyde on the volume and number of granular and pyramidal cells varied by
34 dose and over the two time points. There was a small increase in the volume of the granular cell
35 layer of the dentate gyrus in rats sacrificed on PND 30 ($p < 0.001$) in response to increasing
36 formaldehyde concentration (Aslan et al., 2006), with no significant change in neuron number;

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1 the increased volume (now accompanied by an increase in neuron number) was still evident at
2 the low-exposure level on PND 90 ($p < 0.01$) but not at the high dose. Brain hemisphere volume
3 was decreased at both concentrations on PND 30 ($p < 0.01$) but was increased at both
4 concentrations ($p < 0.01$, with a larger magnitude of effect at 6 ppm) on PND 90 (Sarsilmaz et
5 al., 2007). In the hippocampal cornu ammonis region, the volume of the pyramidal cell layer on
6 PND 30 was increased in low-dose animals ($p < 0.001$) but decreased in high dose animals ($p <$
7 0.001) as compared with control values; neither group was significantly different from controls
8 on PND 90. There was a dose-related decrease in total neuron number in the cornu ammonis on
9 PND 30 ($p < 0.01$ for both doses); on PND 90 the decrease in neuron number remained
10 statistically significant in both treatment groups ($p < 0.01$), but there was no longer any
11 difference in the magnitude of the effect between doses (Sarsilmaz et al., 2007).

12 In a third study from the same laboratory, Songur et al. (2008) used the same exposure
13 paradigm to evaluate changes in oxidant and antioxidant systems in the cerebellum of perinatally
14 exposed rats. Exposure was carried out as in Aslan et al. (2006) and Sarsilmaz et al. (2007),
15 described above. On PND 30 or 90, cerebellums from seven male rats per group were evaluated
16 for levels of malondialdehyde (MDA), NO, superoxide dismutase activity (SOD), and
17 glutathione peroxidase (GPX) activity. Dose-related increases in NO (approximately 20–80%),
18 MDA (100–160%), and GPX (25–60%) and dose-related decreases in SOD (20–30%) were seen
19 on PND 30. In general, the magnitude of change from control levels was maintained on PND 90,
20 with the exception of MDA levels in 6 ppm animals, which appeared to approach control levels
21 at 90 days. The authors stated that these findings indicated that formaldehyde exposure may
22 cause neurotoxicity via the production of oxidative damage in the brain. Persistence of the effect
23 to the 90-day time point (30 days after cessation of exposure) supports the possibility that
24 formaldehyde may cause long-lasting or permanent changes in the brain following early life
25 exposure. These results are consistent with the earlier studies by Aslan et al. (2006) and
26 Sarsilmaz et al. (2007), finding permanent changes in brain structure (although in a different
27 brain region) following early life exposure.

28 29 **4.2.6.1.7. Summary of formaldehyde effects on neurobehavioral and neuropathological** 30 **measures, following exposure via inhalation.**

31 As has been demonstrated in mice (Wood and Coleman, 1995), it is possible that rats
32 experience respiratory tract irritation during low-level formaldehyde exposure. Perturbations in
33 nervous system function reported with formaldehyde exposure include reductions in motor
34 activity, lack of habituation, impairment in acquisition of a new learning task, deficits in
35 retention of a previously learned task, increases in corticosterone levels, sensitization to cocaine-
36 induced locomotor activity, and enhanced fear conditioning using an olfactory CS (see

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1 Table 4-58). Many of these effects were observed at exposure levels at or below 1 ppm, and
2 some persisted days to weeks after termination of exposure.

3 Malek et al. (2004, 2003a, b) detected behavioral changes in rats and mice tested 2 to
4 24 hours postexposure. The mechanism of these behavioral changes is unknown, and available
5 data do not allow dissociation of direct effects on the nervous system and behavioral responses to
6 the irritant effects of formaldehyde (control experiments [e.g., using a different aversive odor
7 with or without irritant properties] were not included). Given that behavioral changes were
8 observed hours to days after cessation exposure (i.e., beyond the time required for formaldehyde-
9 induced irritation to subside), it is unlikely that these behavioral changes were caused by
10 formaldehyde-induced irritation. Similarly, although it is possible that systemic effects of
11 formaldehyde exposure might cause reduced motor activity during or immediately following
12 exposure, it is unlikely that these effects can account for the differences in responses of male rats
13 24 hours after exposure (Malek et al., 2003a). Furthermore, a follow-up study demonstrated
14 reduced motor activity in animals 2 hours after a 2-hour exposure to much lower levels of
15 formaldehyde (0.1 ppm), which fall well below the levels identified by Wood and Coleman
16 (1995) as the AC₅₀ for formaldehyde in mice (Malek et al., 2003b).

17 Two studies reported significant reductions in learning or retention following brief
18 periods of repeated exposure to low levels of formaldehyde (Malek et al., 2003c; Pitten et al.,
19 2000) (see Table 4-58). Malek et al. (2003c) reported an increased number of errors in acquiring
20 a water maze task; testing took place daily 2 hours after termination of a 2-hour exposure. The
21 work of Pitten et al. (2000) revealed that brief exposures over many weeks led to increases in
22 errors performing a previously learned task and that the magnitude of the effect increased over
23 the course of the exposure period. Testing occurred remote from the time of exposure (22 hours
24 after the previous exposure), and the deficits appeared to persist for several weeks after exposure
25 terminated, minimizing the possibility that these effects were related to irritant properties of
26 formaldehyde. Although the exposure levels were moderately high (2.6–4.6 ppm) and continued
27 over several months, the duration of a single exposure event was very brief (10 minutes).

28 Sorg and Hochstatter (1999) and Sorg et al. (2004, 2001) suggest that behavioral
29 sensitization associated with low-level formaldehyde exposure was linked to alterations in HPA
30 control of corticosterone. Cross-sensitization to the locomotor activity-enhancing properties of
31 cocaine and changes in response to a conditioned fear paradigm were observed in animals
32 exposed several weeks earlier to repeated low-level formaldehyde. Direct activation of
33 mesolimbic dopamine pathways or activation of conditioned fear response in the amygdala by
34 formaldehyde could underlie these behavioral effects; these observations were also seen by study
35 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
<i>Irritant detection threshold</i>					
Male Swiss mice	8	0, 1, 1.8, 3, 5.6, or 10 ppm 60-second exposure episode to determine irritant response	<i>Sensitivity of mice to acute formaldehyde levels</i> 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)
<i>Motor activity and habituation</i>					
Male and female LEW.1K rats	10/sex	0, 1, 2.5, or 5 ppm for 2 hours	<i>Reduced horizontal activity</i> : Number of crossed quadrants reduced 2 hours after exposure at all doses for males and females ($p < 0.005$). <i>Reduced habituation</i> : 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	<i>Reduced horizontal activity</i> : 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	<i>Reduced horizontal activity</i> : 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	<i>Reduced horizontal and vertical activity</i> : 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	<i>Reduced horizontal and vertical activity</i> 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	<i>Reduced activity levels on both days</i> . Decreases seen at 5 and 10 ppm; data reported only for 5 ppm group	LOAEL = 5 ppm, 3 hours	Boja et al. (1985)

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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	<i>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 ($p < 0.05$).</i>	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	<i>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.</i>	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	<i>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 ($p < 0.05$, $p < 0.01$).</i>	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	<i>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 ($p < 0.04$).</i>	LOAEL = 2.6 ppm 10 minutes/ 90 days	Pitten et al. (2002)
Ferrets		0.25 ppm	<i>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.</i>	None established	Apfelbach and Reibenspies (1991) (abstract only)
Male juvenile and adult rats	5/group	0.25, 0.5 ppm	<i>Decreases in olfactory thresholds, in juvenile animals but not in adults ($p < 0.002$).</i>	LOAEL = 250 ppm in juveniles	Weiler and Apfelbach (1992) (abstract only)

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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
<i>Neurosensitization endpoints</i>					
Female Sprague-Dawley rats	8–9	0 or 11 ppm 1 hour/day, 7 days	<i>Increase in cocaine-induced activity:</i> 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	<i>Increase in cocaine-induced activity:</i> 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	<i>Increased conditioned fear response</i> 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. <i>Increase in basal corticosterone:</i> 0.7 ppm for 2 or 4 weeks. <i>Hyperresponsive corticosterone response to environment:</i> 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	<i>Increased conditioned fear response</i> to an olfactory cue in formaldehyde-treated, foot-shock-conditioned male rats. Measured as increased time freezing when presented with a novel environment ($p < 0.05$). <i>No effect in female rats.</i>	LOAEL = 2 ppm/4 weeks NOAEL = 2 ppm/4 weeks	Sorg et al. (2004)

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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
<i>Neurochemistry and neuropathology</i>					
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. <i>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.</i>	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)
<i>Neurogenesis</i>					
Neonatal Wistar rats	5	0, 6, or 12 ppm, 6 hours/day, 5 days/week for 30 days	<i>Changes in volume of granular cell layer of the dentate gyrus in the hippocampus at postnatal days 30 and 90 (p < 0.001)</i>	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	<i>Decreases in brain hemisphere volume at PND 30 (p < 0.01)</i> <i>Changes in volume and cell numbers in the CA region of the hippocampus on PND 30 (p < 0.01)</i>	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	<i>Changes in oxidant and antioxidant systems in cerebellum on PNDs 30 and 90 (p = 0.017–0.001).</i> Increases in MDA, NO, and GSH-Px and decreases in SOD at both time points.	LOAEL = 6 ppm/30 days	Songur et al. (2008)

^aTreatment 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.

^bNA = not available.

1 Limited data regarding possible neurochemical changes in the brains of formaldehyde-
2 exposed, immunized mice (Ahmed et al., 2007; Fujimaki et al., 2004b; Hayashi et al., 2004;
3 Kakeyama et al., 2004) and rats (Boja et al., 1985) provided conflicting information, and the
4 implications of these data regarding possible formaldehyde neurotoxicity are difficult to
5 determine.

6 In developmental exposure paradigms, changes in brain structure (Sarsilmaz et al., 2007;
7 Aslan et al., 2006), brain chemistry (Songur et al., 2008), and motor activity (Senichenkova,
8 1991; Sheveleva, 1971) were seen following neonatal or in utero exposure to formaldehyde. In
9 addition, Weiler and Apfelbach (1992) found juvenile animals to be more sensitive to
10 formaldehyde-induced changes in olfactory thresholds when compared with adult animals.
11 These studies raise concern about possible long-lasting neurological effects of early exposure to
12 formaldehyde. It is important to note, however, that exposure levels in these studies were higher
13 (250–6,000 ppb) than those producing the behavioral effects in adults described above.

14 Overall, available data provide substantial evidence of behavioral effects, including
15 motor activity changes and changes in learning and retention, following repeated exposure to
16 relatively low levels of formaldehyde. These effects were seen in multiple laboratories, in
17 studies conducted by different authors, and using different behavioral paradigms. These
18 conclusions are also supported by more limited data, indicating possible developmental effects
19 on the nervous system, including changes in brain structure and in the behavior of offspring; the
20 developmental findings are less robust since they were seen only in individual laboratories and
21 occurred following exposure to higher concentrations of formaldehyde. Studies evaluating
22 developmental neurotoxicity at lower doses, comparable to those used in the adult studies, were
23 not available. None of the available data provide sufficient information to allow a determination
24 of the mechanism for these behavioral changes, although it is unlikely that they are attributable
25 to the irritant properties of formaldehyde. The data regarding behavioral sensitization provide
26 some support for a stress-related mechanism for those findings, but the applicability of this
27 mechanism to the behavioral changes seen in the other studies, including the learning deficits,
28 has not been evaluated.

30 **4.2.6.2. Oral Exposure**

31 Available data regarding neurotoxic effects of formaldehyde exposure following oral
32 exposure are very limited. Several chronic or subchronic oral toxicity studies evaluated changes
33 in brain weight or histopathology in rats or dogs following repeated oral exposures to
34 formaldehyde at doses as high as 300 mg/kg-day, administered in drinking water (Til et al.,
35 1989, 1988; Tobe et al., 1989; Johannsen et al., 1986). Although data were not presented in the

1 publications, all stated that no changes in brain weight or pathology were seen in the standard
2 evaluations performed in these studies.

3 Two studies evaluated changes in behavior following exposure to formaldehyde in
4 drinking water (Venkatakrishna-Bhatt et al., 1997; Venkatakrishna-Bhatt and Panchal, 1992).
5 Venkatakrishna-Bhatt and Panchal (1992) evaluated changes in performance on a conditioned
6 avoidance task in adult male albino rats (five/group). Animals were exposed to formaldehyde in
7 drinking water (10 mg/mL) or by I.P. injection (10 mg/kg) for 60 days. Although it was stated
8 that water consumption was recorded, the data were not presented, and thus actual exposure
9 levels cannot be documented. Prior to the initiation of exposure, rats were trained on the
10 conditioned avoidance task (climbing a wooden pole in response to a warning buzzer, thus
11 avoiding electric shock from a floor grid). Rats were trained to a predetermined performance
12 criterion (not described); animals not achieving the criterion were removed from study. Training
13 and testing conditions (e.g., retest interval and duration of sessions) were not well described.
14 Data were presented as percent response in behavioral performance (apparently separately for the
15 escape or avoidance aspects of the task) or percentage decrease in response. No control data
16 were presented, and pretreatment performance was not described. Figures presented
17 performance at 10-day intervals, starting with day 0, with each data point stated to represent the
18 mean for five experimental sets; again, the interval between experimental sets and the number of
19 trials per set was not specified. Although the authors concluded that a deficit in performance
20 was demonstrated, the data as presented were difficult to interpret and the conclusion could not
21 be verified based on the data as presented.

22 Venkatakrishna Bhatt and Panchal (1992) examined changes in performance on a
23 conditioned avoidance response, presumably using a procedure similar to the one described
24 above. Albino rats (sex not specified, five/group) were exposed to formaldehyde in drinking
25 water at 0.2 or 0.5 mg/mL for 90 days. As described above, rats were trained in performing the
26 task prior to the start of exposure. Venkatakrishna-Bhatt Bhatr and Panchal (1992) stated that
27 there was a dose-related deterioration of performance, but no data were presented to support
28 these conclusions.

29 In summary, available data are insufficient to conduct a reliable assessment of neurotoxic
30 effects of formaldehyde following oral exposure. Limited data suggest a lack of overt
31 neuropathological changes at doses up to 300 mg/kg-day (Til et al., 1989, 1988; Tobe et al.,
32 1989; Johannsen et al., 1986), but detailed information regarding the types of neuropathological
33 evaluations performed in those studies is not available, and thus no firm conclusions can be
34 drawn regarding the potential for neuropathological effects. The two available studies evaluating

1 behavioral changes are not considered to provide useful information, and thus effects on nervous
2 system function could not be evaluated.

4 **4.2.6.3. Summary**

5 Overall, there is strong evidence that formaldehyde exposure via inhalation may cause
6 adverse effects on nervous system function in experimental animals at relatively low levels of
7 exposure (LOAELs as low as 100 ppb). Although human data regarding neurotoxicity following
8 formaldehyde inhalation are limited, available data provide support that the types of effects seen
9 in humans are similar to those found in animal studies. Evidence from available human
10 controlled inhalation exposure studies indicates that humans may be affected at doses similar to
11 those used in animal studies; however, the human data are extremely limited.

12 There are insufficient data to evaluate the potential for neurotoxicity following oral
13 exposure to formaldehyde. Limited evaluations of brain weight or histopathology in available
14 chronic or subchronic oral studies found no evidence of formaldehyde-induced changes (Til et
15 al., 1989, 1988; Tobe et al., 1989; Johannsen et al., 1986). However, reliable studies examining
16 nervous system function or focused studies of neuropathology following oral exposure to
17 formaldehyde are not available.

19 **4.2.6.4. Other Considerations**

20 Major data gaps were found regarding the evaluation of changes in nervous system
21 structure or function following formaldehyde exposure by both the inhalation or oral routes.

22 With respect to inhalation exposure, none of the available human studies resulted in data
23 sufficient to conduct a reliable dose-response assessment for changes in nervous system function.
24 Most of the available animal inhalation studies used short exposure durations (acute or
25 short-term), precluding a reliable evaluation of neurotoxicity following chronic exposure.
26 Available data for neurodevelopmental exposures are also quite limited, consisting of evaluation
27 of neuropathology in only one brain region and functional evaluations focused only on changes
28 in motor activity.

29 Major data gaps also exist regarding neurotoxicity following oral exposure, with no
30 relevant human data and extremely limited animal data. Available oral exposure studies were
31 insufficient to permit a reliable evaluation of the potential for neurotoxicity following oral
32 exposure to formaldehyde.

1 4.2.7. Reproductive and Developmental Toxicity

2 The potential for developmental and reproductive effects after formaldehyde exposure by
3 the inhalation route has generally been considered low, since formaldehyde, as a reactive gas, is
4 not expected to penetrate past the POE (NEG, 2003; IPCS, 2002; Collins et al., 2001).
5 Nevertheless, a number of animal studies have demonstrated effects of formaldehyde on pre- and
6 postnatal development and on the reproductive system. For example, developmental toxicity
7 was observed in two studies that evaluated a standard battery of developmental endpoints
8 resulting from inhalation exposure on GDs 6–10 (Martin, 1990; Saillenfait et al., 1989).
9 Similarly, oral exposures resulted in developmental effects when administered during
10 comparable gestational windows (Marks et al., 1980; Hurni and Ohder, 1973). There have also
11 been reports that identified developmental effects at lower-level formaldehyde exposures that
12 were administered throughout gestation (Senichenkova and Chebotar, 1996; Senichenkova,
13 1991; Kitaev et al., 1984; Sheveleva, 1971; Gofmekler and Bonashevskaya, 1969; Gofmekler,
14 1968; Pushkina et al., 1968). Postnatal functional consequences of developmental exposures
15 have also been identified (Sarsilmaz et al., 2007; Aslan et al., 2006; Weiler and Apfelbach, 1992;
16 Senichenkova, 1991; Sheveleva, 1971). Additionally, a number of studies suggest that
17 formaldehyde adversely affects the male reproductive system after both inhalation and oral
18 exposures (Xing et al., 2007; Zhou et al., 2006; Özen et al., 2005, 2002; Sarsilmaz et al., 1999;
19 Chowdhury et al., 1992; Cassidy et al., 1983; Guseva, 1972). This section reviews the available
20 published studies assessing reproductive and developmental endpoints of formaldehyde.

21 22 **4.2.7.1. *Inhalation Studies Addressing Developmental and Reproductive Toxicity***

23 Saillenfait et al. (1989) reported a comprehensive and well-documented developmental
24 study in Sprague-Dawley rats. Pregnant rats were exposed beginning on GD 6 in order to cover
25 critical stages of development (e.g., implantation and major organogenesis). Female Sprague-
26 Dawley rats (25/group) were exposed to 0, 5, 10, 20, or 40 ppm (0, 6.15, 12.3, 24.6, or
27 49.2 mg/m³) formaldehyde 6 hours/day on GDs 6–20. The onset of pregnancy was determined
28 by the presence of sperm in a vaginal smear. Dams were exposed to formaldehyde in a dynamic
29 flow chamber, and formaldehyde concentrations were determined to be 0, 5.17 ± 0.51, 9.92 ±
30 0.88, 20.04 ± 0.88, and 38.96 ± 3.70 ppm. Dams were weighed on GDs 0, 6, and 21 and
31 sacrificed on day 21. Upon examination, uterine weights, fetal weights, sex ratio, number of
32 implantation and resorption sites, and live and dead fetuses were recorded. Fetuses were
33 examined for external malformations and cleft palate. One-half of viable fetuses were sectioned
34 to assess soft-tissue alterations. The other half were fixed, stained with alizarin red S, and
35 examined for skeletal alterations.

1 Body weight gain of dams and body weight of male and female fetuses were reduced by
2 exposure to 40 ppm formaldehyde to 49, 78, and 81% of control values, respectively ($p < 0.01$)
3 (Saillenfait et al., 1989). Reduced weight gain in dams remained significantly decreased when
4 uterine weight was accounted for ($p < 0.01$). Mean fetal weight of male pups was reduced at
5 maternal exposures of 20 and 40 ppm formaldehyde (5.53 and 4.42 g versus 5.61 g in controls).
6 Decreased fetal body weight in females was only seen at 40 ppm (4.27 g versus 5.24 g in
7 controls). All other pregnancy endpoints were unchanged by formaldehyde exposure (e.g.,
8 uterine weight, implantation and resorption sites, live fetuses, dead fetuses, and sex ratios). No
9 major malformations were noted in fetuses. Some minor soft tissue and skeletal anomalies, such
10 as dilated ureter, missing sternbrae, extra fourteenth rib, and rudimentary thirteenth rib
11 (statistics not given), were reported. However, these effects occurred at similar frequencies in
12 control and treatment groups. The incidence of delayed ossification of the thoracic vertebrae
13 was 8.7% in fetuses from the 40 ppm exposure group versus 1.8% in controls. However, this
14 difference was not statistically significant. Overall, from these results formaldehyde was neither
15 lethal to embryos nor teratogenic, only exhibiting fetotoxic effects at exposures of 20 ppm and
16 above. These are levels where there was a significant decrease in fetal body weight.

17 Martin (1990) conducted a similar study exposing pregnant rats during similar stages of
18 development. Mated female Sprague-Dawley rats (25/group) were exposed to 2, 5, or 10 ppm
19 ($2.46, 6.15, \text{ or } 12.3 \text{ mg/m}^3$) formaldehyde 6 hours/day on GDs 6–15. The study included two
20 control groups: dams placed in the exposure chambers once a day but exposed only to clean air
21 and dams fed and housed similarly to the experimental groups but never put into the inhalation
22 chambers. The method of formaldehyde vapor generation and details of the exposure chamber
23 were not described. Mean formaldehyde exposure concentrations were reported as 1.88, 4.88,
24 and 9.45 ppm (variability not given, analytical method not discussed). Food consumption and
25 body weight were recorded. On GD 20, rats were sacrificed, and the following pregnancy
26 parameters were recorded: live fetuses, dead fetuses and resorptions, number of corpora lutea,
27 fetal weights, sex ratios, and preimplantation and postimplantation losses. Fetuses were
28 examined for major malformations, minor external and visceral anomalies, and minor skeletal
29 anomalies (details not given). Weight gain and food consumption in dams were said to be
30 reduced at 10 ppm ($p < 0.05$). Formaldehyde exposure of the dams at 5 and 10 ppm led to an
31 increased incidence of reduced ossification of the pubic and ischial bones in fetuses on GD 20
32 ($p < 0.05$). Reduced ossification correlated with lower fetal weights, and the author considered
33 both of these findings a result of larger litter size and, therefore, not related to formaldehyde
34 exposure. However, no tables presenting the data or statistical analysis were provided. All other
35 pregnancy parameters and fetal anomalies were described as unaffected by formaldehyde

1 exposure. However, without data presented for the assessed endpoints, background rates of
2 malformations, trends in the data, and variability, it is difficult to evaluate the Martin (1990)
3 comparisons. However, the author's observations of reduced fetal weight and increased
4 incidence of reduced ossification are consistent with the results of Saillenfait et al. (1989).

5 Kilburn and Moro (1985) studied similar endpoints but included formaldehyde exposure
6 during earlier gestational windows. The study report, only available in abstract form and not
7 found as a subsequent published article, does not provide many methodological details. Female
8 rats (number and strain not reported) were exposed to 0 or 30 ppm (0 or 37.2 mg/m³)
9 formaldehyde 8 hours/day during GDs 3–17, 3–12, 8–12, or 9–11. A second experiment
10 included pair-fed controls for dams exposed to 30 ppm formaldehyde during GDs 3–17, 3–12,
11 and 8–12. The authors reported reductions in fetal and maternal weight gain that were greater
12 than decreases in pair-fed controls. Fetal anomalies were noted after 15 days of gestational
13 exposure (e.g., altered organ size and undescended testes). Although the report indicates some
14 maternal toxicity and fetotoxic effects (for example, stunted growth), lack of study details and
15 clear reporting make this report of negligible utility in human health risk assessment.

16 There are several early studies that examined developmental effects of formaldehyde
17 exposure administered throughout gestation (Gofmekler and Bonashevskaya, 1969; Gofmekler,
18 1968; Pushkina et al., 1968). It is unclear if these reports represent the same or overlapping
19 experimental groups. They were performed in the same laboratory and are reported with a
20 similar level of detail. The source of formaldehyde, method of vapor generation, exposure
21 conditions (dynamic versus static), confirmation of exposure concentrations, study design, and
22 data presentation details were not provided. Absence of such critical information detracts from
23 the quality of these studies as a coherent record of experimental information, and, thus, these
24 findings can only be utilized qualitatively in the formaldehyde risk assessment.

25 In the Gofmekler (1968) study, female rats (36, strain not specified) were continuously
26 exposed at 0, low, or high formaldehyde concentrations beginning 10–15 days prior to mating
27 (target concentrations of 0, 0.01, or 0.81 ppm formaldehyde [0, 0.01, or 1 mg/m³]). The author
28 reported a 14–15% increase in pregnancy duration and a decrease in litter size (data not shown).
29 However, males and females were mated 6–10 days, and no information was provided on how
30 mating and conception were confirmed. No external malformations were attributed to
31 formaldehyde exposure. Concentration-dependent increases in pup body weight and decreases in
32 lung and liver weight were attributed to formaldehyde exposure. Pup weights were increased
33 from 5.6 g in controls to 6.0 and 6.3 g in groups 1 and 2, respectively ($p < 0.01$ and $p < 0.001$).
34 Formaldehyde exposure increased pup adrenal weight in both groups and pup thymus and kidney
35 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

^aDams were exposed to formaldehyde continuously from 10–15 days prior to mating. Exposure concentrations were not validated.

^bDifferent from controls, $p < 0.01$.

^cDifferent from controls, $p < 0.001$.

^dDifferent 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

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1 prior to mating (6–10 days) and then throughout gestation. Dams were sacrificed and ascorbic
2 acid and nucleic acid levels determined in harvested organs (methods referenced but not
3 described). No visible malformations in pups were noted. Formaldehyde exposure increased
4 fetal body weight and organ weight in both groups (data not given). There was an average of
5 11.3 fetuses per litter for control dams versus 9.8 and 8.6 for groups 1 and 2, respectively. The
6 authors reported that formaldehyde exposure decreased DNA levels and increased RNA levels in
7 organs (further details not provided). Formaldehyde exposure resulted in lower vitamin C levels
8 in the whole fetus (76 and 75% of controls) and in maternal liver specimens (82 and 88% of
9 controls) for exposure groups 1 and 2, respectively ($p < 0.05$). In contrast, vitamin C was higher
10 in fetal liver (127% of controls) in group 1 ($p < 0.05$). The significance of these differences is
11 unknown. The authors considered the results as general measures of biochemical changes and
12 therefore toxic.

13 The reports of Gofmekler and Bonashevskaya (1969), Gofmekler (1968), and Pushkina et
14 al. (1968) lack key methodological details. As discussed above, exposure conditions and actual
15 formaldehyde concentrations cannot be validated. Although methods were not thoroughly
16 detailed, results were reported in data tables with statistics and detailed descriptions of observed
17 pathological changes. However, without validation of exposure concentrations, these findings
18 can only be considered qualitatively.

19 In another study, Sheveleva (1971) exposed female mongrel (i.e., not a homogeneous
20 genetic strain) white rats to 0, 0.0005, or 0.005 mg/L (0, 0.5, or 5 mg/m³) (0, 0.4, or 4 ppm)
21 formaldehyde on GDs 1–19 (where GD 1 was defined as the day that spermatozoa were detected
22 in vaginal smears) for 4 hours/day. In each group, 15 dams were terminated on GD 20 for
23 evaluation of ovarian corpora lutea, uterine implantation sites, pre- and postimplantation loss,
24 number of live fetuses, fetal length and weight, and external examination for malformations.
25 Additionally, in each group, six dams were allowed to deliver their litters. Developmental
26 landmarks were monitored (i.e., ear and eye opening, incisor eruption, emergence of hair coat),
27 and the pups were further evaluated at 1 and 2 months of age for body weight, threshold of
28 neuromuscular excitability, total oxygen consumption in 1 hour per 100 g of weight, and
29 spontaneous mobility over 10 minutes. Maternal toxicity (recorded on GD 17) included
30 significantly ($p < 0.05$) decreased leukocyte counts in both treated groups and a number of
31 additional findings at 0.005 mg/L (i.e., significant reductions in the threshold of neuromuscular
32 excitability, rectal temperature, and blood hemoglobin level) as well as an increase in
33 spontaneous mobility over 15 minutes. Fetal examinations on GD 20 identified a 50–70%
34 increase in mean preimplantation loss in both formaldehyde-exposed groups. When pups were 1
35 month of age, a reduction in spontaneous mobility was noted in both treated groups; in pups at 2

1 months of age, an increase in mobility was observed in the 0.005 mg/L group. Also, when pups
2 were 2 months of age, there were alterations in hemoglobin levels and leukocyte counts in both
3 treated groups. Detailed descriptions of some study methodologies (particularly in regard to
4 neurological and behavioral assessments) were not provided in the published paper.

5 Based on a review of the work by Gofmekler (1968) and various epidemiologic studies
6 available at the time, Kitaev et al. (1984) hypothesized that formaldehyde may exert toxic effects
7 in the early days of gestation. To study embryotoxic effects of formaldehyde inhalation
8 exposures, mature female Wistar rats (five to nine per group) were exposed to 0.41 or 1.22 ppm
9 (0.5 or 1.5 mg/m³) formaldehyde 4 hours/day, 5 days/week for 4 months (Kitaev et al., 1984).
10 Rats were exposed in dynamic flow chambers and formaldehyde levels measured gravimetrically
11 (but not reported). Females were mated on day 120 of exposure and mating confirmed by the
12 presence of sperm in a vaginal smear. Embryos were harvested on the second or third day of
13 pregnancy (GD 2 or 3) and examined by both light and phase contrast microscopy for changes in
14 morphology (i.e., evidence of embryonic degeneration). Additionally, maternal weight gain and
15 organ weights (ovaries, uterus, and adrenal glands) and blood samples (HCT, Hb, and TP) were
16 monitored as indicators of general toxicity. These parameters were unchanged by formaldehyde
17 exposure. Formaldehyde exposure at 1.22 ppm for 4 months resulted in an increased number of
18 degenerating embryos on GD 3 (14.9 versus 4.4% in controls) and a smaller increase of 10.2%
19 (versus 5.1% in controls; statistical significance not assessed) on GD 2. Indications of
20 degeneration included reduced size and changes in appearance (granulation of the ooplasm,
21 wrinkling and degradation of nuclear material). However, it is unclear if litter effects were
22 accounted for in the statistical analyses, and it is unknown how the affected embryos were
23 distributed between litters. For dams exposed to 0.41 ppm formaldehyde, the number of
24 degenerated embryos was not increased on day 2 (3.8 versus 5.1% in controls) but was increased
25 on day 3 (9.1 versus 4.4% in controls; again, unknown if statistically significant) after maternal
26 exposure to 1.22 ppm formaldehyde. This observation may be coincidental since it was seen in
27 dams sacrificed on GD 2 but not in those sacrificed on GD 3. Kitaev et al. (1984) considered
28 these findings to indicate that repeated exposure to formaldehyde over a 4-month period can
29 disturb reproductive function, resulting in adverse effects early in embryonic development.

30 To further explore the effects of inhalation exposures to formaldehyde on reproductive
31 function, Kitaev et al. (1984) conducted a second series of experiments on 200 similarly treated
32 female rats. After 4 months of repeated formaldehyde exposure at 0.41 or 1.22 ppm as described
33 above, organ weights (ovaries and uterus) and blood levels of gonadotropic hormones and
34 progesterone were determined. However, the day and time of hormone measurement were not
35 given in the report, and normal diurnal variations in these hormones could affect the reported

1 findings if time of day was not accounted for. The length of the estrous cycle was unchanged
2 during exposure. Formaldehyde exposure modulated gonadotropin levels and relative ovarian
3 weight, suggesting low-level effects on the female rat reproductive system prior to mating
4 (Kitaev et al., 1984). Ovarian weight and blood levels of luteinizing hormone (LH) were both
5 significantly increased after exposures at 0.41 ppm formaldehyde but remained at control levels
6 in rats exposed at 1.22 ppm. Blood levels of follicle-stimulating hormone (FSH) were increased
7 approximately 66% from control after 1.22 ppm formaldehyde exposure ($p < 0.05$).
8 Progesterone levels were unchanged by formaldehyde treatment. Kitaev et al. (1984) suggested
9 a role of the hypothalamus-pituitary system based on increased ovary weight, a greater number
10 of degenerated embryos, and increased LH in rats exposed at 0.41 ppm. They postulated that
11 these effects were not seen at 1.22 ppm due to a toxic effect exhibited as embryonic
12 degeneration, thus the absence of a dose-response did not alter the interpretation of the validity
13 of the adverse response. The study NOAEL was not determined, and the study LOAEL was 0.4
14 ppm (0.5 mg/m^3), based upon increased early embryo loss and on maternal outcomes (increased
15 ovarian weight and increased blood LH levels) following 4 months of formaldehyde treatment.
16 For the finding of increase blood FSH levels, the endpoint NOAEL was 0.4 ppm (0.5 mg/m^3)
17 and the LOAEL was 1.2 ppm (1.5 mg/m^3).

18 Senichenkova and Chebotar (1996) and Senichenkova (1991) examined reproductive and
19 developmental effects of daily formaldehyde exposure on GDs 1–19 of pregnancy, including the
20 potential effect of formaldehyde exposure on development early in gestation. Additionally, since
21 anemia adversely affects fetal development, Senichenkova and Chebotar (1996) also examined
22 formaldehyde effects in iron-deficient dams to determine whether coexposure further
23 compromises fetal development. In both studies, female white rats were exposed to 0 or
24 0.41 ppm formaldehyde (0 or 0.5 mg/m^3), 4 hours/day on GDs 1–19. Formaldehyde
25 concentrations in the dynamic exposure chambers were measured gravimetrically to confirm the
26 exposure concentration but were not reported (methods not provided). It is unclear if gravimetric
27 measurements would be sensitive or accurate enough to validate these low-exposure
28 concentrations without a better understanding of the methodology. This uncertainty in exposure
29 conditions should be considered in evaluating the reported results.

30 Mongrel female white rats were exposed at a target concentration of 0 or 0.41 ppm (0 or
31 0.5 mg/m^3) formaldehyde 4 hours/day on GDs 1–19 (Senichenkova, 1991). On GD 20, a subset
32 of the dams was sacrificed and examined for number of corpora lutea, implantation and
33 resorption sites, live/dead fetuses, and fetal weights. Fetuses were examined for gross pathology
34 of the internal organs and skeleton (details not given). Blood pH, partial pressure of CO_2 , and
35 partial pressure of oxygen were measured in both dams and embryos. The remaining dams were

1 brought to term to study postnatal effects of formaldehyde exposure. Rat pups were observed on
2 PNDs 1–25 for viability, physical development, and maturation rate of motor reflexes. Behavior
3 of juvenile offspring (PND 40) was studied in an open field test, and maze learning was tested at
4 sexual maturity. In a follow-up report, Senichenkova and Chebotar (1996) present blood
5 chemistry data, pregnancy outcome, and developmental data for similarly treated dams and their
6 pups in a chemical model of iron deficiency. Intraperitoneal injections of the iron-chelating
7 agent bipyridyl were given on GDs 12–15 at the threshold embryotoxic dose (1 mL, 25%
8 solution). On day 20, the dams were sacrificed and dams and fetuses examined as described
9 above. In addition to blood pH, partial pressure of carbon dioxide and partial pressure of
10 oxygen, acid metabolic products (not detailed), and true bicarbonates were reported for maternal
11 and fetal blood. A review of the data from these reports indicates there may be an overlap of the
12 study groups. Neither paper presents the entire data set; thus, for transparency and brevity, the
13 following text discusses the combined findings from both studies as if they were a single study

14 Formaldehyde exposure did not affect such indicators of pregnancy outcome as number
15 of corpora lutea, implantation and resorption sites, and live and dead fetuses, all of which were
16 unchanged (Senichenkova and Chebotar, 1996; Senichenkova, 1991). Although fetal weight was
17 slightly increased by formaldehyde exposure, 2.35 versus 2.24 g in controls ($p < 0.001$), neither
18 fetal length nor bone length were changed (femur and humerus) (Senichenkova and Chebotar,
19 1996; Senichenkova, 1991). Often, increased fetal weight is the result of early physical
20 development, and other signs of development, such as ossification, would be expected to be
21 enhanced as well. The average number of bone centers per limb was increased by formaldehyde
22 exposure from 2.45 and 2.66 to 2.78 and 2.91 in controls for metacarpal and metatarsal bone
23 centers, respectively ($p < 0.05$) (Senichenkova, 1991); these findings were consistent with
24 increased growth and weight. In contrast, Senichenkova (1991) reported a decrease in the
25 number of embryos with ossification centers in the hyoid bone (100% in controls versus 91% for
26 formaldehyde exposure, $p < 0.05$), consistent with the results of Saillenfait et al. (1989) and
27 Martin (1990). However, litter size, a factor influencing fetal weight, was not provided, and it is
28 unclear if Senichenkova (1991) took litter size into account in the analysis.

29 Senichenkova and Chebotar (1996) reported increased blood acidosis and decreased
30 blood alkaline reserves (bicarbonates and total CO₂) in formaldehyde-treated dams and their
31 embryos ($p < 0.05$). However, this finding should be considered in light of the fact that chronic
32 blood acidosis may increase bone remodeling and decrease bone density in adults. It is unknown
33 if the reported blood acidosis could reduce ossification rates in developing embryos. A better
34 understanding of exposure conditions and the acid metabolic products measured is needed to
35 determine the biological relevance of the reported changes in blood acid balance.

1 Iron deficiency, induced by injections of bipyridyl (an iron-chelating agent), was found to
2 be fetotoxic. Iron-deficient dams with no formaldehyde exposure had higher rates of
3 postimplantation death than controls (12.6 ± 5.5 versus $4.8 \pm 1.3\%$). Formaldehyde exposure in
4 conjunction with iron deficiency increased postimplantation death to $23.1 \pm 5.9\%$. Fetal weight
5 and litter size were not reported for the bipyridyl treatment groups, but bipyridyl treatment in
6 conjunction with formaldehyde resulted in a decreased number of metatarsal bone centers (2.21
7 ± 0.12 versus 2.72 ± 0.08 in controls; $p < 0.001$). This decrease was also significant when
8 compared with formaldehyde or bipyridyl alone ($p < 0.02$). However, all pregnancy outcome
9 parameters were not reported for the bipyridyl treatment.

10 Fetal anomalies were reported after formaldehyde exposure and were increased by iron
11 deficiency. The incidence of litters with internal organ anomalies was increased from 1.4% in
12 controls to 14.2% in formaldehyde-treated dams (Senichenkova, 1991). Undescended testes
13 were the predominant anomaly described: 20.8% in litters from formaldehyde-treated dams
14 versus 1.2% in controls ($p < 0.05$) (Senichenkova, 1991). Similar findings were reported by
15 Senichenkova and Chebotar (1996). Bipyridyl treatment in conjunction with formaldehyde
16 exposure increased the overall incidence of fetal anomalies ($13.8 \pm 2.1\%$ in controls versus $6.6 \pm$
17 1.8% with iron deficiency alone) (Senichenkova and Chebotar, 1996). However, there are
18 discrepancies between the two papers in the reporting of the anomalies, and it is unclear whether
19 the experimental groups overlap between papers, where some parameters are identical (which
20 would lead to double counting of the same animal, including identical SDs) and others are
21 different. Additionally, the reporting is unclear with respect to the basis of the incidence rates
22 reported (for example, overall incidence versus incidence within litter or incidence of litters with
23 anomalies). Unclear reporting, together with some of the uncertainties regarding exposure
24 conditions, suggests that the data may be of limited quality to support risk assessment.

25 In the second phase of the studies, pups were delivered and postnatal development was
26 assessed (Senichenkova, 1991). Eruption of the upper and lower incisors was delayed in pups
27 from formaldehyde-treated dams, occurring on PND 14 versus PND 12 in controls ($p < 0.01$).
28 All other measures of physical postnatal development were unchanged by formaldehyde. To
29 evaluate postnatal functional outcomes following in utero exposure to formaldehyde, an open
30 field test was conducted in juvenile rats on 3 consecutive days (PNDs 40–42). Juvenile rats from
31 formaldehyde-treated dams exhibited increased mobility (crossed squares), rearings, and
32 defecations/urinations compared with control rats on the second and third open field tests
33 ($p < 0.05$). There were no differences seen in the maze-learning test assessed in mature offspring
34 of formaldehyde-treated dams (Senichenkova, 1991).

1 Additional assessments of formaldehyde exposure on neurological development are
2 described above in the section on neurological and behavioral toxicity in animal studies (see
3 Section 4.2.6). In brief, studies conducted by Sarsilmaz et al. (2007) and Aslan et al. (2006)
4 exposed 10 neonatal male Wistar rats/group to 0, 6, or 12 ppm (0, 7.36, or 14.7 mg/m³)
5 formaldehyde 6 hours/day, 5 days/week for 30 days. At that time, five rats/group were killed
6 and subjected to neuropathological examination; the remaining rats were maintained until
7 PND 90, at which time they were killed and evaluated. Aslan et al. (2006) examined the number
8 and volume of granular cells in the hippocampal dentate gyrus, while Sarsilmaz et al. (2007)
9 examined the size and number of the pyramidal cells in the cornu ammonis of the hippocampus.
10 In both studies, lower numbers of cells were observed in both treated groups at PND 90 as
11 compared with PND 30. Although the effects of treatment on the volume and number of
12 granular and pyramidal cells were somewhat inconsistent, a significant decrease in the number of
13 neurons in the pyramidal cell layer of the hippocampal cornu ammonis was observed at both
14 PNDs 30 and 90 (Sarsilmaz et al., 2007).

15 One other study reported effects on nervous system function following exposure to
16 formaldehyde during postnatal development. An abstract by Weiler and Apfelbach (1992)
17 described a study in which juvenile rats (strain not specified) were exposed to 0.25 ppm
18 (0.31 mg/m³) formaldehyde from PNDs 30–160 or adult rats were exposed to 0.5 ppm
19 (0.62 mg/m³) formaldehyde for 90 days. Decreased olfactory sensitivity (i.e., increased olfactory
20 thresholds) was observed and was greater when the exposure was initiated in the young rats, as
21 compared with the adults.

22 Evaluation of offspring following prenatal, perinatal, and/or juvenile inhalation exposures
23 to formaldehyde have also been reported by Kum et al. (2007), Sandikci et al. (2007), and
24 Songur et al. (2005). Kum et al. (2007) exposed female Sprague-Dawley rats (six dams/group)
25 and their offspring to 0 or 6 ppm (0 or 7.4 mg/m³) formaldehyde for 8 hours/day in separate
26 groups with exposures starting on GD 1, on postparturition day 1, or in offspring at 4 weeks of
27 age and continuing for 6 weeks. In another group, exposures were initiated in adult rats. Mean
28 body and liver weights were significantly decreased in the offspring exposed in utero and in
29 early postnatal life, and mean liver weights were also significantly decreased in rats with juvenile
30 exposures. However, neither body weight nor liver weight was affected in the group with
31 exposure initiating at an adult age, suggesting a life-stage-related susceptibility to formaldehyde-
32 induced hepatic toxicity. Evaluation of biomarkers of oxidative stress revealed significantly
33 increased catalase (CAT) and MDA in the livers of offspring that were exposed prenatally,
34 significantly decreased GSH levels in the livers of offspring that were exposed in early postnatal
35 life, and significantly decreased SOD levels in the livers of offspring that were exposed starting

1 at 4 weeks of age. No biomarkers were altered in the livers of rats exposed to formaldehyde only
2 as adults.

3 A similar study design was used by Sandikci et al. (2007) to examine the effects of 0 or
4 6 ppm (0 or 7.4 mg/m³) formaldehyde on bronchus associated lymphoid tissue (BALT)
5 following pre- and perinatal, juvenile, or adult exposures of 6 weeks duration in Sprague-Dawley
6 rats (six/group). The presence of the lysosomal enzyme alpha-naphthylacetate esterase (ANAE)
7 served as a marker of T-lymphocytes in peripheral blood and tissue sections. Significant
8 increases in ANAE-positive T-lymphocytes were found in BALT in all but the in utero exposed
9 groups as compared with control; this outcome is consistent with the postnatal development of
10 BALT in the rat. In peripheral blood, ANAE-positive lymphocyte ratios were significantly ($p <$
11 0.001) increased as compared with controls at all life stages tested.

12 Songur et al. (2005) examined the effect (and reversibility) of formaldehyde exposures
13 during the early postnatal period on zinc, copper, and iron levels and activity of SOD in the lung
14 tissue of Wistar rats. Litters (12–14/group) were exposed to 0, 6, or 12 ppm (0, 7.4, or
15 14.9 mg/m³) formaldehyde 6 hours/day, 5 days/week for 30 days. Trace element and
16 biochemical analyses were conducted on PND 30 or 90. Decreased SOD activity, decreased
17 levels of copper and iron levels, and increased zinc levels were observed in the lungs of treated
18 groups following 30 days of treatment and at 90 days (i.e., 60 days post-treatment). Survival was
19 not affected in neonatal rats. Clinical observations during treatment included evidence of
20 respiratory irritation and toxicity. Body weight and food and water consumption were also
21 nonsignificantly decreased as compared with controls.

22 There are several reports in the literature regarding formaldehyde effects after inhalation
23 exposure on the male reproductive system in animals (Galalipour et al., 2007; Zhou et al., 2006;
24 Özen et al., 2005, 2002; Sarsilmaz et al., 1999; Woutersen et al., 1987; Maronpot et al., 1986;
25 Guseva, 1972). The earliest report examined the effect of simultaneous exposures to
26 formaldehyde from air and water (Guseva, 1972). Male rats ($n = 12$, strain not specified) were
27 coexposed to formaldehyde in air and drinking water 4 hours/day, 5 days/week for 6 months.
28 There were three exposure levels in the experiment of different air and drinking water
29 concentrations: (1) 0.41 ppm (0.5 mg/m³) formaldehyde in air and 0.1 mg/L in water; (2) 0.20
30 ppm (0.25 mg/m³) formaldehyde in air and 0.01 mg/L in water; or (3) 0.10 ppm (0.12 mg/m³)
31 formaldehyde in air and 0.005 mg/L in water. Reproductive function was assessed by mating
32 two females per male. The time for the onset of pregnancy and the number of pregnancies per
33 treatment group were recorded. A subset of dams was sacrificed on GD 20 of pregnancy, and
34 the number and weight of fetuses was determined. Postnatal development of the remaining dams
35 was tracked (e.g., times of eye opening and development of hair coat). Nucleic acid levels were

1 determined in the testes of formaldehyde-exposed rats. Gonadotropic response was assessed by
2 injecting an emulsion of pituitaries from exposed male rats into unexposed infantile females and
3 measuring the weight ratios of the uterus and ovaries. Formaldehyde exposure reduced nucleic
4 acid levels in testes to 88 and 92% of controls in groups 1 and 2, respectively. No other
5 formaldehyde-induced differences were found.

6 Woutersen et al. (1987) and Maronpot et al. (1986) examined tissue sections from testes
7 and ovaries of exposed animals as part of studies primarily addressing respiratory tract toxicity
8 (see Section 4.2.1.2.2.4 for complete study details). Maronpot et al. (1986) exposed female and
9 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/m³)
10 formaldehyde 6 hours/day, 5 days/week for 13 weeks. Decreased weight gain due to
11 formaldehyde exposure was seen in both male and female mice. Additionally, there was 80%
12 mortality at the highest exposure (40 ppm). The authors reported endometrial hypoplasia and
13 lack of ovarian luteal tissue in females exposed to 40 ppm, but no compound-related changes
14 were observed in testes sectioned and viewed by light microscopy.

15 In a study by Appleman et al. (1988), male Wistar rats (40/group) with undamaged or
16 damaged (via bilateral intranasal electrocoagulation) nasal mucosa were exposed for 13 or
17 52 weeks to 0, 0.1, 1, or 10 ppm (0, 0.124, 1.24, or 12.4 mg/m³) formaldehyde 6 hours/day,
18 5 days/week. At study termination, mean body weight was decreased, but relative testes weight
19 was increased in the 10 ppm group (interpreted as a nonadverse outcome that was associated
20 with the decreased body weight). No treatment-related histopathologic findings were reported
21 for male reproductive organs (although it is not clear to what extent they were evaluated since
22 the primary focus of the study was on the nasal epithelium).

23 Following up on earlier reports of decreased Leydig cell quality in rats administered I.P.
24 injections of formaldehyde (Chowdhury et al. [1992], described in Section 5.2.1.8.3), Sarsilmaz
25 et al. (1999) studied the effects of formaldehyde inhalation on Leydig cells. Adult male Wistar
26 rats (30) were exposed to 0, 10, or 20 ppm (0, 12.3, or 24.6 mg/m³) formaldehyde 8 hours/day,
27 5 days/week for 4 weeks. Animals were observed daily and weighed weekly. Rats were
28 sacrificed on day 29 and autopsied, and testes were weighed, fixed, and sectioned for histologic
29 examination. Signs of irritation from formaldehyde exposure were noted (frequent eye blinking,
30 excessive licking, increased frequency of nose cleaning, interrupted breathing, and sneezing).
31 Body weight gain was reduced by formaldehyde exposure from 17.7% gain in control rats to
32 4.66 and 2.63% in rats exposed at 10 and 20 ppm, respectively ($p < 0.001$). As shown in
33 Table 4-60, relative testes weights were unaffected (reported as proportions but more likely to be
34 percentages), although trends and numerical differences were similar to those reported by Özen
35 et al. (2002). Sarsilmaz et al. (1999) found that both Leydig cell quantity and the percentage of

1 cells with normal nuclei were reduced by formaldehyde treatment. Although the dose-dependent
 2 reduction in Leydig cell quantity was statistically significant at both exposure levels, the study
 3 authors considered the data to be within the normal range.

4
 5 **Table 4-60. Formaldehyde effects on Leydig cell quantity and nuclear**
 6 **damage in adult male Wistar rats**
 7

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

8
 9 ^aRats were exposed 8 hours/day, 5 days/week for 4 weeks.

10 ^bStated to represent the ratio of the last day's testicle weight to the body weight but more likely to be the percent
 11 of body weight.

12 ^cCells within 100 defined areas.

13 ^dn = 10.

14 ^eFor each exposure group, 100 defined locations were assessed.

15 ^fn = 100.

16 ^gValues presented as percentage of cells.

17 ^hDifferent from control ($p < 0.05$), as reported by the authors.

18 ⁱDifferent from control ($p < 0.01$), as reported by the authors.

19
 20 Source: Sarsilmaz et al. (1999).

21
 22
 23 It was hypothesized that decreased Leydig cell quality may have been the result of
 24 oxidative stress induced by formaldehyde exposure. Özen et al. (2002), in the same laboratory,
 25 investigated changes in testicular iron, copper, and zinc levels as measures of oxidative stress
 26 and damage. Adult male albino Wistar rats (seven/group) were exposed at 0, 10, or 20 ppm (0,
 27 12.2, or 24.4 mg/m³) formaldehyde 8 hours/day, 5 days/week for either 4 or 13 weeks. Rats
 28 were observed daily and weighed once a week. Rats were sacrificed at the end of the exposure
 29 period and autopsied, and the testes were removed and weighed. Zinc, copper, and iron levels
 30 were determined in testes tissue by using atomic absorption spectrophotometry. Both weight
 31 gain and relative testes weight were decreased in a concentration-dependent and duration-
 32 dependent manner (see Table 4-61). Both zinc and copper levels in rat testes were reduced in a
 33 concentration- and duration-dependent manner by formaldehyde exposure. For example, zinc
 34 was reduced from 277 to 107 mg/kg after a 4-week × 20 ppm exposure and from 260 to
 35 95 mg/kg after a 12-week × 20 ppm exposure ($p < 0.001$) (see Table 4-61). Iron levels in testes
 36 were increased from 30 to 39 mg/kg after a 4-week 20 ppm exposure ($p < 0.01$) and from 33 to

1 58 mg/kg after 12 weeks at 20 ppm ($p < 0.05$). The authors suggested that alterations in trace
 2 element levels in the testes were consistent with oxidative damage and may have contributed to
 3 changes in Leydig cell function. These researchers also reported alterations in trace metals in
 4 lung tissue from Wistar rats exposed to formaldehyde 8 hours/day, 5 days/week for 4 or
 5 13 weeks. Iron levels were increased at 5 ppm for 13 weeks and 10 ppm for either 4 or
 6 13 weeks. Zinc levels decreased for all formaldehyde exposures. In both cases, the authors
 7 attributed elevated iron levels to oxidative stress. In addition to citing the role of zinc as a
 8 cofactor of cytoplasmic Cu-Zn-SOD, the authors suggested that zinc may have been consumed
 9 by increased FALDH activity. Although oxidative stress and increased FALDH activity may be
 10 relevant to the POE and therefore impact the lung, it is less clear how these changes would occur
 11 in the testes. Taken together, the reports of Özen et al. (2002) and Sarsilmaz et al. (1999)
 12 suggested a LOAEL of 10 ppm 8 hours/day for 4 weeks for changes in Leydig cell quantity and
 13 quality, decreased testes weight, and changes in trace metal content (zinc, copper, and iron).

14
 15 **Table 4-61. Formaldehyde effects on adult male albino Wistar rats**
 16

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

17
 18 ^aFormaldehyde exposure was 8 hours/day, 5 days/week for either 4 or 13 weeks. Values are means ± SDs of
 19 seven animals..

20 ^bDifferent from control, $p < 0.001$, as calculated by the authors.

21 ^cDifferent from control, $p < 0.05$, as calculated by the authors.

22 ^dDifferent from control, $p < 0.01$, as calculated by the authors.
 23

24 Source: Özen et al. (2002).
 25
 26

27 In another study that assessed testicular toxicity (Özen et al., 2005), male Wistar rats
 28 (18/group) were exposed by inhalation to 0, 5, or 10 ppm (0, 6.2, or 12.4 mg/m³) formaldehyde
 29 8 hours/day, 5 days/week for 91 days. In-life observations in exposed rats included clinical signs
 30 of respiratory irritation and decreased mean food and water consumption. At study termination,

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1 serum testosterone levels and mean seminiferous tubule diameters were significantly decreased
 2 from control in a dose-responsive manner (see Table 4-62). Immunohistochemical staining of
 3 testis tissues showed increased localization of heat shock protein (Hsp) 70 in the cytoplasm of
 4 spermatogonia, spermatocytes, and spermatids of treated animals compared with controls (not
 5 shown here).

6
 7 **Table 4-62. Formaldehyde effects on testosterone levels and seminiferous**
 8 **tubule diameters in Wistar rats following 91 days of exposure**
 9

Inhalation exposure ^a	Testosterone levels (ng/dL)	Seminiferous tubule diameters (µm)
	<i>n</i> = 6	<i>n</i> = 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

10
 11 ^aFormaldehyde exposure was 8 hours/day, 5 days/week for 91 weeks. Values are means
 12 ± SEMs.

13 ^bDifferent from control, *p* < 0.0001, by one-way ANOVA, as calculated by the authors.

14 ^cDifferent from control, *p* < 0.001, by one-way ANOVA, as calculated by the authors.

15
 16 Source: Özen et al. (2005).

17
 18
 19 Zhou et al. (2006) investigated the effect of formaldehyde on the testes and the protective
 20 effect of vitamin E against oxidative damage by formaldehyde in adult male rats. In this study,
 21 adult male Sprague-Dawley rats (10/group) were treated for 2 weeks in the following groups:
 22 (1) control rats were administered physiological saline by oral gavage, (2) rats were administered
 23 physiological saline by gavage and exposed to 10 mg/m³ (8.05 ppm) formaldehyde by inhalation
 24 for 12 hours/day, and (3) rats were administered daily gavage doses of 30 mg/kg vitamin E and
 25 exposed to 10 mg/m³ (8.05 ppm) formaldehyde by inhalation for 12 hours/day. Formaldehyde
 26 treatment resulted in significantly decreased (*p* < 0.05) mean testis weight. Histopathologic
 27 findings in treated rats included atrophy of seminiferous tubules, decreased spermatogenic cells,
 28 and seminiferous cells that were “disintegrated” and shed into the lumina, which was
 29 azoospermic. Interstitial tissue was edematous with vascular dilatation and hyperemia. In the
 30 formaldehyde-treated group, epididymal sperm count and percentage of motile sperm were
 31 significantly decreased, and the percentage of abnormal sperm was increased (*p* < 0.05), as
 32 compared with control. Evaluation of biochemical markers in testes tissue showed the activities
 33 of testicular SOD, GPX, and GSH were decreased; MDA levels were significantly increased as

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1 compared with control. All observed effects of formaldehyde treatment (i.e., decreased testes
2 weight, biochemical alterations, histopathologic effects, and sperm count, motility, and
3 morphology findings) were attenuated by administration of 30 mg/kg-day vitamin E.

4 In a study by Gokalipour et al. (2007), testicular changes of increasing severity with
5 increasing duration were reported. A total of 28 Wistar rats, aged 6-7 weeks old, were divided
6 into four groups including three formaldehyde treatment groups (4 hours of exposure/day, 4
7 days/week for 18 weeks; 2 hours of exposure/day, 4 days/week for 18 weeks; 4 hours of
8 exposure/day, 2 days/week for 18 weeks) and one untreated control. The three formaldehyde-
9 treated groups were exposed via inhalation to formaldehyde. The mean concentration of
10 formaldehyde vapor, based on three measurements during the study (stated as the beginning,
11 during, and end of the study period), was reported as 1.5 ppm. At the end of the study period,
12 the rats were sacrificed by ether anesthesia and subsequent cervical dislocation. The left testis
13 was dissected from each rat and a specimen was taken from each testis. Tissues were fixed,
14 embedded, sectioned (at 4 μm), and stained with hematoxylin and eosin. Using a light
15 microscope, a histopathological examination was performed on the testes tissues, including
16 morphometric evaluation of the diameter and height of 20 seminiferous tubules/testis.
17 Gokalipour et al. (2007) reported an formaldehyde exposure frequency (or duration)-dependent
18 increase in testicular germ cells and seminiferous tubule defects. In the most frequent duration
19 treatment group, a severe decrease in germ cells in >85% of the seminiferous tubules and
20 arrested spermatogenesis were observed. In the mid-level frequency of duration treatment group,
21 a decrease in the number of germ cells and an increased thickness of the basement membrane of
22 75% of the tubules was observed. In the lowest level duration treatment group, a disruption in
23 the Sertoli and germinal cell arrangement, and increased spacing between germ cells was
24 observed. Further, the seminiferous tubule diameter (STD) and seminiferous epithelial height
25 (SEH) was most decreased among the treatment groups (exhibiting the greatest decrease in the
26 group with the greatest hours and days of exposure) compared to the control (see Table 4-63).
27 The results of this study are consistent with the findings of other studies of male reproductive
28 system outcomes with inhalation formaldehyde exposure (e.g., Özen et al., 2005 and Zhou et al.,
29 2006).

30 Xing et al. (2007) also studied the effects of formaldehyde on sperm development and
31 reproductive capacity in adult male mice. In this study, male mice (12/group, strain not
32 specified) were exposed to 0, 21, 42, or 84 mg/m^3 (0, 16.9, 33.8, or 67.6 ppm) formaldehyde via
33 inhalation for 13 weeks at 2 hours/day, 6 days/week. The males were mated to untreated females
34 in a dominant lethal protocol, and sperm morphology was assessed at study termination. The
35 percent abnormal sperm was increased significantly ($p < 0.05$) in all treated groups, as was the

1 rate of resorptions ($p < 0.01$) (see Table 4-64). The mean number of live fetuses/litter was
 2 decreased in all treated groups, with statistical significance achieved at 84 mg/m³. Although this
 3 study did not assess the number of corpora lutea per dam, thereby precluding the calculation of
 4 preimplantation loss, it is nevertheless indicative of formaldehyde-induced sperm morphology
 5 changes and dominant lethal effects in male mice.

6
 7 **Table 4-63. Effects of formaldehyde exposure on seminiferous tubule**
 8 **diameter and epithelial height in Wistar rats following 18 weeks of exposure**
 9

Inhalation exposure ^a	Seminiferous tubule diameters (µm)	Seminiferous tubule height (µm)
	<i>n</i> = 7	<i>n</i> = 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

10
 11 ^a Values are means ± SEMs.

12 ^b Different from control, $p < 0.05$, as calculated by the authors.

13
 14 Source: Golalipour et al. (2007).
 15

16
 17 **Table 4-64. Incidence of sperm abnormalities and dominant lethal effects in**
 18 **formaldehyde-treated mice**
 19

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

20
 21 ^aSignificantly different from controls ($p < 0.05$), as calculated by the authors.

22 ^bSignificantly different from controls ($p < 0.01$), as calculated by the authors.

23
 24 Source: Xing et al. (2007).
 25
 26

1 **4.2.7.2. Oral Exposure Studies Addressing Developmental and Reproductive Toxicity**

2 No contemporary testing guideline studies, such as a prenatal developmental toxicity
3 study or two-generation reproductive toxicity study, have been performed by the oral route for
4 formaldehyde. However, a number of studies have evaluated developmental toxicity and
5 reproductive parameters in rats, mice, and dogs.

6 Hurni and Ohder (1973) tested the developmental toxicity of formaldehyde administered
7 as a 40% w/v solution containing 11–14% w/w methanol in 9–10 pregnant beagle dogs that
8 received the compound in their diet on GDs 4–56. Commercial grade formaldehyde (as a 40%
9 solution) was sprayed on the pellets prior to feeding. Each animal was allotted a diet of 300 g of
10 chow (reduced to 200 g 1 week prior to term) that was promptly consumed (within
11 5–10 minutes) before the formaldehyde volatilized appreciably. The concentrations of
12 formaldehyde in the chow were 0, 125, or 375 ppm, equivalent to doses of 0, 3.1, or
13 9.3 mg/kg-day, respectively. Dams were allowed to deliver normally and weight gain, gestation
14 length, number of litters, litter size, number of live pups, number of pups surviving through
15 weaning, and pup weights weekly for the first 8 weeks were monitored as indices of the potential
16 reproductive/developmental toxicity of formaldehyde. There were no formaldehyde-related
17 effects in any of the parameters other than progressive pup weights, which were lower by group
18 in litters of dams exposed to formaldehyde (see Table 4-65). A developmental impact of
19 formaldehyde was evident in this strain of dog under the conditions of the experiment. At birth,
20 mean pup body weights were 4 and 8.4% less than control for the low- and high-dose groups,
21 respectively; at 8 weeks of age, the pup weight decrements were 8.3% for the low dose and
22 12.5% for the high dose, as compared with control, and established a LOAEL of 125 ppm. The
23 contribution of methanol, which is a developmental toxin (Deglitz et al., 2004; Rogers et al.,
24 2004) to these outcomes is not known. No internal or skeletal malformations were observed in
25 any of the 264 live-born and 20 still-born pups.

26 Marks et al. (1980) conducted a developmental toxicity study of formaldehyde in CD-1
27 mice in which 29–35 pregnant animals were gavaged on GDs 6–15 with aqueous formaldehyde
28 (containing 10–15% methanol) at 74, 148, and 185 mg/kg-day. Seventy-six controls were
29 gavaged with water alone. All dams were sacrificed on GD 18, and the numbers of implantation
30 sites in each uterine horn were counted. The high dose of formaldehyde was toxic to the dams,
31 as indicated by the deaths of 22 of 34 mice before GD 18. Thus, the dose of 148 mg/kg-day was
32 a NOAEL for maternal toxicity in this study. However, it is unclear to what extent an estimated
33 concurrent dose of up to 75 mg/kg-day methanol may have contributed to this toxic response.
34 To assess the developmental toxicity of formaldehyde, live fetuses were weighed individually,
35 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

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1 rats/group. Testes from these animals and 20 controls were excised and examined for
 2 spermatogenic abnormalities 11 days after dosing. A significant (19%) increase in testicular
 3 sperm head counts was observed in rats exposed to 200 mg/kg-day formaldehyde as compared
 4 with controls (see Table 4-66). The percentage of abnormal sperm heads was also significantly
 5 increased (5%) in the 200 mg/kg-day dose group compared with controls. These data suggest
 6 that formaldehyde can induce morphologic abnormalities in the germ cells of male experimental
 7 animals at dose levels that did not significantly affect testis weights. The contribution of
 8 methanol to these outcomes is unknown.

9
 10 **Table 4-66. Testicular weights, sperm head counts, and percentage incidence**
 11 **of abnormal sperm after oral administration of formaldehyde to male Wistar**
 12 **rats**
 13

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

14
 15 ^aSignificantly different from controls ($p < 0.001$), as calculated by the authors.
 16

17 Source: Cassidy et al. (1983).
 18
 19

20 Postmortem evaluation of the reproductive organs was conducted in a number of oral
 21 studies that ranged between 4 weeks and 2 years in duration (Til et al., 1989, 1988; Tobe et al.,
 22 1989; Johannsen et al., 1986). Johannsen et al. (1986) administered 0, 50, 100, or 150 mg/kg-day
 23 formaldehyde in the drinking water to Sprague-Dawley rats (15/sex/group) for 91 days and 0, 50,
 24 75, or 100 mg/kg-day formaldehyde in basal diet to beagle dogs (4/sex/group) for 91 days; the
 25 study reported no treatment-related effects on absolute or relative gonad weights or
 26 histopathology for either species. In a 4-week drinking water study conducted by Til et al.
 27 (1988), formaldehyde was administered to Wistar rats (10/sex/treated group) at nominal levels of
 28 0, 25, and 125 mg/kg-day; gonad organ weights and histopathology were not affected by
 29 treatment. Tobe et al. (1989) conducted a chronic (24-month) study in Wistar rats
 30 (20/sex/group), with drinking water concentrations of 0, 0.02, 0.1, or 0.5%. According to the
 31 study report, gonad weights were measured and histopathology was conducted, but no treatment-
 32 related findings were noted. In a chronic (105-week) study (Til et al., 1989) in Wistar rats
 33 (70/sex/group), formaldehyde was administered in the drinking water at mean actual levels of 0,
 34 1.2, 15, or 82 mg/kg-day to males and 0, 1.8, 21, or 109 mg/kg-day to females; serial sacrifices

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1 were conducted at 53, 79, and 105 weeks of study. At study termination (105 weeks), mean
2 testes weights were 30% increased ($p < 0.01$) in high-dose males as compared with controls, and
3 histopathology evaluation revealed Leydig cell tumors in treated males (incidences of 0/50, 3/50,
4 3/50, and 2/50 for the control through high-dose groups, respectively; historical control tumor
5 incidence data were not provided). The study authors did not judge these findings to be
6 treatment related. By design, none of the subacute to chronic studies included measures of
7 reproductive function (e.g., estrous cyclicity, sperm measures, or reproductive performance).
8 With the exception of Til et al. (1989), detailed mean organ weight and histopathology incidence
9 data were not provided in the published reports, and Til et al. (1988) only included tumor (not
10 nontumor) data.

11

12 **4.2.7.3. *Intraperitoneal Studies Addressing Developmental and Reproductive Toxicity***

13 Other studies in which formaldehyde was administered by I.P. injection have confirmed
14 the potential effects on the male reproductive system.

15 Chowdhury et al. (1992) administered I.P. injections of 0, 5, 10, or 15 mg/kg-day
16 formaldehyde to Charles foster adult male rats (10/group) for 30 days. On study day 31, blood
17 was collected for serum testosterone measurements and the rats were sacrificed. The testes were
18 removed, weighed, fixed in Bouin's solution, and processed for histopathology. The study
19 authors reported adverse findings in all treated groups, including significant ($p < 0.01$) mean
20 body weight gains, serum testosterone levels, and testes weights. Histopathologic evaluation
21 revealed normal spermatogenic processes and Leydig cells in control animals. However, in
22 treated rats, gradual cellular degeneration in seminiferous tubules and in Leydig cells was
23 observed. Marked nuclear damage was noted in the 10 and 15 mg/kg-day groups, with
24 significantly ($p < 0.001$) decreased Leydig cell populations and nuclear diameters observed in all
25 treated groups. Additionally, a decrease in $3\beta\text{-}\Delta^5$ -hydroxy steroid dehydrogenase was noted in
26 the Leydig cell region of treated rat testes.

27 In a 30-day study performed by Majumder and Kumar (1995), 10 mg/kg-day
28 formaldehyde was administered I.P. to eight male Wistar rats. All animals were sacrificed at
29 term, and the testes, prostate, seminal vesicles, and epididymides were excised and weighed.
30 With the use of methodologies that were not described in the report other than by reference to the
31 *Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus*
32 *Interaction* (WHO, 1987), sperm counts, motility, and viability were compared with those of 10
33 controls (injected I.P. with water alone). As shown in Table 4-67, striking reductions in sperm
34 count and motility were noted in formaldehyde-treated rats compared with controls. Sperm

1 viability was also significantly reduced by formaldehyde treatment, though to a lesser overall
2 extent than sperm count and motility.

3
4 **Table 4-67. Effect of formaldehyde on spermatogenic parameters in male**
5 **Wistar rats exposed intraperitoneally**
6

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

7
8 ^aSignificantly different from controls ($p < 0.0001$), as calculated by the authors.
9

10 Source: Majumder and Kumar (1995).
11
12

13 Majumder and Kumar (1995) also carried out an in vitro experiment in which sperm from
14 normal rats were incubated with different concentrations of aqueous formaldehyde at
15 concentrations ranging from 125 pg/mL to 2.5 µg/mL. Viability of control sperm remained close
16 to 80% for a period of 1 hour, whereas the presence of formaldehyde dose-dependently reduced
17 viability. Thus, only 50% spermatozoa were viable for 30 minutes in the presence of 5 ng/mL
18 formaldehyde compared with 6 minutes in the presence of 500 ng/mL. Sperm motility also was
19 sensitive to the presence of formaldehyde. Less than 10% of sperm was motile for 10 minutes in
20 the presence of 125 pg/mL formaldehyde. The authors of the study considered their data to be
21 good evidence that functional parameters of spermatozoa, such as viability and motility, can be
22 adversely affected by exposure to formaldehyde. Moreover, they suggested that the cumulative
23 effects of I.P. administration of formaldehyde on the male rat reproductive system raise an alert
24 that formaldehyde might impair the reproductive health of males who are occupationally exposed
25 to the compound.

26 Odeigah (1997) conducted two short-term in vivo assays to examine sperm head
27 abnormalities and dominant lethal mutations. In the sperm assessment, five daily I.P. injections
28 of 0, 0.125, 0.25, or 0.5 mg/kg formaldehyde were administered to male albino rats (six/group;
29 strain not specified). The rats were killed 3 weeks after the last injection, and epididymal sperm
30 counts and abnormalities were assessed. A dose-related decrease in sperm count was observed,
31 and significantly increased incidences of sperm head abnormalities were found at all treatment
32 levels (see Table 4-68).
33

1 **Table 4-68. Incidence of sperm head abnormalities in formaldehyde-treated**
 2 **rats**
 3

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

4
 5 ^aSignificantly different from controls ($p < 0.05$), as calculated by the authors.

6 ^bSignificantly different from controls ($p < 0.001$), as calculated by the authors.

7
 8 Source: Odeigah (1997).
 9

10
 11 In the dominant lethal assay (Odeigah, 1997), five daily I.P. injections of 0, 0.125, 0.25,
 12 or 0.5 mg/kg formaldehyde were administered to male rats (5 control rats, 12/treated group).
 13 Subsequently, each male was mated with two untreated virgin females per week for
 14 3 consecutive weeks. The females were killed 13 days after the midpoint of the mating period
 15 and evaluated for live and dead uterine implants. In general, the number of live embryos was
 16 decreased with treatment, and the number of dead implants was increased (see Table 4-69).
 17 Additionally, there was a reduction in fertile matings in females mated 1–7 days after the males
 18 had been treated. This study did not assess the number of corpora lutea and therefore precluded
 19 the determination of preimplantation loss. Nevertheless, it is indicative of dominant lethal
 20 effects on the male germ cells.
 21

22 **4.2.7.4. Dermal Exposure Studies Addressing Developmental and Reproductive Toxicity**

23 In a study designed to assess the embryotoxic effects of dermal exposure to
 24 formaldehyde, Overman (1986) applied 0.5 mL of a 37% formaldehyde solution directly to the
 25 dorsal skin of female Syrian golden hamsters (four–six/group) on GDs 8, 9, 10, or 11 for 2
 26 hours. To prevent grooming during the treatment period, the animals were anesthetized with
 27 Nembutal. At the end of the 2-hour treatment period, the application site was washed thoroughly
 28 to remove any remaining formaldehyde. The dams were terminated on GD 15 (i.e., one day
 29 prior to expected delivery, since the typical gestation period for the Syrian golden hamster is
 30 16–18 days). The fetuses were removed and fixed in either Bouin’s solution or 95% ethanol for
 31 visceral or skeletal evaluation, respectively. The uteri were examined for implantation sites.
 32 Fixed fetuses were weighed, measured (crown-rump), and examined for external abnormalities;
 33 fetuses that had been placed in Bouin’s fixative were evaluated for visceral anomalies by using a

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1 **Table 4-69. Dominant lethal mutations after exposure of male rats to**
 2 **formaldehyde**
 3

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

4
 5 ^aNumber of females with implants presented in parentheses.

6 ^bDominant lethal mutation index:

7
$$\text{Index} = 1 - \frac{(\text{Live implants experiment group per female})}{(\text{Live implants of control group per female})} \times 100$$

8
 9
 10 Source: Odeigah (1997).

11
 12
 13 free-hand sectioning technique, and those that were placed in ethanol were macerated, stained,
 14 and cleared for skeletal examination. In this study, the dams exhibited signs of dermal irritation
 15 and irritability, but the author reported no treatment-related effects on maternal body weight
 16 gain. The percent of resorption sites was increased (although not significantly) in treated litters
 17 as compared with control (0, 4.2, 8.1, 4.6, and 3.2% resorbed implantation sites for control and
 18 GDs 8, 9, 10, and 11 treatment groups, respectively). No treatment-related effects on fetal
 19 weight, length, or visceral or skeletal development were observed.

20
 21 **4.2.7.5. Summary of Reproductive and Developmental Effects**

22 Formaldehyde exposures up to 40 ppm 6 hours/day from GDs 6–15 or 6–20 did not
 23 result in external or internal malformations (Martin, 1990; Saillenfait et al., 1989). Martin
 24 (1990) reported delayed skeletal ossification and dose-dependent decreases in fetal body weight
 25 at 5 ppm. Formaldehyde exposure at 40 ppm to pregnant female Sprague-Dawley rats reduced
 26 fetal body weights in male and female progeny and in male pups of dams exposed to 20 ppm

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1 formaldehyde (Saillenfait et al., 1989). Based on these studies (see Table 4-70), the LOAEL for
2 developmental effects in rats is 5 ppm, with a NOAEL of 2 ppm for decreased fetal weight and
3 delayed skeletal ossification, based on inhalation exposures during GDs 6–20.

4 Developmental studies during earlier gestational windows of inhalation exposure to
5 formaldehyde have reported additional adverse health effects, including delayed ossification,
6 changes in relative organ weight, undescended testes, biochemical changes (e.g., ascorbic acid
7 and nucleic acids), and blood acidosis (Senichenkova and Chebotar, 1996; Senichenkova, 1991;
8 Kilburn and Moro, 1985; Gofmekler and Bonashevskaya, 1969; Gofmekler, 1968; Pushkina et
9 al., 1968). Kitaev et al. (1984) hypothesized that formaldehyde may affect reproductive function
10 by stimulating the hypothalamo-pituitary-gonadal (HPG) axis based on their observations of
11 increased ovary weight, increased number of ovulating cells, and changes in blood levels of
12 gonadotropins (LH and FSH). Evidence of preimplantation loss, which may be related to HPG
13 disruption, was observed in this study and by Sheveleva (1971). Additional studies are needed to
14 better understand developmental effects of formaldehyde exposure during early gestational
15 windows.

16 The prenatal developmental toxicity of oral and dermal exposures to formaldehyde has
17 not been thoroughly studied. Reductions in postnatal growth in beagle pups was observed by
18 Hurni and Ohder (1973) following in utero exposure to 125 ppm maternal dietary formaldehyde
19 during GDs 4–56 in beagle dogs. However, gavage dosing during gestation of mice to overtly
20 maternally toxic doses (Seidenberg and Becker, 1987; Marks et al., 1980) (see Table 4-71) and
21 dermal application during gestation to hamsters at a dose that caused dermal irritation and
22 irritability (Overman, 1986) did not result in any observed fetal toxicity (see Table 4-72).

23 Few studies identified effects on maternal toxicity or female reproductive capacity. As
24 summarized in Table 4-70, exposure of rat dams at 10–40 ppm formaldehyde during pregnancy
25 has been shown to result in significantly decreased weight gain (Martin, 1990; Saillenfait et al.,
26 1989; Kilburn and Moro, 1985). Maronpot et al. (1986) reported endometrial hypoplasia with a
27 lack of ovarian luteal tissue in female rats exposed at 40 ppm but not at 20 ppm. Changes in LH
28 and FSH levels were reported in dams exposed to 0.41 ppm formaldehyde by Kitaev et al.
29 (1984), establishing an unbounded LOAEL for maternal toxicity.

30 Studies designed to assess male reproductive system endpoints in rats following repeated
31 inhalation exposures to formaldehyde have shown concentration-dependent decreases in Leydig
32 cell number and quality, effects on seminiferous tubules, decreases in testes weight, alterations in
33 sperm measures, decreased testosterone levels, alterations in trace metals in the testes, and/or
34 dominant lethal effects (Zhou et al., 2006; Özen et al., 2005, 2002; Zhou et al., 2006; Sarsilmaz et
35 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 120 th 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 2 nd day preg.) At 1.2 ppm: ↑ no. degenerating embryos (stat. sig. in 3 rd 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 formaldehyde 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 ¹	0 or 0.41 ppm (reported as 0.5 mg/m ³) formaldehyde (also a 3 rd 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/m³ to ppm: 1 ppm = 1.23 mg/m³; 1 mg/m³ = 0.813 ppm.

^aTreatment 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.

^bStudies with negative findings are included.

^cL: LOAEL; N: NOAEL.

^dExposure concentrations not validated; details of formaldehyde vapor generation not reported; exposure during gestation not well characterized in study report.

^eNo statistics provided.

^fLack of study details.

^gSee Table 4-73 for reproductive effects.

^hGofmekler and Bonashevskaya (1969) seem to report on different findings from the same study (i.e., same animals) as Gofmekler (1968).

ⁱNumber/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.

^aStudies with negative findings are included.

^bL: LOAEL; N: NOAEL.

^cReported dose units.

^dmg/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)

^aL: 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 120 th 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 ^d	L: ND N: 10 ppm	Appleman et al. (1986)

^aTreatment is given as the formaldehyde concentration in air (ppm) with the length of exposure each day and the duration of treatment in days.

^bStudies with negative findings are included.

^cL: LOAEL; N: NOAEL.

^dGuseva (1972) was a drinking water and inhalation study.

Table 4-73. Summary of reported reproductive effects in formaldehyde inhalation studies (continued)

^eDevelopmental effects shown in Table 4-70.

^fStatistics not provided in study report.

^gFocus 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/m³) and the NOAEL was 0.4 ppm (0.5 mg/m³).

ND: not determined; NR: not reported.

To convert concentrations in air (at 25°C) from mg/m³ to ppm: 1 ppm = 1.23 mg/m³; 1 mg/m³ = 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)

^aStudies with negative findings are included.

^bL: LOAEL; N: NOAEL.

^cFocus 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.

^dActual 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	

^aStudies with negative findings are included.

^bL: LOAEL; N: NOAEL.

ND = not determined; NR = not reported; T = testosterone.

1 facilitate the formation of adducts between other chemicals or drugs (endogenous or xenobiotic)
2 and DNA (Fennell, 1994; Koppel et al., 1991; Casanova et al., 1989; Heck and Casanova, 1987;
3 Lam et al., 1985; Casanova-Schmitz et al., 1984a; Casanova-Schmitz and Heck, 1983; Ohba et
4 al., 1979; Dönecke, 1978; Brutlag et al., 1969).

5 The high reactivity of formaldehyde results in little specificity in reaction sites, indicating
6 that a range of adducts and cross-links might be expected. However, the spectrum of
7 formaldehyde-DNA reaction products is difficult to quantify in vivo as many of these products
8 are labile and difficult to measure directly (Fennell, 1994; Casanova et al., 1989). Additionally,
9 formaldehyde is metabolically incorporated into nucleic acids, and therefore DNA and RNA
10 assays incorporating radiolabeled formaldehyde need careful interpretation to distinguish
11 between covalently bound and metabolically incorporated formaldehyde (Casanova et al., 1989;
12 Heck and Casanova, 1987; Casanova-Schmitz et al., 1984a, b; Casanova-Schmitz and Heck,
13 1983). Hence, reports of formaldehyde-DNA reactivity in cell-free system results may not
14 provide a useful measure of exposure (Fennell, 1994). Besides, the question of biological
15 relevance must also be considered. On the other hand, methods used to extract and measure
16 formaldehyde-DNA reaction products after in vivo exposures should be evaluated to ensure that
17 formaldehyde reaction products are neither created nor removed during sample preparation
18 (Fennell, 1994; Casanova et al., 1989).

20 **4.3.1.1. DNA-Protein Cross-Links (DPXs)**

21 Formaldehyde forms DPX by reacting with the amino or imino groups of proteins (e.g.,
22 lysine and histidine side chains) or of nucleic acids (e.g., cytosine) resulting in a Schiff base
23 formation which then reacts with another amino group (McGhee and von Hippel 1975a, b).
24 Evidence from numerous experimental models, ranging from cell-free systems to single cells and
25 in vivo animal and human exposures, suggests that formaldehyde reacts readily with DNA
26 forming DPXs (Reviewed in Conaway et al 1996; IARC 2006, 1995). As shown in Table 4-76,
27 cross-links between histones and DNA have been demonstrated in isolated chromatin samples on
28 exposure to formaldehyde from earlier studies (Ohba et al., 1979; Dönecke, 1978; Brutlag et al.,
29 1969). Several in vitro studies demonstrated induction of DPX by formaldehyde exposure in
30 bacteria (Wilkins and McLeod 1976), yeast (Magana-Schwencke and Ekert, 1978) and
31 mammalian cells including animal cells (Chinese hamster ovary [CHO] cells, Chinese hamster
32 V79 lung epithelial cells, mouse leukemia L1210 cells, mouse hepatocytes, rat Yoshida
33 lymphosarcoma cells, rat C18 tracheal epithelial cells, hepatocytes, nasal, tracheal, buccal
34 epithelial cells, kidney cells and aortic endothelial cells) and human cells (lung and bronchial
35 epithelial cells, fibroblasts, white blood cells, peripheral blood lymphocytes, Epstein-Barr Virus
36 (EBV)-Burkitt's lymphoma cells, Jurkat E6-1 cells, HeLa cells, lymphoblastoid cells, gastric

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Table 4-76. Formaldehyde-DNA reactions (DPX formation)

Species/Strain	Cell/Strain	Result	References
<i>DNA Interaction</i>			
DPX formation in vitro			
In vitro	Nucleohistone	+	Brutlag et al., 1969
		+	Döenecke, 1978
		+	Ohba et al., 1979
Bacteria	<i>Escherichia coli</i>	+	Wilkins and McLeod, 1976
Yeast	<i>Saccharomyces cerevisiae</i>	+	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

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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		+	Quiévryn 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	

2

3

^a indicates that DNA-protein cross-links formed at cytotoxic concentrations.

4

^b used homogenates of nasal mucosa.

5

‘+’ indicates a positive test result.

6

‘-’ indicates a negative test result.

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1 mucosal cells and whole blood cultures) and in vivo studies involving experimental animals and
2 occupationally exposed human populations as summarized in Table 4-76. Some relevant studies
3 are described here.

4 Ross and Shipley (1980) showed that formaldehyde induces single strand breaks (SSBs)
5 and DPXs in mouse leukemia cells; SSBs are formed at concentrations $>200\ \mu\text{M}$ and a reduction
6 of radiation-induced breaks (indirect measure of DPXs) at $50\ \mu\text{M}$. The authors used a [^{14}C]-
7 thymidine-incorporated mouse L1210 cell line to monitor formaldehyde-induced DNA strand
8 breaks and DPXs. They exposed cells to varying concentrations of formaldehyde for 2.5 hours.
9 An alkaline-elution technique in the presence or absence of proteinase K was used to measure
10 strand breaks. In order to detect DPXs, some cells were exposed to 300 R of X-rays immediately
11 after formaldehyde treatment. Formaldehyde-induced DPXs were repaired 24 hours after the
12 compound was removed from the culture (Ross and Shipley, 1980).

13 Casanova-Schmitz and Heck (1983) have shown that homogenates of rat nasal mucosa
14 incubated with formaldehyde in vitro followed by extraction with a strong aqueous-immiscible
15 organic solvent demonstrated increased DPX formation in DNA obtained after enzymatic
16 proteolysis from the aqueous-organic interface (IF), termed as “interfacial DNA” (IF-DNA).

17 Bermudez and Delehanty (1986) observed the formation of DPXs, scheduled (S) and
18 unscheduled DNA synthesis (UDS), and synthesis of RNA when cultured F344 rat nasal
19 epithelial cells from the naso- and maxillary turbinates were incubated with formaldehyde.
20 Unscheduled and scheduled DNA synthesis was stimulated ($0.05\text{--}0.1\ \text{mM}$) and then inhibited
21 ($0.1\text{--}1\ \text{mM}$), depending on the formaldehyde concentration. Experiments by Cosma et al. (1988)
22 and Cosma and Marchok (1988) showed the induction of DPXs and DNA SSBs in cultured C18
23 rat tracheal epithelial cells exposed to $200\ \mu\text{M}$ formaldehyde for 90 minutes (Cosma et al., 1988;
24 Cosma and Marchok, 1988).

25 Several human cells (epithelial cells, fibroblasts, buccal cells) or cell lines
26 (lymphoblastoid cells) exposed to formaldehyde have been shown to form DPX (Craft et al
27 1987; Costa et al 1997; Emri et al 2004; Li et al 2004).

28 Craft et al. (1987) detected DPXs by alkaline elution in TK6 human lymphoblastoid cells
29 immediately after a 2-hour exposure (zero time) to 0, 15, 50, 75, 100, 150, 300, and $600\ \mu\text{M}$
30 formaldehyde with a significant nonlinear increase in DPXs above $50\ \mu\text{M}$ concentration, which
31 correlated with the onset of cytotoxicity, but DPXs were completely removed in cultures held for
32 24 hours before processing. In the zero-time sample, significant increases in DPXs were first
33 observed at $50\ \mu\text{M}$ and increased linearly up to $150\ \mu\text{M}$. In cells held for 24 hours, there was no
34 detectable increase in DPXs.

1 However, Costa et al. (1997) detected DPXs with paraformaldehyde (which dissociates to
2 release formaldehyde) at doses that were cytotoxic (>0.003%) but could not discriminate
3 between the DPX-inducing and cytotoxic effects of this chemical in EBV-human Burkitt's
4 lymphoma cells (Costa et al., 1997). Grafström et al. (1983) reported that the number of DPXs
5 induced by 100 µM formaldehyde in vitro in human epithelial cells and fibroblasts of bronchial
6 origin was similar and that the frequency of these cross-links was proportional to the
7 concentration of formaldehyde. Besides the bronchial epithelial cells and fibroblasts, the authors
8 also noted that formaldehyde exposure resulted in DPXs and DNA SSBs in skin fibroblasts and
9 DNA excision repair-deficient skin fibroblasts. However, formaldehyde was only moderately
10 cytotoxic to normal bronchial epithelial cells and fibroblasts at concentrations that induced
11 substantial DNA damage. Repair of the formaldehyde-induced DNA SSBs and DPXs appeared
12 to be inhibited by the continued presence of formaldehyde in the culture medium (Grafström et
13 al., 1984).

14 Emri et al. (2004) detected a significant increase in DPX formation in primary human
15 skin fibroblasts and keratinocytes at 8 hours of exposure in vitro to formaldehyde at 25 µM with
16 an approximately linear increase up to 100 µM. These cells were exposed to 0, 12.5, 25, 50, and
17 100 µM formaldehyde for 8 hours and then exposed to 250 µM methyl methane sulfonate
18 (MMS) for 2.5 hours. The induction of DPX formation was measured by the ability of
19 formaldehyde to reduce DNA migration in the comet assay induced by MMS in this study (Emri
20 et al., 2004).

21 Li et al. (2004) measured DNA damage in primary human buccal cells by using the
22 comet assay. The appearance of SSBs, suggesting compound-induced fragmentation of DNA,
23 occurred at formaldehyde concentrations of 5 and 7.5 µM. At higher concentrations, the
24 response slope decreased, indicating formation of DPXs or DDXs (Li et al., 2004). The same
25 laboratory reported similar findings in primary human peripheral blood lymphocytes and HeLa
26 cells (Liu et al., 2006). Peak response for SSBs was seen at 10 µM in both cells, with higher
27 concentrations resulting in cross-link formation. SSBs in HeLa cells induced by 10 µM
28 formaldehyde were repaired by 60 minutes after cells were washed to remove formaldehyde.

29 Schmid and Speit (2007) tested formaldehyde for its ability to induce DPXs in blood
30 cultures. They used an indirect method to monitor DPX formation in which the extent of DNA
31 migration in the comet assay in response to γ radiation was compared in formaldehyde-treated
32 cultures versus controls. A concentration of 25 µM was required for DPX formation, and repair
33 of these lesions was rapid, with DPXs induced by concentrations of formaldehyde up to 100 µM
34 and completely removed after 8 hours. Overall, several in vitro studies in cultured mammalian

1 cells demonstrated the formation of DPX and SSB formation confirming the genotoxicity of
2 formaldehyde.

3 Several in vivo studies involving rodents, monkeys and humans have demonstrated DPX
4 formation following formaldehyde exposure (see Table 4-76). As mentioned earlier, in vitro
5 studies by Casanova-Schmitz and Heck (1983) have shown that reaction of formaldehyde with
6 cellular macromolecules in tissue homogenates causes a decrease in the extractability of DNA
7 into aqueous phase during solvent extraction and the formaldehyde-bound DNA-protein complex
8 migrates into the interface. In the same study Casanova-Schmitz and Heck (1983) have also
9 shown that DNA isolated from the nasal, but not olfactory, mucosa of rats exposed to
10 formaldehyde (2, 6, 15, and 30 ppm 6 hours/day for 2 days) via inhalation showed significant
11 increase in DPXs in the interfacial DNA ≥ 6 ppm, which was shown to be linear in the exposure
12 range of 2–30 ppm (2.45–36.8 mg/m³). However, DNA in the aqueous phase did not show DPX
13 formation. Thus, the cross-linked DNA that could be extracted from the interface after
14 proteolysis was considered to be supporting evidence of chemically induced DPX formation.
15 The inability of this study to detect DPXs at lower levels of formaldehyde exposure is likely be
16 due to the protective mechanism of GSH, which catalyzes the conversion of formaldehyde to
17 formate. Later, Lam et al., (1985) have shown that coexposure of rats with 2 ppm acrolein and 6
18 ppm formaldehyde for 6 hours resulted in higher DPX in the nasal mucosa of rats compared to
19 the rats given formaldehyde alone, suggesting that GSH depletion by acrolein enhanced the DNA
20 binding of formaldehyde. In this study inhalation exposure of rats to acetaldehyde alone at 0,
21 0.1, 0.5, 1 and 2.5 ppm for 6 hours induced a concentration-dependent depletion of GSH in rat
22 nasal mucosa with no detectable DPX levels at 2 ppm dose. However, acetaldehyde forms DPX
23 (as interfacial DNA) in rat nasal mucosa at much higher concentrations (100-1000 ppm)
24 compared to formaldehyde.

25 So, in a later study, Casanova and Heck (1987) reported that GSH depletion caused an
26 increase in DPX formation in the IF-DNA in the nasal mucosa of F344 rats when a dual-isotope
27 (³H/¹⁴C) method was used. The dual isotope method helps in making the distinction between
28 metabolic incorporation and covalent binding of formaldehyde. Oxidation by removal of one
29 hydrogen atom is required for metabolic incorporation of formaldehyde into cellular
30 macromolecules, but not in the formation of DNA adducts or DNA-protein crosslinks. Thus, the
31 ratio of ³H/¹⁴C of DNA containing DPX will be higher than the macromolecules where
32 formaldehyde is metabolically incorporated. However, the authors further demonstrated that,
33 when the double isotope method was used, the ³HCHO is oxidized significantly more slowly
34 than H¹⁴CHO under these conditions, resulting in an overestimate of the concentration of cross-
35 links due to an isotopic effect on the oxidation of ³HCHO catalyzed by formaldehyde

1 dehydrogenase (FDH). Besides, this method leaves residual formaldehyde that is likely to form
2 DNA adducts by reacting with deoxyribonucleosides in the DNA hydrolysates (Heck and
3 Casanova, 1987).

4 To overcome this, Casanova et al. (1989) used an improved method, which is based not
5 on the analysis of residual formaldehyde bound to deoxyribonucleosides in DNA hydrolysates
6 but on the determination of the total ¹⁴C-formaldehyde bound to DNA. This study showed that
7 formaldehyde was exclusively bound to interfacial DNA, indicating the formation of DPXs.
8 Hydrolysis of DPXs in different samples quantitatively released formaldehyde. Besides, DPX
9 formation was detectable at all concentrations of exposure to formaldehyde (0.3–10 ppm for
10 6 hours). Overall, these studies clearly show that formaldehyde induces DPXs in nasal epithelial
11 cells of rodents. However, there are no published rodent studies that assess DPXs beyond the
12 nasal passages of the upper respiratory tract.

13 Formaldehyde-induced DPXs were also found in the nasal mucosa and extranasal tissues
14 of rhesus monkeys exposed to 0, 0.71, 2, or 6 ppm (0, 0.86, 2.45, or 7.36 mg/m³) formaldehyde
15 6 hours/day for 3 days (Casanova et al., 1991). These data were used as a basis for cross-species
16 prediction of formaldehyde-induced DPXs in humans. The presence of DPXs in rhesus monkeys
17 confirms formaldehyde's DNA reactivity as a general effect. Additionally, DPXs were detected
18 in the larynx/trachea/carina (pooled sample) and in intrapulmonary airways of monkeys exposed
19 to 2 or 6 ppm formaldehyde. These data demonstrate direct effects of formaldehyde on DNA in
20 tissues that correspond to observed tumor sites in humans (nasal and nasopharynx).

21 22 **4.3.1.2. DNA Adducts**

23 In addition to the formation of DPX, there is evidence that formaldehyde forms
24 hydroxymethyl (hm) DNA adducts in vitro in a variety of cell-free systems (Zhong and Que Hee,
25 2004a; Cheng et al., 2003; Kennedy et al., 1996; Fennell, 1994; Beland et al., 1984) and nasal
26 epithelial cells (Zhong and Que Hee 2004b). In cell-free systems, formaldehyde directly reacts
27 with DNA forming hmDNA adducts (Cheng et al., 2003; Kennedy et al., 1996; Fennell, 1994;
28 McGhee and von Hippel, 1977a, b, 1975a, b). The results on formaldehyde-induced DNA
29 adduct formation are summarized in Table 4-77.

30 Beland et al. (1984) first reported hmDNA adducts in Chinese hamster ovary (CHO) cells
31 incubated with 1 mM of radiolabeled formaldehyde. After a 2-hour incubation, small amounts of
32 N⁶-hydroxymethyldeoxyadenosine (N⁶-hmdA) adducts were detected with concomitant
33 metabolic incorporation of formaldehyde. Besides N⁶-hmdA, various other forms of hmDNA
34 adducts, including N⁴-hydroxymethyldeoxycytidine (N⁴-hmdC), and
35 N² hydroxymethyldeoxyguanosine (N²-hmdG), were detected by high performance liquid
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Table 4-77. Formaldehyde-DNA reactions (DNA adduct formation)

Species/Strain	Cell/Strain	Result	References
<i>DNA Interaction</i>			
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			
<i>Drosophila</i>	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

3

4 '+' indicates a positive test result.

5

5 '-' indicates a negative test result.

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8 chromatography (HPLC) following in vitro reaction between formaldehyde and calf thymus
9 DNA or individual deoxynucleotides.

10

10 Since the formaldehyde adducts are labile, Fennel (1994) developed a method by
11 derivatizing them with sodium bisulfite to their sulfomethyl form, whereby he detected

11

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1 N⁶-sulfomethyldeoxyadenosine (SOMedA) and N²-sulfomethyldeoxyguanosine (SOMedG) by
2 using HPLC. However, the levels of SOMedA in DNA isolated following incubation of
3 radiolabeled formaldehyde with isolated rat hepatic nuclei were similar to those in control nuclei.
4 And in human TK6 lymphoblastoid cells treated with formaldehyde, detection of SOMedA
5 adducts was precluded by additional radioactive spots. These observations suggest that N⁶-
6 sulfomethyldeoxyadenosine adducts are formed at very low levels in formaldehyde-incubated rat
7 hepatic nuclei and that measurement of hydroxymethyldeoxyadenosine would not provide a
8 useful measure of formaldehyde exposure. Fennel (1994) also reported that ³²P-postlabeling
9 studies (Gupta et al. 1982) allowed for much greater analytical sensitivity but did not confirm the
10 level of SOMedA adduct detected by HPLC. However, either estimate of adduct formation is
11 much less than the estimate of DPX formation (120 pmol/mg DNA) in similarly treated rat
12 nuclei (Heck and Casanova, 1987).

13 Casanova et al. (1989) demonstrated that detection of hmDNA adduct formation was
14 sensitive to the methodology used, particularly the buffer used for sample preparation.
15 Specifically, Tris buffer can prevent hmDNA adduct formation due to the abundance of
16 formaldehyde-reactive primary amine sites in the buffer. In contrast, the tertiary amine sites that
17 predominate in Bis-Tris buffer do not react with formaldehyde.

18 Zhong and Que Hee (2004a) observed hmDNA adducts (N⁶-hmdA, N²-hmdG, and
19 N⁴-hmdC) in placental DNA exposed to 100 ppm formaldehyde in vitro for 20 hours at 37°C
20 followed by hydrolysis of formaldehyde-reacted DNA using bis-Tris buffer. However,
21 deoxythymidine did not form hydroxymethyl derivatives in this study (Zhong and Que Hee,
22 2004a). On the other hand, the same investigators were able to detect N⁶-hmdA and N²-hmdG
23 adducts in human nasal epithelial cells cultured in the presence of 0, 10, 25, 50, 100, 250, 400, or
24 500 µg/mL formaldehyde and using Tris buffer during hydrolysis of adducted DNA. The
25 toxicity threshold for <90% viability appeared to be between 100 and 250 µg/mL initial
26 formaldehyde culture concentration, and even at 500 µg/mL concentration it was not toxic, with
27 a viability was 70% in this study (Zhong and Que Hee, 2004b).

28 There are only a few reports of formaldehyde-induced hmDNA adducts in vivo.
29 Alderson (1985) hypothesized that a N⁶-hydroxymethyl adenosine, an RNA adduct formed in
30 the reaction of formaldehyde and adenosine is likely to play a mutagenic role in *Drosophila*,
31 based on the observation that flies fed on a medium containing formalin was mutagenic only
32 when adenosine or adenylic acid, but not adenine was present in the medium. An indirect
33 evidence from the study of Wang et al., (2007) has shown that N⁶-hydroxymethyl-
34 deoxyadenosine (N⁶-hmdA) adducts were formed in hepatic and pulmonary DNA from rats
35 exposed to *N*-nitrosodimethylamine (NDMA) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-

1 butanone (NNK), suggesting that these two nitrosamines could be a source of formaldehyde-
2 DNA adducts. The same group recently has shown that formaldehyde-DNA adducts are
3 detectable in the leukocytes of smokers and nonsmokers. More recently, Lu et al (2010) have
4 shown that in rats exposed to formaldehyde by inhalation deoxyguanosine monoadducts and
5 DDX were detected in nasal epithelium, while endogenous formaldehyde monoadducts and
6 DDX were detectable in white blood cells and several internal organs including liver, lung,
7 spleen, thymus and bone marrow.

8
9 **4.3.1.3. DNA-DNA Cross-Links (DDXs)**

10 Formaldehyde, besides forming DPXs and DNA adducts, has also been shown to form
11 DDX in vitro. Li et al. (2004) showed that formaldehyde induces DNA strand breaks at low-
12 exposure concentration and DDXs and DPXs at higher concentrations in buccal cells. The
13 authors also showed that formaldehyde induces DDXs in human peripheral blood lymphocytes
14 exposed in vitro when the concentration was more than 25 µM. Recently, Lu et al (2010)
15 reported DDXs in the nasal epithelium of F344 rats that were exposed by nose-only to 10 ppm
16 formaldehyde for 1 or 5 days (6 hrs/day). The relevance of these modifications in formaldehyde-
17 induced genotoxicity is not known at the moment.

18 Overall, formaldehyde forms predominantly DPXs that are detected in cell-free systems
19 and single cells in vitro and in animal and human tissues in vivo. In rodents, DPXs are formed in
20 nasal epithelia but not in extranasal passages, which are completely removed within a day after
21 formation. The DPXs are detected in nasal and extranasal tissues of monkeys, suggestive of
22 direct effects of formaldehyde in tissues that correspond to observed tumor sites (nasal and
23 nasopharynx) in humans. Besides, this is used as a basis for cross-species comparison with
24 humans. Formaldehyde-DNA adducts are labile, constituting a minor fraction of the DNA-
25 reaction products. DPXs but not DNA adducts appear to play an important role in the
26 genotoxicity of formaldehyde.

27
28 **4.3.1.4. Single Strand Breaks**

29 Formaldehyde has been shown to induce DNA single strand breaks in a number of
30 mammalian cell systems in vitro as well as in vivo as shown in Table 4-78. In the in vitro
31 studies, most studies were positive, a few were negative and one study was equivocal for strand
32 breaks induced by formaldehyde. Among the in vivo studies, Im et al (2006) observed a dose-
33 dependent increase in DNA damage, analyzed by the Oliver tail movements in the comet assay
34 in both blood lymphocytes as well as livers of rats exposed to 5 and 10 ppm formaldehyde. Sul
35 et al (2007) also observed a dose-dependent increase in SSBs of Sprague-Dawley rats exposed to

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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	

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‘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.

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1 xeroderma pigmentosum) or cell lines hypersensitive to DNA-DNA crosslinks (e.g., Fanconi's
2 anemia) (Speit et al 2000) have been shown that formaldehyde causes DNA repair inhibition at a
3 concentration range of 0.125 mM to 10 mM. Emri et al (2004) have shown that DNA repair was
4 inhibited in human keratinocytes and fibroblasts after irradiation with UVB and UVC, but not
5 UVA followed by treatment with low concentrations of formaldehyde (10 µM). UVA, UVB,
6 and UVC induced wavelength- and time-specific changes of DNA migration in comet assay.
7 They observed that DNA SSB induced by UVB or UVC irradiation alone were repaired within
8 3-6 hours of exposure, while cells with UV irradiation followed by formaldehyde exposure still
9 had the strand breaks at the same timepoints suggesting that formaldehyde is likely to contribute
10 to UV-induced carcinogenesis.

11
12 4.3.2. In Vitro Clastogenicity

13 Clastogenic effects, including increased MNs, CAs, and SCEs are also reported in a range
14 of in vitro study systems as shown in Table 4-80.

15 Several studies demonstrated formaldehyde-induced chromosomal aberrations (CAs) in a
16 variety of mammalian cells, such as CHO cells (Garcia et al 2009; Natarajan et al 1983), Chinese
17 hamster lung fibroblasts (Ishidate et al., 1981), Syrian hamster embryo cells (Hagiwara et al.,
18 2006; Hikiba et al., 2005), mouse lymphoma cells (Speit and Merk 2002) and human peripheral
19 blood lymphocytes (Dresp and Bauchinger, 1988; Schmid et al 1986; Miretskaya and
20 Shvartsman 1982) and fibroblasts (Levy et al., 1983).

21 Miyachi and Tsutsui (2005) measured the induction of sister chromatid exchanges
22 (SCEs) in Syrian hamster embryo (SHE) cells. Cells were exposed to 0, 3.3, 10, and 33 µM
23 formaldehyde for 24 hours. SCE levels after 3.3 µM formaldehyde were not different from
24 controls, but significant increases were observed at both 10 and 33 µM. Toxicity as measured by
25 reduced cloning efficiency was seen only at 33 µM (Miyachi and Tsutsui, 2005). The same
26 laboratory used SHE cells to measure the induction of CAs (Hikiba et al., 2005). Cells were
27 exposed to 0, 33, 66, and 99 µM formaldehyde for 24 hours prior to staining for analysis and the
28 percentages of aberrant metaphases were 0, 6, 6, and 71, respectively. The aberrations were
29 predominantly chromosome gaps and chromosomal breaks and exchanges. The relative colony-
30 forming efficiency remained high (at least 85%) for the concentrations of formaldehyde used in
31 the experiment (Hikiba et al., 2005).

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Table 4-80. In vitro clastogenicity of formaldehyde

Species	Cell/Tissue origin	Without activation	With activation	References
<i>Cytogenetic Assays</i>				
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

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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 μ M, 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 μ M 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."

1 Indicative that formaldehyde was inducing a clastogenic (rather than aneugenic) effect, 81% of
2 MNs in binucleated cells were centromere-negative.

3 In summary, formaldehyde forms MNs, SCEs, and CAs in isolated animal and human
4 cells following in vitro exposure (see Table 4-80).

6 4.3.3. In Vitro Mutagenicity

7 Mutations may occur during repair of formaldehyde-induced DNA damage (DPXs, DNA
8 adducts, SSBs, or clastogenic effects) or as a result of replication errors during mitogenesis. The
9 in vitro evidence for formaldehyde-induced mutations is strengthened by examining the
10 correlation between these genotoxic and clastogenic events and induction of mutations.

11 Therefore studies are presented with respect to relevance to one or more of the following lines of
12 evidence for mutagenicity recommended for consideration in the EPA guidance (U.S. EPA,
13 2005a): (1) that the chemical is DNA reactive and/or has the ability to bind to DNA, (2) that the
14 chemical generates positive results in in vitro mutagenic test systems (specifically gene
15 mutations and CAs), and (3) that the chemical induces indications of genetic damage in in vivo
16 tests (specifically gene mutations and CAs). Numerous studies have demonstrated
17 formaldehyde-induced DNA mutations under a variety of experimental conditions (reviewed in
18 IARC 1995, 2006; Ma and Harris 1988; Auerbach et al. 1977; Conaway et al 1996; NTP 2009).

20 4.3.3.1. Mutagenicity in Bacterial Systems

21 A number of research reports describe the mutagenicity of formaldehyde in bacterial test
22 systems using reverse and forward mutation assays as well as specific strains detecting deletions,
23 insertions and point mutations. Among the bacterial strains, *Salmonella typhimurium* TA102 and
24 the *Escherichia coli* strains containing an AT base pair at the primary reversion site are often
25 used to detect oxidative compounds, cross-linking agents and hydrazines. In an early National
26 Toxicology Program (NTP) collaborative study with three laboratories, formaldehyde
27 consistently tested positive for mutagenicity in *Salmonella typhimurium* strain TA100 in the
28 presence of a rat or hamster liver S9 activating system (Haworth et al., 1983). Formaldehyde
29 was mutagenic with and without metabolic activation in a number of other studies using reverse
30 mutation assays with *S. typhimurium* strains TA98, TA100, TA102, TA104, TA2638, and
31 TA2638a and *E. coli* strains WP2 (pkM101), WP2 *uvrA* (pkM101), and hrs/r30R (Ryden et al.,
32 2000; Dillon et al., 1998; Watanabe et al., 1996; Le Curieux et al., 1993; O'Donovan and Mee,
33 1993; Zielenska and Guttenplan, 1988; Schmid et al., 1986; Connor et al., 1983, 1985; Orstavik
34 and Hongslo, 1985; Takahashi et al., 1985; Fiddler et al., 1984; Frei et al., 1984; Donovan et al.,
35 1983), while other studies (Muller et al., 1993; Jung et al., 1992; Wilcox et al., 1990; Marnett et

1 al., 1985) show both positive and negative results. These results are summarized in Table 4-81
2 and some of the studies are described in greater detail.

3 Formaldehyde has been shown to be mutagenic in forward mutation assays using *S.*
4 *typhimurium* (Couch et al 1982; Donovan et al 1983; Temcharoen and Thilly 1983) as well as in
5 *E. coli* (Bosworth et al 1987). Temcharoen and Thilly (1983) examined the toxicity and
6 mutagenicity of *S. typhimurium* strain TM677, using forward mutation to 8-azaguanine
7 resistance, and have shown that formaldehyde induced both toxicity and mutagenicity at
8 minimum concentrations of 0.17 mM (-S9) and 0.33 mM (+S9). It has also been shown that
9 formaldehyde formed as an intermediate by oxidation at the methyl group of
10 *N*-nitrodimethylamine, a biologically active *N*-nitramine of environmental significance, is
11 mutagenic to *S. typhimurium* TA100 strain at low concentrations and toxic above 2 μ mol/plate
12 (Frei et al., 1984).

13 Bosworth et al (1987) developed a forward mutation assay in *E. coli* D494 *uvrB* strain
14 transformed with a multi-copy mutator plasmid pGW1700, in which mutations are scored by an
15 increase in ampicillin-resistant colonies after exposure of bacterial cells during the logarithmic
16 growth by the test chemicals. This assay is more sensitive to base-pair substitutions, but less
17 sensitive to frameshift mutations compared to Salmonella/microsome-based assays. In this
18 assay, the authors (Bosworth et al 1987) observed positive curvilinear response to formaldehyde
19 exposure. Crosby et al (1988) used four *E. coli* strains GP120, GP120A, 7-2, and 33694
20 containing the xanthine guanine phosphoribosyl transferase (*gpt*) gene (which detects point
21 mutations, deletions and insertions) tested the mutagenicity of formaldehyde by exposing for 1
22 hour at 4 and 40 mM concentrations. They observed 41% large insertions, 18% large deletions
23 and 41% point mutations. However, at 40 mM dose there were 92% point mutations, a majority
24 of them (62%) being transition mutations at a single AT base pair in the *gpt* gene. In the same
25 study they observed frameshift mutations in *E. coli* that was transformed with naked pSV2gpt
26 plasmid DNA exposed to 3.3 or 10 mM formaldehyde. Thus, the mutation pattern appear to
27 differ depending on the concentration of formaldehyde exposure to the bacterial strain as well as
28 the nature of DNA.

29 Formaldehyde has also been shown to induce primary DNA damage in *E. coli* and
30 mutagenic activity in the Ames fluctuation test in *S. typhimurium* TA100, TA102, or TA98
31 strains (Le Curieux et al., 1993).

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Table 4-81. Summary of mutagenicity of formaldehyde in bacterial systems

Species	Strain	Metabolic activation		References
		+S9	-S9	
<i>Mutagenicity Assays</i>				
Reverse Mutation				
<i>S. typhimurium</i>	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
<i>E. coli</i>	WP2, WP2 <i>uvrA</i>	ND	+	Takahashi et al., 1985
	H/R30R, HS30R <i>uvrA</i>	ND	+	Takahashi et al., 1985
	NG30 <i>recA</i> , O16 <i>polA</i>	ND	-	Takahashi et al., 1985
<i>S. typhimurium</i>	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
<i>E. coli</i>	WP2 <i>uvrA</i> /(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	
<i>S. typhimurium</i>	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
<i>E. coli</i>	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
<i>S. typhimurium</i>	TA102, 2638	ND	+	Watanabe et al., 1996
<i>E. coli</i>	WP2 (pKM101), WP2uvrA (pKM101)	ND	+	Watanabe et al., 1996
Mutagenicity Assays				
<i>S. typhimurium</i>	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
<i>E. coli</i> (Lac+ reversion)	WP3101P, WP3106P	+		Ohta et al 1999
<i>S. typhimurium</i>	TA102, 2638 ^d	ND	+	Ryden et al., 2000
Forward Mutation				
<i>S. typhimurium</i>	TM677	ND	(+)	Couch et al., 1982
		+	+	Donovan et al., 1983
		+	+	Temcharoen and Thilly, 1983
<i>E. coli</i>	D494uvrB	+		Bosworth et al 1987

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Table 4-81. Summary of mutagenicity of formaldehyde in bacterial systems (continued)

Species	Strain	Metabolic activation		References
		+S9	-S9	
Deletion Mutation				
<i>E. coli</i>	GP120, GP120A 7-2, 33694	ND	+ ^f	Crosby et al., 1988
Point Mutation				
<i>E. coli</i>	GP120, GP120A 7-2, 33694	ND	+	Crosby et al., 1988
Insertion Mutation				
<i>E. coli</i>	GP120, GP120A 7-2, 33694	ND	+	Crosby et al., 1988

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^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 WP2uvrA(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 *hisG* and *trpE* 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 (WP2uvrA). 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).

1 Dillon et al. (1998) employed salmonella strains TA100, TA102, and TA104 because of
2 the latter two strains being more sensitive to oxidative mutagens. Formaldehyde was clearly
3 mutagenic between 6 and 50 µg/plate in all three strains with and without metabolic activation
4 using Aroclor-induced S9 from male F344 rats or male B6C3F1 mice, except for an equivocal
5 response in TA102 with mouse S9 (Dillon et al., 1998). Using a set of six tester strains
6 (WP3101–WP3106) of *E. coli*, each reversible by a mutation involving a single DNA base pair
7 substitution, Ohta et al. (1999) determined that formaldehyde preferentially induced GC to TA
8 transversion mutations. Ryden et al. (2000) demonstrated a statistically significant increase in
9 the number of revertants in *S. typhimurium* TA102 (2.5-fold) and TA2638a (3-fold) strains by
10 formaldehyde at ≥17 µg/plate compared with solvent controls.

11 In summary, formaldehyde induces mutations in several bacterial strains containing an
12 AT base pair at the primary reversion site that are used to detect oxidative compounds and cross-
13 linking agents without metabolic activation by exogenous enzyme-activating systems. This
14 evidence is strengthened by examining the correlation between genotoxic and clastogenic events
15 and mutation induction.

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17 **4.3.3.2. Mutagenicity in Other Nonmammalian Cell Systems**

18 Formaldehyde has been shown to be mutagenic in several nonmammalian systems also.
19 It has been shown to cause gene conversion, strand breaks, crosslinks, homozygosis and related
20 damage in yeasts (*Saccharomyces cerevisiae*), forward and reverse mutations in molds
21 (*Neurospora crassa*), micronuclei formation in spiderworts (*Tradescantia pallida*), DNA
22 damage and mutations in several plants, genetic cross-over or recombination, sex-linked
23 recessive lethal mutations, dominant lethal mutations, heritable translocations and gene
24 mutations in insects (*Drosophila melanogaster*) and recessive lethal mutations in nematodes
25 (*Caenorhabditis elegans*), but failed to show micronuclei formation in newt larvae (*Pleurodeles*
26 *waltl*) (Reviewed in Conaway 1996; IARC 2006).

27 **4.3.3.3. Mutagenicity in Mammalian Cell Systems**

28 Several studies demonstrated the mutagenicity of formaldehyde in mammalian cells. In
29 its report, the Federal Panel on Formaldehyde underlined the role of formaldehyde as an inducer
30 of gene mutations and CA in a variety of test systems (Report of the Federal Panel on
31 Formaldehyde, 1982). Results from several studies are summarized in Table 4-82.

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Table 4-82. Mutagenicity in mammalian cell systems

Species/Strain	Cell/Strain	In Vivo test	Metabolic activation		References
			-S9	+S9	
<i>Mutagenicity Assays</i>					
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 (<i>Hprt</i> locus)	no	-		Merk and Speit 1998
	V79 cells <i>Hprt</i> 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

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Table 4-82. Mutagenicity in mammalian cell systems (continued)

Species/Strain	Cell/Strain	<i>In Vivo</i> <i>test</i>	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
<i>Mutagenicity Assays</i>					
Cell Transformation					
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,

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1 indicating that the mutagenicity of formaldehyde may be due to covalent alteration of DNA
2 bases. They have also shown that formaldehyde-induced DNA damage in human fibroblasts was
3 not susceptible to repair by the typical “long patch” excision repair mechanism.

4 Craft et al. (1987) measured the induction of mutations at the thymidine kinase (*tk*) locus
5 or at the ouabain resistance (*Oua^r*) locus in TK6 human lymphoblastoid cells. The *tk* mutations
6 can result from a variety of mutational events, including base pair substitution, small and large
7 deletions, and chromosome exchange events, while mutations to *Oua^r* require specific base pair
8 substitutions. Single treatment of formaldehyde (0, 15, 30, 50, 125, and 150 μM) for 2 hours
9 resulted in a nonlinear increase in *tk* mutagenesis with increasing slope $>125 \mu\text{M}$ (see
10 Figure 4-31). To explore a dose-response effect, cells were also exposed as follows: three
11 treatments of 50 μM for 2 hours or five treatments of 30 μM or 10 treatments of 15 μM for
12 2 hours (treatments were spaced 2–4 days apart) with multiple treatments causing an increase in
13 *tk* mutations, although their combined effect was less than a single treatment of equivalent $C \times t$
14 (150 μM for 2 hours). Lymphoblasts given four treatments of 150 μM formaldehyde for 2 hours
15 failed to induce mutations at the *Oua^r* locus. Dose-response increases were seen in all exposure
16 scenarios, with 30 μM being the level of statistical significance. There was little indication of a
17 dose-response effect until the cumulative concentration was greater than 100 μM .
18 Formaldehyde-induced DPXs were no longer evident after 24 hours of exposure; mutants
19 induced in the TK6 lymphoblast cell line showed a similar dose-response curve to the DPXs
20 measured immediately after exposure ended (Craft et al., 1987).

21 The same group also studied mutations induced at the X-linked hypoxanthine-guanine
22 phosphoribosyl transferase (HPRT) locus by eight repetitive treatments of 150 μM formaldehyde
23 in TK6 human lymphoblast cell line by Southern blot analysis, wherein half (14/30) of induced
24 mutants contained partial or complete deletions with most of the partial deletions showing
25 unique deletion patterns, while only a third (5/15) of spontaneous mutants had partial or
26 complete deletions, indicating that formaldehyde can induce large losses of DNA in human
27 lymphoblast cells (Crosby et al., 1988).

28 Liber et al. (1989) followed up the findings of Crosby et al. (1988) by performing
29 Southern blot, Northern blot, and DNA sequence analysis on the 16 induced and 10 spontaneous
30 human lymphoblast mutants not showing deletions. Northern blot analysis showed that the point
31 mutations fell into four categories: normal size and amount of RNA, normal size but reduced
32 amounts of RNA, reduced size and amounts of RNA, and no RNA. Sequence analysis of
33 recombinant DNAs from *hpert* mRNA in formaldehyde-induced mutants showed a preferential
34 AT to CG transversion at a specific site, with other changes represented to a lesser degree (Liber
35 et al., 1989).

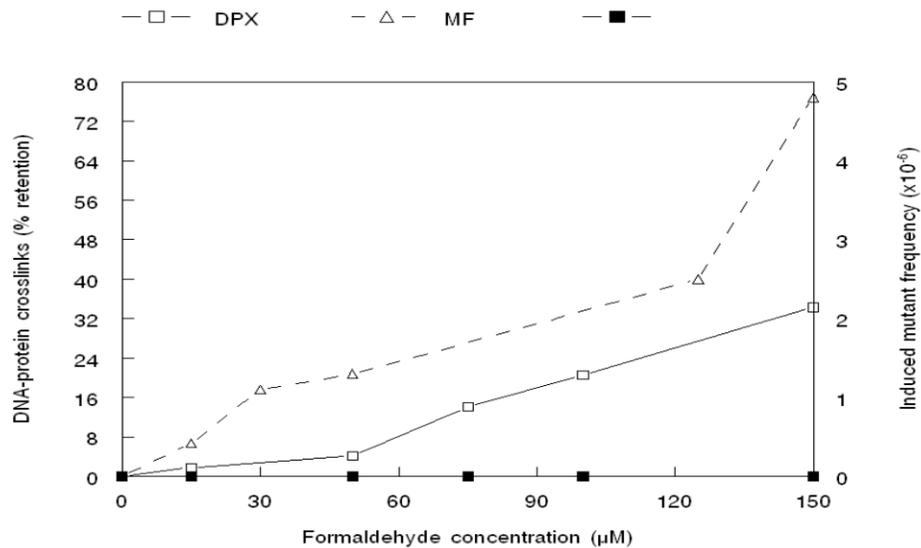


Figure 4-31. DNA-protein cross-links (DPX) and thymidine kinase (*tk*) mutants in TK6 human lymphoblasts exposed to formaldehyde for 2 hours.

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*[±] 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

1 human cell lines was consistent with the findings of Craft et al. (1987), ranging from 11.6 to
2 13 hours (Quievryn and Zhitkovich, 2000). In the same report, removal of DPXs from human
3 peripheral lymphocytes was much slower, with a half-life of 18.1 hours. This difference was
4 primarily in slower active repair of DPXs, with a $t_{1/2}$ of 66.6 hours for human lymphocytes
5 versus 23.3 hours for human cell lines (Quievryn and Zhitkovich, 2000).

6 Since DPX repair involves proteolytic removal of proteins from the DNA, Speit et al.
7 (2000) hypothesized that single peptides or small peptide chains cross-linked to the DNA are
8 critical to formaldehyde-induced mutation. However, these authors did not find significant
9 difference in the induction and repair of DPXs in normal and DNA repair-deficient cell lines but
10 observed increased susceptibility of the repair-deficient cell lines to formaldehyde-induced MN
11 induction. In this study, a normal human cell line (MRC5CV1), a xeroderma pigmentosum cell
12 line deficient in nucleotide excision repair (NER), and a Fanconi anemia cell line, which has a
13 genetic defect leading to hypersensitivity towards DDXs, were exposed to 125, 250, and 500 μ M
14 formaldehyde for 2 hours. The authors suggest that more than one repair pathway is involved in
15 the repair of cross-links and that the altered NER pathway has more severe consequences to
16 formation of CAs than disturbed cross-link repair (Speit et al., 2000).

17 The correlation of early DPX formation and mutation is at first counterintuitive since the
18 cross-linking of protein to DNA inhibits DNA replication. Without active DNA replication,
19 formaldehyde-DNA adducts and DPXs would not induce replication error and would be unlikely
20 to result in a change in DNA sequence or mutation. Recent evidence indicates that residual
21 peptides and short polypeptides that remain cross-linked to DNA after DPX removal may in fact
22 be the cause of DPX-associated, formaldehyde-induced mutation (Speit et al., 2000).

23 A study by Merk and Speit (1998) indicated that formaldehyde-induced DPXs did not
24 result in direct gene mutations in the *hprt* locus of V79 Chinese hamster cells, leading the
25 authors to speculate that formaldehyde was not mutagenic. Since, the *hprt* locus in the V79
26 Chinese hamster cell line is primarily sensitive to point mutations and other studies show the
27 formation of deletion mutations by formaldehyde at the same locus in human lymphoblasts
28 (Crosby et al., 1988), Merk and Speit (1998) concluded that the *hprt* mutation assay is insensitive
29 to deletion mutations.

30 Later, using the mouse lymphoma assay, Speit and Merk (2002) demonstrated that
31 exposure to formaldehyde for 2 hours was mutagenic in a concentration-dependent manner in the
32 L5178Y mouse lymphoma cells, which was mainly contributed by a strong increase in small
33 colony mutants, suggestive of CAs (Speit and Merk, 2002). Detailed analysis of both
34 spontaneous and formaldehyde-induced lesions indicates that recombination or deletion of DNA
35 from the *tk* locus was primarily responsible for the loss of heterogeneity, thereby leading to the

1 observed mutant phenotype. Therefore, it is believed that formaldehyde is mutagenic in the
2 L5178Y cell mouse lymphoma system by a clastogenic mechanism rather than through point
3 mutations. This finding is consistent with that of Craft et al. (1987), who demonstrated
4 formaldehyde mutagenicity at the *tk* locus of TK6 cells, and also with the findings of Grafström
5 et al. (1984), who demonstrated increased SSB formation in formaldehyde-exposed cell lines.

6 Formaldehyde has also been shown to induce cell transformation in mouse embryo
7 fibroblasts (Ragan and Boreiko 1981; Boreiko et al 1983; Frazelle et al 1983) . At low
8 concentrations of 0.017 mM formaldehyde has shown to cause cell transformation in C3H10T1/2
9 mouse cells (Ragan and Boreiko 1981) and hamster kidney cells in vitro (Plenser and Hansen
10 1983).

11 More recently, Shaham et al. (2003) examined the frequency of DPXs and the incidence
12 of mutant versus wild type p53 tumor suppressor genes in the peripheral blood lymphocytes of a
13 cohort of workers exposed to formaldehyde. The adjusted mean levels of DPXs were greater in
14 the lymphocytes of exposed subjects compared with those of unexposed subjects, and exposure
15 to formaldehyde increased the likelihood of their having a higher level of pantropic p53
16 (>150 pg/mL). The authors speculated on a possible causal relationship between DPXs and
17 mutations in p53. Recio et al (1992) demonstrated point mutations in the p53 tumor suppressor
18 gene in 45% (5 out of 11) of the primary nasal squamous cell carcinomas (SCCs) obtained from
19 F344rats that were chronically exposed to 15 ppm formaldehyde for 2 years (Recio et al ., 1992).

20 In summary, the results of in vitro experiments demonstrate the mutagenicity of
21 formaldehyde. Mutagenicity is observed below levels of significant cytolethality in mammalian
22 cell lines. Formaldehyde is clearly a DNA-reactive genotoxicant inducing lesions (DPXs) that
23 show clastogenicity (SSBs, MNs, etc.). The experiments by Speit and Merk (2002) explore
24 mechanistic links between DPXs, clastogenicity, and the observed locus-specific mutations in
25 the mouse lymphoma in vitro testing system.

26 27 4.3.4. In Vivo Mammalian Genotoxicity

28 **4.3.4.1. Genotoxicity in Laboratory Animals**

29 As discussed above, formaldehyde is clearly reactive at the POE in animal studies,
30 resulting in increased DPXs in the nasal mucosa. Despite formaldehyde's reactivity and
31 mutagenicity in isolated mammalian cells, clear evidence of mutagenicity does not emerge from
32 animal bioassays (see Table 4-83).

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Table 4-83. Genotoxicity in laboratory animals

Species/Strain	Cells/Organ/Tumor	Result	References
<i>Cytogenetic Assays</i>			
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

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‘+’ indicates a positive test result.

‘-’ indicates a negative test result.

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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

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1 positive control group dosed with 100 mg/kg cyclophosphamide (CP). Mice from all groups
2 were administered 1 mg/kg colcemid (which arrests cells in metaphase), 28 h after dosing with
3 test compounds. Two hours after colcemid treatment, mice were sacrificed; bone marrows from
4 femurs were prepared and analyzed the metaphase chromosomes for structural aberrations. They
5 reported that formalin, methanol, water and CP, respectively produced structural chromosomal
6 aberrations (%) such as aneuploidy (101, 65, 6, 3), breaks (3, 3, 1, 32), exchanges (40, 24,
7 10,17), aberrant chromosomes with gaps (45, 32, 2.1, 63) and without gaps (43, 27, 1.7, 55) in
8 bone marrow cells of male B6C3F1 mice. The cytogenetic effects seen in bone marrow suggest
9 that formalin or other chemicals given at a single high dose (gavage) were able to reach bone
10 marrow and induce genotoxicity, specifically, aneuploidy and sister chromatid exchanges. It is
11 recognized that the results reported were preliminary; however, they are significant in terms of
12 genotoxic potential of formaldehyde exposure via oral route of exposure.

13 In a different study Natarajan et al. (1983) failed to detect significant differences in MN
14 induction in bone marrow cells or CAs in spleen cells of male and female CBA mice given I.P.
15 6.25, 12.5, and 25 mg/kg formaldehyde compared with saline-treated controls. However, the
16 same study showed a positive induction of MNs and CAs in vitro. The authors suggest that the
17 lack of genotoxicity in vivo may be due to the inability of formaldehyde to reach the target cells
18 in sufficient quantity to induce biological effects.

19 Kligerman et al. (1984) also found no difference in the incidence of SCEs or
20 chromosome breakage in the peripheral lymphocytes of male and female F344 rats exposed to
21 formaldehyde in air at 0.5, 6, or 15 ppm (0.61, 7.36, or 18.4 mg/m³) 6 hours/day for 5 days.
22 However, in a different study (Migliore et al., 1989), clastogenic effects, such as increased MNs
23 and CAs, were reported in GI epithelial cells of male Sprague-Dawley rats after oral exposures to
24 200 mg/kg formaldehyde. In this study, micronucleated cells and nuclear anomalies were
25 increased in a time-dependent manner in the stomach, duodenum, ileum, and colon of rats, and
26 the mitotic index was unchanged for these cells compared with controls at 16, 24, and 30 hours.
27 These clastogenic effects were seen without regenerative cell proliferation, supporting
28 formaldehyde-induced mutations as primary effects of formaldehyde rather than secondary to
29 regenerative cell proliferation.

30 Kitaeva et al. (1990) observed cytopathological and cytogenetic effects of formaldehyde
31 chronic inhalation in 0.5 and 1.5 mg/m³ doses in the female rat's germ cells and bone marrow
32 cells, where formaldehyde-induced harmful effects were seen in germ cells at <1.5 mg/m³ doses,
33 while the reliable clastogenic and cytogenetic effects on the marrow cells were induced even at
34 the 0.5 mg/m³ dose, suggesting differences among effects of small doses of formaldehyde on
35 different cell systems.

1 Dallas et al. (1992) observed a slight increase (7.6 and 9.2%) in CAs in pulmonary lavage
2 cells from male Sprague-Dawley rats exposed to 15 ppm (18.4 mg/m³) formaldehyde in air
3 6 hours/day, 5 days/week for 1 or 8 weeks by inhalation compared with corresponding controls
4 (3.5 and 4.8%), respectively. However, the small study, limited as it was to five animals/group,
5 showed statistically significant increase at the highest dose tested (15 ppm) but not at lower
6 doses (0.5 and 3 ppm). In the same study, no clastogenic effects were seen in bone marrow,
7 which is consistent with formaldehyde acting primarily at the site of first contact.

8 Speit et al (2009) investigated the genotoxicity of formaldehyde in peripheral blood
9 samples of Fischer-344 rats exposed to 0 to 15 ppm formaldehyde by whole-body inhalation for
10 4 weeks (6 h/day, 5 days/week). In this study, the authors found no significant increase in the
11 genotoxic assays such as comet assay with or without gamma-irradiation of blood samples (DNA
12 migration as determined by tail movement or intensity), sister chromatid exchange (SCE) assay
13 and micronucleus test (MNT) compared to controls. However, rats given 50 mg/kg
14 methylmethane sulfonate (MMS) by gavage for 4 hrs (positive control for Comet and SCE
15 assays) or 10 mg/kg cyclophosphamide (CP) given twice orally (positive control for MNT)
16 induced significant increase in genotoxicity in this study. The lack of genotoxicity in this study
17 is not surprising since earlier studies by Casanova-Schmitz et al (1984a) have shown that
18 formaldehyde does not cause toxicity to bone marrow possibly due to the inability of this
19 chemical to reach the bone marrow. Although MMS and CP used in this study were positive in
20 the genotoxicity assays, the data from positive controls can not be used for validation since the
21 exposure routes of formaldehyde (inhalation) and the positive controls (oral) were different.

22 No animal studies have examined clastogenic effects of formaldehyde in nasal or
23 respiratory epithelial cells. Therefore, it is unknown whether similar changes would occur in
24 response to exposure to formaldehyde via inhalation. However, the negative finding in bone
25 marrow cannot be considered definitive evidence on the question of the mutagenic potential of
26 formaldehyde for cells present at the POE. With weak positive results in pulmonary lavage cells
27 and clear clastogenicity in GI epithelial cells below exposures that trigger regenerative cell
28 proliferation, the existing evidence, however incomplete, supports the concept of genotoxic
29 action of formaldehyde at the POE.

30 31 **4.3.4.2. Genotoxicity in Humans**

32 The majority of the studies on the effects of formaldehyde in exposed humans have
33 measured various cytogenetic endpoints, such as MNs, SCEs, or CAs in nasal and oral mucosal
34 cells (considered to be in direct contact with formaldehyde) as well as peripheral lymphocytes.
35 Since genotoxicity at the proximal sites (oral, nasal) can be readily linked to the reactive nature

1 of formaldehyde, these studies are discussed first, noting where researchers also collected blood
2 lymphocyte samples. A subsequent discussion is focused on results in blood lymphocytes.
3 Finally, the few studies that measured DPXs in exposed humans are discussed. Table 4-89
4 provides a summary of human cytogenetic studies of formaldehyde.

6 **4.3.4.2.1. Nasal, buccal, and oral mucosal cells.**

7 Epithelial cells of the URT and oral cavity are potential targets of formaldehyde's DNA
8 reactivity and genotoxicity. Several studies indicate that formaldehyde exposure results in
9 measurable increases in SCEs, MN formation, and DPXs in nasal, buccal, and oral mucosal cells;
10 however, these genotoxic effects vary with the type of exposure. Study quality, sample size,
11 availability of exposure measurements, and assay methodology may in part contribute to
12 variability in study findings. The studies fall into three general categories: workers (industrial or
13 professional), students and staff attending anatomy and mortuary science courses, and subjects in
14 a controlled clinical trial.

15 Ballarin et al. (1992) observed significantly higher frequency of micronucleated cells in a
16 formaldehyde exposed group in a plywood factory compared with controls (0.9 ± 0.47 versus
17 0.25 ± 0.22 , $p < 0.01$). In this study, the frequency of MNs and cytology of respiratory nasal
18 mucosal cells was examined in 15 nonsmokers exposed to levels of formaldehyde that ranged
19 between 0.1 and 0.39 mg/m³ (~0.32 ppm) for an average of 6.8 years. Exposed subjects were
20 compared with age- and sex-matched controls.

21 Ye et al. (2005) reported significant increases in MNs per thousand cells in nasal mucosal
22 cells for 18 nonsmoking workers (2.70 ± 1.50) in a formaldehyde manufacturing plant in the
23 Hubei province of China as compared with controls (1.25 ± 0.41). In addition, higher
24 frequencies of SCEs in peripheral lymphocytes of workers were also reported (8.24 ± 0.89 versus
25 6.38 ± 0.41). In this study, the average age of workers was 29 ± 6.8 years, the average duration
26 at work was 8.5 years (range 1–15 years), and the reported 8-hour TWA was 0.985 mg/m³
27 (0.8 ppm). The control group consisted of 23 undergraduate students with an average age of
28 19 ± 2.3 years. The 8-hour TWA in the student dormitories was 0.011 mg/m³ (9 ppb). A group
29 of 16 waiters with an average exposure duration of only 12 weeks and an 8-hour TWA of
30 0.107 mg/m³ (90 ppb) was also included in the study. The incidence of MNs and SCEs in the
31 waiters was the same as that in controls. Overall, results from this study suggest that the
32 genotoxic potential of high-level formaldehyde exposure may have occupational risks in long-
33 term exposure.

34 However, in a different study, Speit et al. (2007b) showed that formaldehyde did not
35 induce MNs in exfoliated buccal mucosa cells of humans exposed up to a maximum of 1 ppm

1 and a cumulative exposure of 13.5 ppm-hours over 2 weeks. In this study, volunteers exposed to
 2 formaldehyde in closely controlled conditions (4 hours/day for 10 days) with a complex
 3 exposure schedule, amounting to a cumulative total of 13.5 ppm-hours (16.6 mg/m³-hours), were
 4 used. Samples of the buccal mucosa were taken from subjects 1 week before the start of the
 5 experiment, at the start of the experiment, at the conclusion of the series of exposures, and at 7,
 6 14, and 21 days after the completion of exposure. Thus, the subjects served as their own
 7 controls. Two thousand cells per data point were assessed for the frequency of MNs on slides
 8 that were coded by an independent quality assurance organization. As shown in Table 4-84, the
 9 frequency of MN formation was statistically unchanged from that in controls. The apparent
 10 slight increase in subjects evaluated at the conclusion of exposure was caused by frequencies of
 11 MNs in two subjects (5.0 and 4.5 MNs per 1,000 cells). The data as reported show a high
 12 variability, where the SD approaches or exceeds the mean for each sample point, suggestive of
 13 data with an asymmetrical distribution.

14
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Table 4-84. MN frequencies in buccal mucosa cells of volunteers exposed to formaldehyde

Sampling point	Group	MN/1000 cells (± SD)
<i>Control data</i>		
1 week before exposure	1	0.95 ± 0.67
Immediately before exposure series	2	0.86 ± 0.84
<i>Test data</i>		
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

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^aStatistically 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

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1 containing formaldehyde following an 85-day exposure period (see Table 4-85). Overall,
 2 students were exposed to 0.33 ppm (0.4 mg/m³) formaldehyde as an 8-hour TWA on days when
 3 embalming was performed (an average of 6.9 embalming). Blood, oral, and nasal samples were
 4 collected pre- and postexposure. As shown in Table 4-85, nasal epithelial MNs increased by
 5 22% (frequency of micronucleated lymphocytes increased by 28%). By contrast, SCE frequency
 6 decreased by 7.5% after formaldehyde exposure.

7
 8 **Table 4-85. MN and SCE formation in mortuary science students exposed to**
 9 **formaldehyde for 85 days**
 10

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

11
 12 ^aStatistically significant ($p < 0.05$), as calculated by the authors.
 13 Source: Suruda et al. (1993).
 14
 15

16 Another group (Titenko-Holland et al., 1996) also reported a significant increase in MN
 17 frequency of buccal, but not nasal, epithelial cells from mortuary students exposed to embalming
 18 fluid. In this study, 28 out of 35 students were sampled before and after a 90-day embalming
 19 class. The mean formaldehyde exposure for the subjects providing data on buccal cell MNs was
 20 14.8 ± 7.2 ppm-hours (18.2 ± 8.8 mg/m³-hours) for the entire 90-day period and 16.5 ± 5.8 ppm-
 21 hours (20.3 ± 7.1 mg/m³-hours) for students providing data on nasal cell MNs. Cells were
 22 recorded as having either whole chromosomes with centromeres (MN⁺) or acentric fragments
 23 and no centromeres (MN⁻). Cells with multiple nuclei were present only in samples taken after
 24 exposure to embalming fluid. There was a ninefold increase in the MN frequency in buccal cells
 25 ($p < 0.5$) and only a twofold increase ($p > 0.05$) in nasal cells. In addition, there was a twofold
 26 increase in the MN⁺ frequency in buccal cells (see Table 4-86). The authors suggested that
 27 chromosomal breakage appears to be the primary mechanism of MN formation.

28 Ying et al. (1997), however, observed higher frequencies of MNs in the nasal exfoliative
 29 cells (3.85 ± 1.48 versus 1.20 ± 0.676, paired t-test, $p < 0.001$) and oral exfoliative cells (0.857 ±
 30 0.558 versus 0.568 ± 0.317, $p < 0.001$) after formaldehyde exposure, although there was no
 31 significant increase in the frequency of lymphocyte MNs ($p > 0.05$) in students exposed to
 32 formaldehyde in anatomy classes (three classes per week for 3 hours over an 8-week duration).
 33 In this study, blood samples and nasal swabs were collected before and after the study. The
 34 TWA concentration of formaldehyde in anatomy laboratories and student dormitories was 0.508

1 $\pm 0.299 \text{ mg/m}^3$ and $0.012 \pm 0.0025 \text{ mg/m}^3$, respectively, suggesting that nasal mucosa cells
 2 exposed through respiration are the primary target of formaldehyde-induced genotoxicity.

3
 4 **Table 4-86. Incidence of MN formation in mortuary students exposed to**
 5 **formaldehyde for 90 days**
 6

Sampling point	Buccal cells (<i>n</i> = 19)			Nasal epithelial cells (<i>n</i> = 13)		
	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
<i>p</i> value	0.007	0.08	0.005	0.20	0.31	0.03

7
 8 ^aStatistically significant at the level shown, as calculated by the authors.
 9

10 Source: Titenko-Holland et al. (1996).
 11
 12

13 Ying et al. (1997), however, observed higher frequencies of MNs in the nasal exfoliative
 14 cells (3.85 ± 1.48 versus 1.20 ± 0.676 , paired t-test, $p < 0.001$) and oral exfoliative cells ($0.857 \pm$
 15 0.558 versus 0.568 ± 0.317 , $p < 0.001$) after formaldehyde exposure, although there was no
 16 significant increase in the frequency of lymphocyte MNs ($p > 0.05$) in students exposed to
 17 formaldehyde in anatomy classes (three classes per week for 3 hours over an 8-week duration).
 18 In this study, blood samples and nasal swabs were collected before and after the study. The
 19 TWA concentration of formaldehyde in anatomy laboratories and student dormitories was 0.508
 20 $\pm 0.299 \text{ mg/m}^3$ and $0.012 \pm 0.0025 \text{ mg/m}^3$, respectively, suggesting that nasal mucosa cells
 21 exposed through respiration are the primary target of formaldehyde-induced genotoxicity.

22 In a different study (Ying et al., 1999), however, the same group showed that exposure to
 23 formaldehyde affected the composition of lymphocyte subsets (B cells, total T cells, T helper-
 24 inducer cells, T cytotoxic-suppressor cells), but no significant difference was reported between
 25 lymphocyte proliferation rate and SCEs at the given levels and durations of formaldehyde
 26 exposure. This study involved 23 nonsmoking students exposed to $0.508 \pm 0.299 \text{ mg/m}^3$
 27 formaldehyde for a period of 8 weeks (3 hours, 3 times per week).

28 Burgaz et al. (2002) reported significantly ($p < 0.05$) higher mean MN frequencies in
 29 buccal mucosal cells from shoe workers as well as anatomy and laboratory workers ($0.62 \pm$
 30 0.45% and $0.71 \pm 0.56\%$, respectively) compared with unexposed controls ($0.33 \pm 0.30\%$). In
 31 this study, the measured air concentrations of formaldehyde in the breathing zone of the anatomy
 32 and pathology laboratory workers were between 2 and 4 ppm (2.5 and 5 mg/m^3). MN count per

1 3,000 cells was measured in buccal smears from shoe workers and from anatomy and pathology
2 staff, and eighteen male university staff were used as controls.

3 In a critical review, Speit and Schmid (2006) examined data from studies that have
4 reported the formation of MNs in nasal or buccal cells of persons either environmentally or
5 occupationally exposed to formaldehyde. The authors identified a number of issues relating to
6 study design, exposure regimen, and confounding factors, including MN levels in nasal and
7 buccal cells well below established background levels, reports limited by the number of cells
8 observed, variation in standard techniques, and nonconcordance between buccal and nasal
9 findings. However, the authors concluded that, despite these limitations, the weight of evidence
10 supports the finding that formaldehyde may be genotoxic in human cells in direct contact with
11 formaldehyde.

12 13 **4.3.4.2.2. *Peripheral blood lymphocytes.***

14 Mature lymphocytes are present at the POE as intraepithelial lymphocytes and within
15 germinal centers in the mucosa. Because more lymphocytes may be available in the nasal
16 mucosa than the oral mucosa, mouth versus nose breathing may contribute to variability in
17 findings. Since some of the lymphocytes traffic around the body, it is reasonable to find
18 clastogenic effects in these relatively long-lived cells reflected in peripheral blood lymphocytes.
19 Thus, lymphocytes proliferating in response to antigen would be more vulnerable to DNA
20 reactivity of formaldehyde and to the clastogenic effects in general.

21 A cytogenetic evaluation by Fleig et al. (1982) of 15 employees exposed for an average
22 of 28 years in a formaldehyde manufacturing plant revealed no statistically significant increase
23 in the frequency of CAs in peripheral blood lymphocytes compared with a matched control
24 group. Although some of the workers are smokers in this study, smokers did not show an
25 increased incidence of CAs. Likewise, in a different study (Thomson et al., 1984), no exposure-
26 related differences were evident in the frequency of CAs and MNs in lymphocytes from six
27 pathology workers and five unexposed controls. This study did not account for differences in
28 smoking.

29 Bauchinger and Schmid (1985) observed an increased incidence of CAs (dicentric and
30 ring chromosomes) in the peripheral lymphocytes of 20 male paper mill workers and supervisors
31 exposed to formaldehyde (average exposure of 14.5 ± 7.2 years) compared with unexposed
32 workers. When workers and supervisors were analyzed separately, significant increases were
33 only seen for supervisors. The average length of exposure for supervisors ($n = 11$) and workers
34 ($n = 9$) was 18.9 years and 7.2 years, respectively. Information regarding formaldehyde
35 concentrations for the two groups was not provided. However, the incidence of SCEs among

1 workers was actually slightly lower than among the 20 controls. In contrast, Ye et al., (2005)
2 observed that the frequency of SCEs in peripheral lymphocytes of 18 nonsmoking formaldehyde
3 factory workers (mean exposure duration of 8.6 years) was significantly increased over
4 nonsmoking controls (8.24 ± 0.89 versus 6.38 ± 0.41) (described in Section 4.3.4.2.1).

5 Vargová et al. (1992) observed that the percentage of aberrant cells and number of breaks
6 per cell in the peripheral blood lymphocytes of formaldehyde-exposed workers was 3.08 and
7 0.045 versus 3.6 and 0.030 in controls in a pressed board factory, respectively, suggesting both
8 groups to be at an increased risk. However, normal unexposed population had only 1–2%
9 aberrant cells. The authors also noted that the mitotic index was significantly decreased in
10 exposed workers compared with controls. This study did not specify about the smoking
11 background of the two groups, however, the authors indicate that both exposed and unexposed
12 workers have similar habits and social status.

13 Kitaeva et al. (1996) evaluated the genotoxic effects of formaldehyde among 15
14 industrially exposed workers and 8 academic laboratory instructors and observed an increase in
15 the frequencies of CAs and MNs in the lymphocytes of exposed subjects compared with
16 6 unexposed controls. This study did not provide information about smoking history.

17 Shaham et al. (1996, 1997) found significantly higher levels of DPXs and SCEs in
18 peripheral blood lymphocytes of workers occupationally exposed to formaldehyde (physicians
19 and technicians) compared with unexposed control workers. The authors also observed a linear
20 relationship between years of exposure to formaldehyde and levels of DPXs and SCEs.
21 Although both studies have smokers and nonsmokers, smoking did not influence DPX formation
22 in either of the studies (Shaham et al 1996; 1997).

23 Formaldehyde-induced genotoxicity has also been reported in peripheral blood
24 lymphocytes of anatomy class students and mortuary workers. Vasudeva and Anand (1996) did
25 not observe significant differences in the incidences of CAs between the formaldehyde exposed
26 students and the matched, unexposed controls. In this study, peripheral blood lymphocytes from
27 30 medical students exposed to formaldehyde in a gross anatomy laboratory for 15 months with
28 average exposures of less than 1 ppm (1.23 mg/m^3) formaldehyde were used. No smoking
29 history is available for this study.

30 He et al. (1998) used the cytokinesis-blocked MN (CBMN) assay to detect the frequency
31 of micronucleated peripheral lymphocytes in 13 students exposed to formaldehyde during a
32 12-week (10 hours/week) anatomy class. Sampling of breathing zone air showed a mean
33 concentration of 2.37 ppm (3.17 mg/m^3). Ten students from the same school, without exposure
34 to formaldehyde, were used as controls. All study subjects were nonsmokers. CAs and SCEs
35 were observed in both groups, and there were significant increases ($p < 0.01$) in the frequencies

1 of micronucleated cells and CAs in the formaldehyde-exposed group compared with the control
2 group.

3 In a study involving 97 plasticware workers (34 males and 63 females) exposed to 0.5 to
4 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
5 25 years, Lazutka et al (1999) observed significantly higher CAs than controls (nonexposed
6 donors matched by age and similar smoking habits as the exposed workers). Although workers
7 with short and long exposures showed significant increases in the frequency of CAs, the
8 cytogenetic damage did not increase with exposure duration.

9 Sari-Minodier et al. (2001), using the CBMN assay in anatomy/pathology laboratory
10 workers who were occupationally exposed to formaldehyde for a mean exposure period of 9 yrs
11 (range 1-16 yrs), reported higher frequency of micronucleated peripheral blood lymphocytes
12 ($18.8\% \pm 13.1$) than in matched controls ($8.8\% \pm 4.4$).

13 Shaham et al. (2002) observed a mean \pm S.E. number of 0.27 ± 0.003 SCEs per
14 chromosome in the peripheral lymphocytes of a hospital pathology department workers with a
15 mean exposure period of 15.4 yrs (range 1-39 yrs) compared with 0.19 ± 0.002 SCEs in control
16 subjects from the administrative staff of the same hospital ($p < 0.01$) after adjusting for age, sex,
17 smoking habits, etc. This study involved 90 individuals employed in 14 hospital pathology
18 laboratories and 52 unexposed controls.

19 Yu et al. (2005) reported dose-dependent increase in MNs and comet assay parameters
20 (olive tail moment and comet tail length) in peripheral lymphocytes in 151 workers from two
21 plywood factories compared with 112 unexposed controls. Both exposed and control subjects
22 are nonsmokers in this study. The TWA exposure level in the working environment was
23 $0.1\text{--}7.88 \text{ mg/m}^3$ ($0.08\text{--}6.42$ ppm) formaldehyde compared with a background level of <0.01
24 mg/m^3 (<0.008 ppm) formaldehyde applicable to controls. In the comet assay, the authors
25 observed olive tail moments averaging 0.93 (0.78–1.1), 3.03 (2.49–3.67), and 3.95 (3.53–4.43)
26 for control, low-, and high-exposure individuals, respectively. For the same subjects, comet tail
27 lengths were 6.78 (6.05–7.6), 11.25 (10.12–12.5), and 12.59 (11.8–13.43), respectively. In the
28 CBMN assay, MNs/100 cells were 0.27 ± 0.13 , 0.41 ± 0.25 , and 0.65 ± 0.36 , respectively, for
29 control, low-, and high-exposure individuals.

30 In a population of 18 workers exposed to formaldehyde at a plant in China, with a mean
31 employment of 8.5 years (range 1 to 15 years), Ye et al (2005) examined nasal epithelial cells
32 and lymphocytes for cytogenetic effects. This study also included a second group of 16 waiters
33 who worked in a newly fitted ball room for 12 weeks with a low level exposure to formaldehyde
34 from building material, tobacco smoke and furniture and a group of 23 college students as a
35 control group. The background indoor air concentration of 0.009 ppm formaldehyde was

1 reported in students' dorms. Significantly increased frequencies of MNs in the nasal mucosal
2 cells and SCEs in peripheral blood lymphocytes were reported for the workers, but not the
3 waiters in this study.

4 Orsière et al. (2006) reported no apparent effect on the DNA damage in peripheral blood
5 lymphocytes as assessed by a chemiluminescence microplate assay in pathology and anatomy
6 laboratory workers ($n = 59$) before and after a 1-day exposure to formaldehyde. This study had
7 59 exposed workers and 37 controls with similar smoking history. However, with the CBMN
8 assay, the authors reported statistically significant differences in the frequency of binucleated
9 micronucleated cells (1.69 ± 0.93 versus $1.11 \pm 0.6\%$) in exposed versus control subjects.

10 Discrimination between clastogenic and aneugenic events by using fluorescence *in situ*
11 hybridization (FISH) technique with a pan-centromeric DNA probe resulted in a higher rate of
12 binucleated micronucleated cells (1.91 ± 1.01 versus $1.19 \pm 0.56\%$ in controls) and showed that
13 the frequency of centromeric nuclei was higher in the exposed group than in controls, though not
14 significantly. Among the centromeric MNs, the frequency of MNs with only one centromere
15 (C1+MN) was significantly greater in pathologists/anatomists than in controls (1.1 ± 0.62 versus
16 $0.31 \pm 0.24\%$, $p < 0.001$). The authors interpreted their data on monocentromeric nuclei in
17 anatomists/pathologists as an indication that formaldehyde exposure might be associated with
18 aneugenic (rather than clastogenic) events.

19 Based on pooled analysis of two reports (Iarmarcovai et al., 2006a, b) (see Table 4-87),
20 MN frequency ratios in the peripheral lymphocytes of cancer patients, welders, and
21 anatomists/pathologists were significantly increased compared with the corresponding controls.
22 The data were taken from three biomonitoring studies by using CBMN/FISH. The incidence of
23 MNs was scored and then evaluated further for the presence of centromere-negative MNs
24 (C-MNs), centromere-positive MNs (C+MNs), and, for the latter case, those containing a single
25 centromere (C1+MNs) and those containing two or more centromeres (Cx+MNs). Applying
26 their findings to considerations of the aneugenic mechanism of action of formaldehyde, the
27 authors hypothesized that the use of centromeric signals enables the identification of endpoints
28 representing impaired chromosomal migration (with C1+MN formation) or centrosome
29 amplification (with Cx+MN formation).

30 Recently, Costa et al. (2008) observed a significant increase in the genotoxicity of
31 formaldehyde-exposed pathological anatomy laboratory workers ($n = 30$) compared with
32 controls ($n = 30$) in cytogenetic assays. In this study, the authors evaluated the level of exposure
33 to formaldehyde near the breathing zone of workers, and TWA of exposure was calculated for
34 each subject, giving a mean level of exposure to be 0.44 ± 0.08 ppm (range: 0.04–1.58 ppm). As
35 compared with control subjects, peripheral blood lymphocyte cultures of formaldehyde exposed

Table 4-87. Multivariate regression 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)

^aBolded 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 \pm SEM (range)	Mean SCE \pm SEM (range)	Mean TL (μ M) \pm 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).

1 4.3.5. Summary of Genotoxicity

2 Formaldehyde's genotoxicity has been demonstrated in a variety of in vitro and in vivo
3 test systems including bacteria (*S. typhimurium* and *E. coli*), molds (*N. crassa*), yeasts (*S.*
4 *cerevesiae*), fruit flies (*Drosophila*), plants, several mammalian primary cells and cell lines,
5 tumor cell lines, human lymphoblasts and lymphocytes, in vivo studies in rodents (different
6 strains of mice, rats and hamsters), nonhuman primates (monkeys) and peripheral blood
7 lymphocytes of occupationally exposed workers (industrial as well as professional) workers.
8 Among these test systems several genotoxicity endpoints, such as DPXs (or DPCs), DNA
9 adducts, DDXs, SSB, cytogenetic endpoints (MNs, SCEs, CAs), mutations (point, dominant
10 lethal, deletion, heritable), *p53* mutations and mutant *p53* expression in rat nasal SCCs, other
11 genetic endpoints such as UDS, DNA repair inhibition, etc), and clastogenic effects in human
12 buccal, nasal and peripheral blood lymphocytes and chromosomal changes (monosomy and
13 trisomy) in lymphocytes.

14 Formaldehyde forms predominantly DPXs that are detected in cell-free systems and
15 single cells in vitro. DPXs are also formed in nasal epithelia but not in extranasal passages of
16 rodents, which are completely removed within a day after formation. In vivo data in human and
17 mammalian cells demonstrate that formaldehyde is genotoxic at the site of first contact,
18 including cells of the mouth or the nose. DPXs are also detected in nasal and extranasal tissues
19 of monkeys, suggestive of direct effects of formaldehyde in tissues that correspond to observed
20 tumor sites (nasal and nasopharynx) in humans. In addition, this is used as a basis for cross-
21 species comparison with humans. Formaldehyde-DNA adducts are labile and constitute a minor
22 fraction of the DNA-reaction products and are less likely to play an important role in the
23 genotoxicity of formaldehyde. There is limited literature on the formation of DDXs after
24 formaldehyde exposure, but its role in genotoxicity is not clear.

25 Formaldehyde clastogenicity has been demonstrated by the induction of SCEs, SSBs,
26 MNs, and CAs in cultured mammalian cells. Formaldehyde induces mutations in salmonella and
27 escherichia bacterial strains that contain an AT base pair at the primary reversion site that is used
28 to detect oxidative compounds and cross-linking agents without metabolic activation by
29 exogenous enzyme-activating systems. Formaldehyde induces mutations in cultured mammalian
30 cells at levels that do not cause significant toxicity.

31 Formaldehyde exposure causes differential induction of MNs in human nasal epithelial
32 and buccal epithelial cells, which is significant in industrial exposure workers and students
33 working in anatomy or mortuary science, respectively. However, recent data and data from
34 larger studies support a finding of increased MNs in blood lymphocytes, although the issue
35 remains controversial because of issues relating to study design, exposure regimen, and

1 confounding factors, including MN levels in nasal and buccal cells well below established
2 background levels, reports limited by the number of cells observed, variation in standard
3 techniques, and nonconcordance between buccal and nasal findings (Speit and Schmid, 2006).
4 Several clastogenic effects, such as induction of MNs, SCEs, and CAs, were seen in human
5 peripheral blood lymphocytes; also a recent study showed increased monosomy 7 and trisomy 8
6 in the lymphocytes of formaldehyde exposed workers. Formaldehyde exposure also caused *p53*
7 mutations in rat nasal carcinomas with the expression of mutant *p53* protein.

8 Overall, induction of DPXs as a predominant lesion in vitro and in vivo, clastogenicity,
9 and mutagenicity with locus-specific mutations in nonhuman and human cells supports the
10 concept of genotoxic action of formaldehyde at the POE.

11 A summary of the genotoxicity of formaldehyde in humans is presented in Table 4-89.

12 4.4. SYNTHESIS AND MAJOR EVALUATION OF NONCARCINOGENIC EFFECTS

13 The adverse health effects due to formaldehyde exposure have been extensively studied
14 in humans and in animal models. Studies of human exposure include occupational exposures,
15 environmental exposures, and clinical studies of intentionally exposed subjects (see Section 4.1).
16 Occupational exposures are primarily due to inhalation and dermal contact. Animal studies are
17 available for a variety of routes of exposure, including inhalation, oral, dermal, and intravenous
18 and I.P. injections (see Section 4.2). Additionally, as discussed in Chapter 3, in vitro studies
19 address biological activity and the metabolic fate of formaldehyde.
20

21 Taken together, the human and animal studies support numerous health effects, not only
22 at the POE as expected for a reactive gas but also on pulmonary function, neurobehavioral
23 function, reproduction, development, immunomodulation, and sensitization (atopy, asthma). The
24 discussion below provides a description of the adverse effects seen in each area, summarizing the
25 data for both human and animal studies. MOA data are discussed where information regarding
26 formaldehyde's biological activity may be linked to the observed adverse health effects.
27

28 4.4.1. Sensory Irritation

29 Sensory irritation of the eyes, nose, and throat is reported in humans upon direct contact
30 with formaldehyde gas during inhalation exposures (Holmström and Wilhelmsson, 1988;
31 Ritchie and Lehman, 1987) and includes irritation resulting from acute exposures (Lang et al.,
32 2008; Yang et al., 2001; Krakowiak et al., 1998; Kulle, 1993; Green et al., 1989, 1987; Kulle et
33 al., 1987; Sauder et al., 1987, 1986; Schachter et al., 1987, 1986; Witek et al., 1987; Day et al.,
34 1984; Bender et al., 1983; Weber-Tschopp et al., 1977). Controlled exposures in inhalation
35 chambers confirm the specificity of these responses to formaldehyde exposure and allow for
36 assessment of these symptoms through both subjective and objective measures (Kulle, 1993;

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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	
<i>Analyses of nasal and/or buccal cells</i>									
Plywood workers	15	2–19	6.8	0.32–0.83	(1)			+ nasal	Ballarin et al. (1992)
Age and sex matched controls	15								
Male mortuary science students	22	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)
Female mortuary science students	7								
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	8	NA	23.6	NA	NA			+ buccal – buccal	Kitaeva et al. (1996)
Male anatomy faculty	5		25.6						
Controls (Females)	7								
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	28	1–13	4.70	2–4	NA			+ buccal	Burgaz et al. (2002)
Controls (University staff)	18								
Workers at a formaldehyde plant	18	1–15	8.5		0.8			+ nasal	Ye et al. (2005)
Controls	23								
Volunteers	21	10 days		13.5 ppm-hours				– buccal	Speit et al. (2007b)
<i>Analyses of peripheral lymphocytes</i>									
Manufacturing workers	15	23–35	28		<5 1971	–			Fleig et al. (1982)
Age and sex matched controls	15				<1 later				

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	6	4-11			0.9-5.8			-	Thomson et al. (1984)
Controls	5								
Anatomy students ^c	8	10-week class		1.08-1.99 ^d	1.2		+		Yager et al. (1986)
				0.08-0.6 ^e	0.3				
Papermakers	20	2-30	14.4	<3	NA	+ ^f	-		Bauchinger and Schmid (1985)
Controls	20								
Wood workers	25	<5 to <16		0.45-8.6	NA	-			Vargová et al. (1992)
Controls	19								
Male embalming students	22	Blood sampled before and after first 9 weeks of embalming course		0.15-4.3	1.4		-	+	Suruda et al. (1993)
Female embalming students	7						-	-	
Manufacturing workers	15		10	Up to 4	NA	+		+	Kitaeva et al. (1996)
Anatomy faculty	8		17	NA	ND				
Controls	6								
Medial students	30	Sampled near end of 15-month term		<1	NA	-			Vasudeva and Anand (1996)
Controls	30				-				
Anatomy students	13	12-week class			2.37 ^g	+	+	+	He at al. (1998)
Controls (students)	10								
Physicians	6	2-24	10	3.1-2.8	1.6		+		Shaham et al. (1997)
Technicians	7	2-25	15				+		
Controls (age matched/unexposed)	20								
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				

^aSame 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.

^bNot dose related; both low- and high-exposure groups had same SCE increase.

^cEach student sampled before and after 10-week anatomy class.

^dBreathing zone samples.

^eRoom air samples.

^fIncrease only in 11 supervisors. See text for details.

^gAverage breathing zone during dissection procedure.

^hExposed and controls from 14 hospitals.

ⁱLow- and high-exposure groups established but numbers not provided.

^jNot dose related; both low and high groups had same SCE increase.

^kDescribed 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.

1 Holness and Nethercott, 1989; Green et al., 1987; Kulle et al., 1987; Sauder et al., 1986; Weber-
2 Tschopp et al., 1977). Eye irritation may be reported as itching, burning, and general discomfort.
3 Tearing, redness of the eyes, and increased blink frequency are observed and may be quantified
4 in exposure under controlled conditions (Lang et al., 2008; Yang et al., 2001; Andersen and
5 Molhave, 1983; Weber-Tschopp et al., 1977; Schuck et al., 1966). Eye irritation appears to be
6 the most sensitive endpoint in most individuals and may be observed after short exposures
7 (195 minutes at 0.5 ppm: Lang et al. [2008]; 30 seconds at 1.65 ppm: Yang et al. [2001]).

8 Itching, burning, and discomfort of the nose, which may be accompanied by increased
9 mucous production (runny nose), are reported by individuals exposed via inhalation (Krakowiak
10 et al., 1998; Kulle, 1993; Green et al., 1987; Kulle et al., 1987; Weber-Tschopp et al., 1977).
11 Throat irritation may also be described subjectively as itching and burning and is often
12 accompanied by a cough (Krakowiak et al., 1998). Symptoms of eye and mucous membrane
13 irritation are also reported in numerous rodent studies and support the health effects reported in
14 humans (see Section 4.1.1.1). Although dermal contact may result in dermatitis and an apparent
15 hypersensitivity reaction, symptoms do not present upon contact as sensory irritation. There are
16 no human or animal data that assess sensory irritation from oral exposures.

17 The time to onset of sensory irritation symptoms and severity of the sensory irritation are
18 a function of both the air concentration and duration of exposure. Additionally, nose and throat
19 irritation becomes more prominent at higher exposures and longer duration of exposure (Kulle,
20 1993; Kulle et al., 1987). Controlled human laboratory exposures (Yang et al., 2001; Kulle,
21 1993; Kulle et al., 1987; Cain et al., 1986; Andersen and Molhave, 1983) provide more direct
22 exposure-response evidence for sensory irritation. These studies are limited to healthy
23 nonsmoking individuals. Two studies (Cain et al., 1986; Andersen and Molhave, 1983)
24 document discomfort and irritation of the eye in response to acute exposures as low as 0.25 ppm.
25 Dose-response relationships are reported in a number of different ways: as an incidence of the
26 reported symptom among subjects, as a score for severity of the symptom, or in some cases as a
27 subjective measure, such as blink frequency for eye irritation.

28 Symptoms of sensory irritation, including eye irritation (burning watering, increased
29 blinking), nasal irritation (rhinitis, itching/burning), throat/respiratory tract irritation (wheezing,
30 coughing, phlegm production), have been reported in numerous worker cohorts. Occupational
31 exposure environments include hospital and medical settings, students, and industrial workers
32 (Takahashi et al., 2007; Takigawa et al., 2005; Krakowiak et al., 1998; Akbar-Khanzadeh et al.,
33 1994; Uba et al., 1989; Horvath et al., 1988; Schachter et al., 1987). Formaldehyde levels often
34 vary in a work environment and peak as well as average exposures may be used to report
35 occupational exposures. Although sensitive individuals often remove themselves from an

1 irritating workplace (the healthy worker effect), eye, nose, and throat symptoms are still reported
2 in this environment. Among workers in a plant where formaldehyde resins were used, those
3 exposed to an average of 210 ppb formaldehyde (range of 40-400 ppb) reported increased
4 symptoms, including nasal and eye irritation, above those in the control population (Holmström
5 and Wilhelmsson, 1988).

6 These effects have been noted in students, particularly medical students, who are exposed
7 to formaldehyde in cadaver labs. In a study of 24 formaldehyde-exposed anatomy students
8 (personal breathing zone samples 0.73 ppm, range 0.49–0.93) (Kriebel et al., 1993), eye, nose,
9 and throat irritation was present when comparing rates of irritation from the end or middle of
10 class to before the start of class. Takahashi et al. (2007) showed that 143 medical students
11 reported various symptoms (including eye and throat irritation) and that the percentage of
12 students reporting symptoms increased between the beginning (measured after the first day of
13 class) and the end of the course (2 months later). After the first day of class, approximately 35%
14 of students reported eye soreness and about 15% reported throat irritation.

15 Sensory irritation has also been reported in occupational settings, including particleboard
16 manufacturing, woodworking, and embalming, with average formaldehyde concentrations of
17 300-400 ppb. Symptoms of irritation were observed at a frequency of 40-65% among exposed
18 workers compared to 6-25% among unexposed comparison groups (Horvath et al., 1988;
19 Alexandersson and Hedenstierna, 1988; Holmström and Wilhelmsson, 1988; Holness and
20 Nethercott, 1989).

21 Eye, nose and throat irritation also were observed in association with residential exposure
22 to formaldehyde. Studies with formaldehyde measurements and analyses that adjusted for
23 potential confounders (e.g., age, gender and smoking status) observed a clear exposure-response
24 relationship for average formaldehyde concentrations or cumulative exposure (Ritchie and
25 Lehnen, 1987; Hanrahan et al., 1984; Liu et al., 1991).

26 Ritchie and Lehnen (1987) surveyed residents in 2,000 homes classified as having
27 formaldehyde concentration <0.1 ppm, 0.1–0.3 ppm, and >0.3 ppm. Increases in the prevalence
28 of eye irritation of 1-2%, 12-32%, and 86-93%, respectively were observed with increasing
29 categories of formaldehyde exposure. Liu et al. (1991) report irritant effects associated with
30 formaldehyde exposure in mobile homes, where formaldehyde concentrations ranged from the
31 0.01 ppm detection limit to 0.46 ppm. Eye irritation (60%), nose/throat irritation (10–20%), or
32 headache (<10%) were reported in residents.

33

1 **MOA**

2 The mucosae of the URT, oral cavity pharynx, and upper airways are complex tissues,
3 where epithelial and goblet cells predominate. In addition, the nasal mucosa is highly enervated.
4 The main nerves include the trigeminal nerve and olfactory sensory cells (olfactory epithelium,
5 the vomeronasal organ, and the organ of Masera) (Feron et al., 2001). A possible MOA for
6 sensory irritation includes formaldehyde-induced stimulation of the trigeminal nerve (though
7 whether formaldehyde acts as a direct agonist is unknown). Trigeminal nerve stimulation in the
8 nasal passages transmits signals to the CNS, which then sends efferent signals back to the nasal
9 tissues, causing sensory irritation, and possibly systemically via vagal nerve stimulation,
10 resulting in more systemic effects.

11 Animal studies are potentially useful models for understanding mechanisms of toxicity,
12 especially where sufficient human data do not exist. While experimental animal studies provide
13 a model of secondary effects, rodents also demonstrate RB, an effect not seen in humans. Thus,
14 species that exhibit bradypnea (like mice and rats) may not be appropriate for assessing
15 respiratory endpoints. The mechanism underlying RB includes formaldehyde binding to the
16 sensory nerve endings of the trigeminal nerve, where signals travel to the CNS. The vagus nerve
17 transmits the efferent signal to produce smooth muscle contraction. The animals become
18 inactive, their core temperatures decrease by several degrees C, and their respiratory rates and
19 minute volumes decrease. However, this is not to say that trigeminal nerve stimulation is not an
20 appropriate potential mechanism of action in other species or in humans. Since trigeminal nerve
21 stimulation has been independently confirmed in species without RB, this mechanism may be a
22 viable explanation for the observed effects.

23

24 4.4.2. Pulmonary Function

25 Several adequately conducted and reported studies evaluated chronic effects of
26 occupational (Malaka and Kodama, 1990; Herbert et al., 1994; Horvath et al., 1988; Holness and
27 Nethercott, 1989; Alexandersson et al., 1982; Alexandersson and Hedenstierna, 1989) or
28 residential exposure to formaldehyde (Krzyzanowski et al., 1990; Franklin et al., 2000). Small
29 decreases in preshift lung function relative to unexposed groups were reported in several
30 different occupational settings including plywood, oriented strand board and carpentry (Malaka
31 and Kodama, 1990; Herbert et al., 1994; Alexandersson et al., 1982; Alexandersson and
32 Hedenstierna, 1989). The studies included appropriate comparison groups, formaldehyde
33 measurements, detailed reporting of methods, and adjustment for potential confounding
34 variables, including age and smoking status (in some studies weight and ethnicity). Lung
35 function values were presented as percent of expected based on age, height, and sex or adjusted

1 for these variables in the analysis. Studies of long-term exposure also reported increased
2 respiratory symptoms such as cough, increased phlegm, asthma, chest tightness and chest colds
3 in exposed workers (Malaka and Kodama, 1990; Herbert et al., 1994; Pourmahabadian et al.,
4 2006, Alexandersson et al., 1982; Alexandersson and Hedentierna 1989).

5 Other studies evaluated pulmonary function among occupational groups with exposure to
6 formaldehyde but the results of these studies are less informative because spirometric values
7 were not adjusted for age, height or gender, statistical analyses were not reported, or the studies
8 suffered from small sample size, methodological, analytical or reporting deficiencies
9 (Khamgaonkar and Fulare, 1991; Pourmahadadian et al. (2006); Kilburn et al., 1985; Main and
10 Hogan, 1983; Ostojic et al., 2006; Holmström and Wilhelmsson, 1988; Nunn et al., 1990). Also,
11 occupationally exposed groups compared to nonoccupational referent groups may have exhibited
12 a healthy worker effect masking any formaldehyde related lung function decrements (Holness
13 and Nethercott, 1989).

14 A longitudinal study by Alexandersson and Hedenstierna (1989) documented an
15 association of lung function decrements between 1980 and 1984 with time-weighted average
16 occupational formaldehyde concentrations of 0.42–0.5 mg/m³ (340–400 ppb). Statistically
17 significant annual decreases in FEV₁/FVC and FEF_{25–75%} were noted over 5 years in nonsmokers
18 after correction for normal aging and reference lung function spirometry values. The decrease in
19 FEF_{25–75%} was 0.212 ± 0.066 L/second (mean ± SD) for each year of exposure and was
20 significant ($p < 0.01$). The decrease in FEV₁/FVC was 0.4 ± 0.2 ($p < 0.01$). A cross-sectional
21 study with a participation rate of 93% among carpentry workers observed statistically significant
22 decrements in FEV₁, FEV₁/FVC, and FEF_{25–75%} associated with an 8-hour time-weighted
23 average formaldehyde exposure of 1.13 ppm (Malaka and Kodama, 1990). In multiple
24 regression models adjusting for age, height, weight, cigarettes/day, and dust, formaldehyde as a
25 continuous variable was a significant predictor for FEV₁, FEV₁/FVC, and FEF_{25–75%}. Each unit
26 increase in formaldehyde (ppm-years) was associated with a decrease of 0.015 liters, 0.347%,
27 and 0.043 l/s in FEV₁, FEV₁/FVC, and FEF_{25–75%}, respectively. The strongest response was for
28 FEF_{25–75%}, which showed a 12% drop in observed function compared with expected function in
29 the unexposed.

30 Kryzanowski et al. (1990) described a well-designed and executed cross-sectional study
31 of residential formaldehyde exposure in a large, representative sample that provided clear
32 evidence of a linear relationship between increased formaldehyde exposure and decreased peak
33 expiratory flow rate (PEFR) among children. A statistically significant linear relationship
34 between increased household mean formaldehyde exposure and decreased PEFR was reported in
35 children ($\beta = -1.28 \pm 0.46$ L/minute per ppb formaldehyde). The average formaldehyde

1 concentration was 26 ppb, with a maximum sample value of 140 ppb. Decrements in PEF
2 associated with increasing formaldehyde concentrations also were observed among adults
3 beginning at an average concentration of 40 ppb. While Franklin et al. (2000) did not observe an
4 association between a one-time measurement of FVC or FEV₁ among children aged 6–13 years
5 and indoor concentrations of formaldehyde, levels of exhaled nitric oxide (NO) were higher in
6 children exposed to average formaldehyde levels ≥ 0.05 ppm compared to < 0.05 ppm. These
7 findings indicate that formaldehyde may increase lower airway inflammation at concentrations
8 associated with effects on pulmonary function.

9 The pulmonary function measures associated with formaldehyde exposure are consistent
10 with bronchial constriction, inflammation, or chronic obstructive lung disease. Decreases in
11 spirometric values, including vital capacity (VC), forced expiratory volume (FEV), forced vital
12 capacity (FVC) and FEV/FVC have been documented. Decreases in lung volume (FEV₁, FVC)
13 indicate possible pulmonary obstruction (narrowing of the airways during exhalation) (Pellegrino
14 et al., 2005). Early changes in small airways are observed as reductions in expiratory flow in the
15 terminal portion of the spirogram (PEF, FEF_{75%}, MEF_{25%-75%}). These changes may be observed
16 even if FEV₁ is not affected.

17 Lung function deficits have been reported in pre- versus postshift measurements among
18 occupational groups (Herbert et al., 1994; Horvath et al., 1988). Students have also shown
19 decrements in lung function that are associated with exposure to formaldehyde in laboratories
20 Kriebel et al., 1993; Kriebel et al., 2001. Kriebel and colleagues (1993) observed a 2% cross-lab
21 decrement in PEF among anatomy students during the first two weeks of a 3-hour laboratory that
22 they attended once per week. The cross-lab pulmonary response was attenuated over the 10-
23 week duration of the course (formaldehyde geometric average concentration of 0.73 ppm).
24 While the acute effects of formaldehyde exposure appeared to diminish after several weeks of
25 exposure, the effect on prelaboratory PEF across 10 weeks was a 2.7 ± 1.1 L/minute per week
26 drop that was statistically significant ($p < 0.01$) in a model adjusting for random person effects,
27 asthma, interaction between time and asthma, and eye as well as nose symptoms of irritation.
28 Prevalence of eye and nose symptoms was associated with decreased PEF ($p < 0.02$).

29 Overall, acute formaldehyde exposures (0.5–3 ppm) have not induced significant
30 pulmonary deficits in healthy, nonexercising volunteers in controlled human exposure studies
31 (Kulle et al., 1987; Schachter et al., 1986; Schachter et al., 1987; Witek et al., 1986; Day et al.,
32 1984; Andersen and Molhave, 1983). However, it is unclear whether the data analysis in these
33 reports had the statistical power to substantiate the small deficits reported in occupational and
34 student studies. The studies exposed small numbers of diverse individuals, often including males
35 and females of varying age, and some included current smokers. Some studies report the

1 absolute values of the lung function parameters without adjustment to individual expected
2 function or the unexposed baseline for each individual (Kulle et al., 1987; Andersen and
3 Molhave, 1983; Day et al., 1984). In other studies that reported lung function values as a percent
4 of baseline, the variation of the mean change in lung function parameters is quite large, nearly
5 equaling the reported value and exceeding it in several cases (Witek et al., 1986; Schachter et al.,
6 1986; 1987). The absence of normalized raw data, combined with large individual variation,
7 limit the interpretation of these studies.

8 Small but statistically significant deficits in pulmonary function (e.g., decreased FEV₁,
9 FVC₁, FEV₃, specific airways conductance) due to acute formaldehyde exposure (2 or 3 ppm)
10 have been reported in healthy volunteers in controlled human exposure studies using exercise
11 (Green et al., 1987, 1989; Sauder et al., 1986;). Although changes in lung function parameters
12 averaged over experimental groups were generally small, some individuals exhibited clinically
13 significant deficits, even after only 2 hours of exposure (Green et al., 1987). This differential
14 response suggests susceptibility in certain subjects (Green et al., 1987). Other studies that
15 included an exercise component did not report a difference in response among healthy volunteers
16 (Schachter et al., 1986; Kulle et al., 1987). Acute controlled studies that evaluated responses
17 among asthmatics reported no changes in pulmonary function associated with formaldehyde
18 exposure (Sheppard et al., 1984, Ezratty et al., 2007; Harving et al., 1990; Green et al., 1987,
19 1989; Sauder et al., 1987; Witek et al., 1987, 1986; Krakowiak et al., 1998). These findings
20 suggest that a brief exposure to formaldehyde may not trigger a response in the airways of
21 asthmatic individuals in the absence of allergen. However, the large variation in pulmonary
22 response among the individuals (healthy and asthmatic) that participated in the experimental
23 exposure studies suggests that some individuals may be more sensitive to formaldehyde.

24 Several animal studies document increased airway resistance and bronchial constriction
25 following inhalation exposure to formaldehyde (Nielson et al., 1999; Swiecichowski et al., 1993;
26 Biagini et al., 1989; Amdur et al., 1960). A study using cynomolgus monkeys (Biagini et al.,
27 1989) demonstrated that methacholine-induced bronchial constriction can be similarly induced
28 by acute formaldehyde exposure (10 minutes at 2.5 ppm). Thus, formaldehyde exposure
29 simulated bronchial constriction observed after methacholine challenge, but these effects may
30 not occur by a similar MOA. Similar results were reported in guinea pigs (Swiecichowski et al.,
31 1993; Amdur et al., 1960), rats (Ohtsuka et al., 1997), and mice (Nielson et al., 1999).

32 Taken as a whole, studies of occupational exposure to formaldehyde, as well as
33 residential exposure to low indoor formaldehyde concentrations support an association with
34 deficits in pulmonary function among adults and children. Respiratory symptoms also were
35 reported at the same exposure levels. A longitudinal study that documented a progressive

1 decrease in pulmonary function among nonsmokers over 5 years (Alexandersson and
2 Hedenstierna, 1989) supports the conclusion that formaldehyde exerts a progressive chronic
3 effect on pulmonary function. Formaldehyde exposures used in controlled human chamber
4 studies have not caused functional deficits among healthy, nonexercising volunteers or among
5 asthmatics, although wide variability in responses suggests that some individuals may be
6 sensitive responders. A few studies that incorporated exercise reported small decreases in
7 pulmonary function related to formaldehyde exposure. These effects have been corroborated in
8 animal studies exposed to formaldehyde.

9 10 **MOA**

11 Formaldehyde-induced inflammation of the airways may contribute to observed
12 decreases in measures of pulmonary function. Even short-term inflammatory reactions could
13 reduce the effective diameter of the conductive airways, resulting in lower respiratory volumes in
14 a number of functional tests. Formaldehyde-induced trigeminal nerve stimulation contributes to
15 airway inflammation, which in turn would reduce airway function. Chronic exposures may
16 result in increased sensitization or chronic inflammatory responses, which could contribute to the
17 effects seen in the worker and residential populations.

18 Formaldehyde-induced pulmonary function deficits may also be in part a result of smooth
19 muscle contraction in repose to trigeminal nerve stimulation. Trigeminal nerve stimulation
20 transmits signals to the CNS. The resulting efferent signal from the vagal nerve produces
21 smooth muscle contraction and may result in decreased pulmonary function. Efferent signaling
22 has also resulted in release of substance P and other neuromodulatory compounds, which may
23 contribute to BC and sensitization of pulmonary responses (asthma, atopy).

24 25 4.4.3. Hypersensitivity and Atopic Reactions

26 A large number of studies have investigated the potential association between
27 formaldehyde exposure and a continuum of adverse health effects ranging from decrements in
28 pulmonary function to asthma. In general, epidemiologic studies of adults have reported varied
29 results between null findings and positive findings but have not consistently distinguished
30 between studies in which formaldehyde may be causing an increase in the incidence of asthma
31 (e.g., phenotypic switching), increasing the prevalence of asthma, initiating an asthma attack or
32 worsening the severity of an attack. Formaldehyde may itself be an allergen or it may potentiate
33 the ability of other allergens to cause phenotypic switching or increase the sensitivity of atopic
34 individuals. Thus formaldehyde exposure among nonatopic individuals could theoretically cause
35 phenotypic switching in the presence or absence of allergens possibly resulting in a diagnosis of

1 asthma. Formaldehyde could also cause an asthma attack or potentiate the influence of other
2 stimuli on the risk of asthma attacks.

3 Asthma is a specific manifestation of IgE-mediated hypersensitivity, characterized by
4 BHR and airway inflammation, resulting in lower airway obstruction (Fireman, 2003; Kuby,
5 1991). A variety of hypersensitivity reactions have been reported following exposure to
6 formaldehyde. Rashes and skin reactions have been reported in some individuals after dermal
7 exposures to formaldehyde. Increased expression of Th-2 cytokines in the lymph nodes of mice
8 given dermal applications of formaldehyde does indicate the involvement of an immune
9 component to the observed sensitization (Dearman et al., 2005; Hilton et al., 1998; Arts et al.,
10 1997). However, the response does not appear to be IgE mediated (Arts et al., 1997; Lee et al.,
11 1984). Gorski et al. (1992) observed an increase in formaldehyde-mediated neutrophil burst in
12 dermatitis patients exposed in a controlled chamber study and suggests a putative role of
13 oxidative stress and reactive oxygen species (ROS). Some case reports of bronchial asthma in
14 occupational settings suggest direct respiratory tract sensitization to formaldehyde gas (Lemiere
15 et al., 1995; Burge et al., 1985; Hendrick et al., 1982; Hendrick and Lane, 1977, 1975; Stenton
16 and Hendrick, 1994, Nordman et al., 1985). However, the development of an allergic asthmatic
17 response to formaldehyde appears to be a rare occurrence.

18 The few studies that reported an association of formaldehyde exposure with a new
19 diagnosis of asthma are consistent with the notion that formaldehyde may be an allergen or that
20 formaldehyde exposure may result in sensitization to common allergens among nonatopic
21 individuals, referred to as phenotypic switching (Rumchev et al., 2001; Smedje and Norback,
22 2001). Rumchev et al. (2002) reported that residential formaldehyde exposure was associated
23 with an increased risk of incident asthma in a population-based case-control study of 192
24 children aged 6 months to 3 years. The asthma diagnosis was based on a recent discharge from
25 the emergency department of a children's hospital in Perth, Australia, with a primary diagnosis
26 of asthma. The assessment of formaldehyde exposure was based on in-home measurements (8-
27 hour passive sampler) in two seasons and associations with asthma were adjusted for several
28 potential confounding factors including, other indoor air pollutants, house dust mite
29 concentrations, humidity and temperature, family history of asthma and allergy, smoking, and
30 other risk factors and demographic variables. Multiple regression models indicated a 3%
31 increased risk with each $10 \mu\text{g}/\text{m}^3$ increase in formaldehyde concentration in the home (OR
32 reported per unit formaldehyde increase 1.003 [95% CI:1.002-1.003]). When formaldehyde was
33 categorized into four exposure groups, odds ratios were increased at 50-50 $\mu\text{g}/\text{m}^3$ and above 60
34 $\mu\text{g}/\text{m}^3$ compared to 10-29 and 30-49 $\mu\text{g}/\text{m}^3$. A 39% increased risk was observed at the highest
35 exposure category ($p < 0.05$). Asthma incidence also was evaluated in a four year follow-up of

1 1732 students, aged 7-13 years, from 39 schools in Sweden who completed a mailed
2 questionnaire in 1993. Among the 1347 students who responded in 1997 (78%), 56 (4.5) were
3 diagnosed with asthma by a physician. While asthma incidence was not associated with
4 formaldehyde exposure in the entire cohort (OR 1.2 [95% CI: 0.8-1.7]), among 22 students with
5 no history of atopy at baseline, a higher concentration of formaldehyde was associated with an
6 odds ratio for incident asthma of 1.7 (95% CI: 1.1-2.6). Mold concentration also was associated
7 with incident asthma. The authors did not discuss the correlation of formaldehyde and mold
8 concentrations and do not appear to have adjusted for mold concentrations in the formaldehyde
9 analyses. However, when the units for both mold and formaldehyde are standardized to the
10 geometric mean standard deviation, the magnitude of the formaldehyde effect is larger.
11 Therefore, it is unlikely that the association with formaldehyde was the result of uncontrolled
12 confounding by mold concentrations.

13 Other studies of asthma prevalence have observed an association with indoor
14 formaldehyde concentrations among children exposed at home (Krzyzanowski et al., 1990;
15 Garrett et al., 1999) and at school (Zhao et al., 2008). A large, representative study of 202
16 households (mean formaldehyde level of 26 ppb) found that among children aged 6–15 years old
17 and exposed to environmental tobacco smoke, the prevalence of physician-diagnosed asthma was
18 45.5% for those with measured levels of formaldehyde in the kitchen >60 ppb ($N = 11$). The
19 prevalence of asthma dropped to 0% for levels 41–60 ppb ($N = 12$) and 15.1% for levels ≤ 40 ppb
20 ($N = 106$) (chi-squared trend test $p < 0.05$). No trend in asthma prevalence was seen for children
21 who were not exposed to environmental tobacco smoke (Krzyzanowski et al., 1990).
22 Krzyzanowski et al. also reported a statistically significant linear relationship between increased
23 household mean formaldehyde exposure and decreased peak expiratory flow rates among
24 children. PEFr is a diagnostic tool used to identify new asthma cases. Two studies of self-
25 reported asthma reported null results (Gee et al., 2005; Tavernier et al., 2006; Palczynski et al.,
26 1999). However, Tavernier et al. did not report the range of formaldehyde concentrations
27 evaluated so the variation of the concentration of formaldehyde is not known. Among the 187
28 children aged 15 years or less studied by Palczynski et al., only 9 were defined as having asthma.

29 Ambient formaldehyde concentrations outdoors on school grounds also were associated
30 with cumulative asthma in a cross-sectional study of students at 10 schools in China (Zhao et al.,
31 2008). Ambient formaldehyde concentrations of $5.8 \mu\text{g}/\text{m}^3$ were associated with an odds ratio
32 for cumulative asthma of 4.6 (95% CI:1.1-19.5). The odds ratio was adjusted for age, gender,
33 parental asthma or allergy and home factors including environmental tobacco smoke. Another
34 study that evaluated ambient formaldehyde exposure reported an increase in the severity of
35 asthma symptoms in a panel of 22 Hispanic children, 10-16 years old, with a minimum one year

1 history of doctor diagnosed asthma (Delfino et al., 2003). A 24-hour average formaldehyde
2 concentration at a central site monitor of 7.21 ppb (range 4.27-14.02 ppb) was associated with an
3 increase in asthma symptom scores on the next day (OR 1.37 (95% CI:1.04-1.89).

4 A recent meta-analysis of formaldehyde exposure supports an association with asthma in
5 children (McGwin et al., 2010). Of the seven studies that were included in the meta-analysis, six
6 reported increased risks of asthma associated with exposure to formaldehyde. The results of the
7 random-effect model results showed an overall effect estimate of OR = 1.17 (95% CI: 1.01-
8 1.036). The three studies with the highest statistical weights based on the inverse of the variance
9 of the study ORs were for the studies by Rumchev et al. (2002), Garrett et al. (1999) and
10 Krzyzanowski et al. (1990).

11 A cross-sectional study of residential exposure to formaldehyde among 88 adults from
12 Sweden reported an increase in reports of nocturnal breathlessness in association with mean
13 bedroom formaldehyde concentrations of 29 $\mu\text{g}/\text{m}^3$ compared to 17 $\mu\text{g}/\text{m}^3$ (Norback et al., 1995).
14 The adjusted odds ratio was 12.5 (95% CI:2.0-77.9) per a 10-fold increase in the indoor
15 concentration, higher in magnitude than the odds ratios for toluene, terpenes and volatile organic
16 compounds. This finding is supported by the observation by Krzyzanowski et al. (1990) of
17 decreased morning peak expiratory flow rate among adult smokers exposed to >40 ppb
18 formaldehyde in their homes. Another study of pregnant women found no association with
19 asthma prevalence for personal exposure to ≥ 47 ppb compared to < 18 ppb (Matsunaga et al.,
20 2008). However, the number of women with a diagnosis of asthma was small ($N = 21$) and the
21 confidence limits were wide (OR 2.65 [95% CI: 0.63-11.11]).

22 The epidemiologic literature also indicates that formaldehyde may increase the
23 prevalence of allergic sensitization to common allergens, as well as the severity of responses
24 (Garrett et al., 1999; Delfino et al., 2003; Cassett et al., 2006).

25 Garrett et al. (1999) reported that the prevalence and severity of allergic sensitization to
26 12 common allergens was increased in relation to formaldehyde levels in the homes of 148
27 children, aged 7-14 years, living in Victoria, Australia based on the highest of four seasonal
28 4-day formaldehyde measurements in the home ($p < 0.001$). The proportion of atopic children
29 between groups of the highest recorded formaldehyde level was 0.33, 0.64, and 0.75 for <20
30 $\mu\text{g}/\text{m}^3$, $20-50 \mu\text{g}/\text{m}^3$, and $>50 \mu\text{g}/\text{m}^3$, respectively (test for trend, $p < 0.001$). The differences in
31 prevalence were statistically significant ($p = 0.001$). In logistic regressions, the crude association
32 for atopy with an increase in bedroom formaldehyde concentration per $10 \mu\text{g}/\text{m}^3$ was OR = 1.34
33 which increased when adjusted for parental asthma and gender to an odds ratio of 1.40 per 10
34 $\mu\text{g}/\text{m}^3$ (95% CI: 0.98-2.00). Thus, parental asthma was not a confounder of the association
35 between formaldehyde and prevalence of atopy. The adjusted odds ratio for atopy with an

1 increase in highest recorded formaldehyde per 20 $\mu\text{g}/\text{m}^3$ was 1.42 (95% CI: 0.99–2.04). Passive
2 smoking, the presence of pets, indoor nitrogen dioxide concentrations, airborne fungal spores
3 and house-dust-mite allergens also did not influence the effect estimates and were unlikely to be
4 confounders. The authors reported that mean respiratory symptom scores adjusted for parental
5 asthma and parental allergy increased with increasing formaldehyde exposure categories (<20
6 $\mu\text{g}/\text{m}^3$, 20-50 $\mu\text{g}/\text{m}^3$, >50 $\mu\text{g}/\text{m}^3$, $p < 0.05$). Delfino et al. (2003) also reported an association of
7 the severity of respiratory symptoms with ambient formaldehyde concentration on the previous
8 day among asthmatic children. The association between formaldehyde concentrations and
9 severity of allergic sensitization was analyzed using two measures, number of positive skin prick
10 tests and the ratio of wheal diameters after skin pricks with allergens compared to the histamine
11 wheal size. Average levels of both measures of severity were higher in the two higher
12 formaldehyde groups compared to the lowest group ($p < 0.05$). Further, both measures were
13 linearly related to increasing formaldehyde categories in regression models controlling for
14 parental asthma and allergy and sex. The role of formaldehyde in exacerbating allergic
15 responses to common allergens among atopic individuals was demonstrated in a controlled
16 human exposure study by Cassett et al. (2006) using formaldehyde concentrations of 100 $\mu\text{g}/\text{m}^3$
17 and a 30 minute cross-over exposure protocol. Another study using different allergens,
18 dosimeters, and study protocol did not report an effect of formaldehyde on allergic responses
19 (Ezratty et al., 2007).

20 Exacerbation of response after formaldehyde exposure has been demonstrated in animal
21 studies as well. Sadakane et al. (2002) demonstrated that formaldehyde exposure exacerbated
22 sensitization and challenge with Der f and suggested that formaldehyde exposure may aggravate
23 eosinophilic infiltration and goblet cell proliferation that accompanies allergic responses.
24 Several animal studies report increased airway resistance and BC due to inhalation exposures to
25 formaldehyde (Nielsen et al., 1999; Swiecichowski et al., 1993; Biagini et al., 1989; Amdur,
26 1960). Changes in pulmonary resistance were observed as early as 10 minutes after exposure
27 (Biagini et al., 1989), and reported effect levels ranged from 0.3 to 13 ppm. BHR is commonly
28 associated with allergic Type I hypersensitivity reactions but is not sufficient to demonstrate that
29 an agent induces Type 1 hypersensitivity.

30 In conclusion, the epidemiologic studies of formaldehyde exposure among children
31 support the finding that low indoor and outdoor concentrations result in increased asthma
32 incidence and prevalence. Formaldehyde exposure is associated with increased prevalence of
33 allergic sensitization to common allergens and with increased severity of asthmatic symptoms
34 and the allergic response to allergen challenge. The studies of formaldehyde exposure among

1 adults is sparse but suggest that exposure increases the prevalence of respiratory symptoms.
2 These effects are supported by findings of experimental animal studies.

4 **MOA**

5 The MOA underlying this response has not been elucidated. Formaldehyde-induced IgE
6 production has been reported in some studies (Vandenplas et al., 2004; Wantke et al., 1996a).
7 Other studies suggest that this effect does not appear to be immunogenic in nature (Fujimaki et
8 al., 2004; Lee et al., 1984). Although formaldehyde exposure has been reported to alter cytokine
9 levels and immunoglobulins in some experimental systems (Fujimaki et al., 2004a; Ohtsuka et
10 al., 2003), these immunomodulatory effects do not support immunogenically mediated type 1
11 hypersensitivity.

12 These decrements may be mediated via neurogenic potentiation (Sadakane et al., 2002;
13 Riedel et al., 1996; Tarkowski and Gorski, 1995). Tarkowski and Gorski (1995) suggest that
14 formaldehyde may increase permeability of respiratory epithelium and destruction of
15 immunologic barriers. Tachykinin NK1 receptor and various neuropeptides (NGF and substance
16 P) have been implicated in formaldehyde-induced sensitization and lend weight of evidence to a
17 neurogenic MOA (Van Schoor et al., 2000; Ito et al. 1996).

19 4.4.4. Upper Respiratory Tract Histopathology

20 Several studies in occupational workers have reported increased squamous cell
21 metaplasia and reduced mucociliary clearance in nasal and buccal swabs from humans
22 occupationally exposed to formaldehyde (Holmström et al., 1989; Holmström and Wilhelmsson,
23 1988). Evidence of genotoxic effects include increased MNs and CAs in nasal and buccal
24 epithelial cells from both workers and students exposed to formaldehyde (Ying et al., 1997;
25 Titenko-Holland et al., 1996; Suruda et al., 1993) and suggest a potential association between
26 genotoxicity and altered histopathology.

27 Numerous animal experimental studies in multiple strains of rats, mice, hamsters, rabbits,
28 and monkeys describe formaldehyde-induced URT pathology (Fló-Neyret et al., 2001; Roemer
29 et al., 1993; Reuzel et al., 1990; Monticello et al., 1989; Zwart et al., 1988; Wilmer et al., 1987;
30 Morgan et al., 1986b, 1983; Swenberg et al., 1986; Buckley et al., 1984). Effects are first
31 observed in the anterior respiratory mucosa and progress through the nasal passages with
32 increasing exposure concentration and time. The first observed effect includes damage to the
33 mucociliary apparatus of the nasal passages in response to formaldehyde. Studies conducted
34 both in vivo and in vitro demonstrate that formaldehyde disrupts mucus flow and ciliary beat that
35 are dependent on concentration and duration of exposure. Mucociliary apparatus deficits have

1 been recorded even after 18 hours of recovery following formaldehyde exposure. The
2 breakdown of the mucociliary apparatus may allow for increased infection and allow the
3 underlying epithelium to come into contact with exogenous chemicals.

4 Formaldehyde is highly reactive and may impact all cells in the nasal mucosa, including
5 epithelial cells (ciliated, columnar, and cuboidal), goblet cells, sensory neurons, and
6 intraepithelial lymphocytes. The histologic changes of these processes have been described in all
7 laboratory animals examined and progress from the anterior nares to the posterior regions of the
8 nasal passages, including the ETs and olfactory epithelium if the concentration and duration of
9 exposure are great enough.

10 Humans and nonhuman primates have significantly less complex nasal passages than
11 rodents. Formaldehyde has lower peak flux in human nasal tissues compared with rodents,
12 which are obligate nose breathers, but will penetrate more deeply into the human respiratory tract
13 than in rodents, since humans lack the autonomic RA response. Additionally, humans may
14 switch to mouth breathing in the presence of an irritant gas, thus bypassing the sensitive nasal
15 passages and increasing the tissue dose in the mouth and throat. These differences have been
16 demonstrated by using nonhuman primates where, at comparable concentrations, tissue
17 pathology and increased cell proliferation progressed further into the respiratory tract than in
18 rodents (Monticello et al., 1989). Nonhuman primates share common structural respiratory
19 components and patterns of breathing and do not have a reflex autonomic apnea response.

20 Despite the anatomical and physiological differences in breathing patterns and different
21 exposure parameters between humans and rodents, similar toxic effects are reported in tissues at
22 the POE in humans and laboratory animals. Several occupational studies have reported
23 increased squamous cell metaplasia in nasal and buccal samples in response to formaldehyde
24 exposure (Ballarin et al., 1992; Boysen et al., 1990; Holmström et al., 1989), paralleling the
25 histologic effects seen in experimental animal studies. A few human epidemiology studies
26 suggest increased NPCs (see Section 4.5) as well as oral/buccal tumors in response to
27 formaldehyde exposure (Shangina et al., 2006; Laforest et al., 2000).

28 The observed formaldehyde-induced URT toxicity is related to its high reactivity and
29 solubility. Moreover, additional interspecies differences in the surface area and configuration of
30 the nasal passages and upper airways will influence the areas of high formaldehyde flux in POE
31 tissues.

32 33 **MOA**

34 Formaldehyde-induced damage to the mucociliary apparatus of the nasal passages may
35 occur because formaldehyde may disrupt mucus flow and ciliary beat that is dependent on

1 concentration and duration of exposure. Formaldehyde reacts with the mucosal glycoproteins
2 and thus may contribute directly to the breakdown of the mucus layer. As formaldehyde reaches
3 the cells of the pseudostratified epithelium in the nasal passages, it exerts a range of effects from
4 direct damage to cell membrane, intracellular proteins, and DNA to alterations in GSH pools and
5 increased ROS. Adaptive effects include increased mucus flow and goblet cell proliferation as
6 well as the transition of respiratory epithelium to more insensitive cuboidal cells. With
7 continued exposure at sufficient concentration, squamous metaplasia develops, creating a
8 protective layer of keratinized cells. Gradually, this damage exceeds the cell's ability to
9 compensate for and repair damage; chronic nasal lesions develop, and the cells die both through
10 general necrosis as well as programmed cell death, depending on the severity of the cellular
11 damage (Monticello et al., 1989; Swenberg et al., 1983).

12 Genotoxic effects have been reported in nasal and buccal lesions taken from both workers
13 and students exposed to formaldehyde (Ying et al., 1997; Titenko-Holland et al., 1996; Suruda et
14 al., 1993). MN formation occurs in the more sensitive pseudostratified epithelium of the nasal
15 passages, nasopharynx, and upper airways, since there is only one layer of epithelial cells that are
16 constantly regenerating. However, the genotoxicity observed in buccal cells is more difficult to
17 explain, since buccal basal cells are usually covered by protective keratinized cell layers. Cuts,
18 sores, or other buccal lesions would increase basal epithelial cells' vulnerability to direct
19 exposure to formaldehyde.

20 21 4.4.5. Toxicogenomic and Molecular Data that may Inform MOAs

22 Over the past several years, studies have begun to examine the effects of formaldehyde
23 exposure on gene and protein expression. These include studies on in vivo and in vitro changes
24 in the global expression of mRNA (transcriptomics) and proteins (proteomics) in the tissues and
25 cells of humans and rodents exposed to formaldehyde. Currently, nine "-omics" studies from
26 five research groups are available. These studies are summarized in Section 5.2 and are
27 evaluated and discussed in the context of their relevance to informing MOAs and the dose-
28 response characterization briefly here.

29 In 2002, EPA released the *Interim Policy on Genomics* (U.S. EPA, 2002c), which
30 addresses how to use genomic data in regulatory decision making. Although the policy
31 encourages research in genomics, it places limits on its use, stating that genomic data alone are
32 not sufficient as a basis for decision making. These data thus cannot currently be utilized as the
33 critical effect in a chemical risk assessment but can be utilized in a weight-of-evidence approach
34 on a case-by-case basis. The Science Policy Council developed a white paper entitled *Potential*
35 *Implications of Genomics for Regulatory and Risk Assessment Applications at EPA* (U.S. EPA,

1 2004). This report described three areas where genomic data might be applied in risk assessment
2 at EPA: MOA analysis, susceptible population, and mixtures assessments. The genomic data on
3 formaldehyde thus may be applied to a discussion of MOA.

4 Toxicogenomics studies have investigated the gene and protein expression changes
5 resulting from formaldehyde exposure in a variety of respiratory tissues, including nasal tissue
6 (Andersen et al., 2008; Thomas et al., 2007; Hester et al., 2005, 2003), and, in lung tissue (Lee et
7 al., 2008; Li et al., 2007; Sul et al., 2007, 2006; Im et al., 2006) used human tracheal cell lines to
8 study genomic changes after exposure to formaldehyde in vitro. Unfortunately, these studies are
9 not directly comparable because different gene chip technology platforms were used in different
10 tissues, in both in vivo and in vitro study designs. In general, the gene and protein expression
11 changes reflect changes in apoptotic pathway genes, oxidative stress, and tissue remodeling.
12 Andersen et al. (2008) concluded that there was a threshold level where exposure to
13 formaldehyde (6 ppm) does not elicit changes in nasal epithelium of F344 rats. Overall,
14 Andersen et al. (2008) concluded that genomic changes were no more sensitive than tissue
15 responses and that formaldehyde, being an endogenous chemical, is well handled until some
16 threshold is achieved when toxicity rapidly ensues with genomic and histologic changes. At
17 about 6 ppm, this largely involves tissue remodeling (and protection), but regenerative
18 hyperplasia occurs at higher doses. Andersen et al. (2008) conclude that there is a threshold
19 where exposure to formaldehyde does not elicit changes in F344 nasal epithelial tissue over the
20 duration examined in this study (i.e., 15 days). Andersen et al. (2008) argue that this is
21 consistent with bioassays that indicate no tumor formation in rodents below 6 ppm
22 formaldehyde.

23 The primary conclusion in the Andersen et al. (2008) paper is that genomic changes,
24 including those suggestive of mutagenic effects, did not temporally precede or occur at lower
25 doses than phenotypic changes in the tissue. The authors stated as follows:

26
27 “The genomic signatures related to these transitions are for cell membrane and
28 extracellular components, then inflammation and cellular stress, and eventually
29 apoptosis. Importantly, these hierarchical models indicate that the tissue
30 responses at low dose concentrations are qualitatively different from those at high
31 concentrations and linear extrapolations or extrapolations that specify similar
32 modes of action at high and low doses would be inappropriate.”
33

34 4.4.6. Noncancer Modes of Actions

35 Noncancer health effects of interest span numerous organ systems and include
36 reproductive and developmental effects, neurological/neurobehavioral effects, and a complex
37 interaction between inflammation and immune and adverse pulmonary function. To date, no

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1 -omics studies have examined changes in reproductive tissue or altered gene expression in
2 developing animals. In regard to neurological/behavioral effects, one study (Lu et al. [2008],
3 described in Section 4.1.1.6) has reported elevations in the mRNA for NMDA receptor subunits
4 in brain homogenates following exposure to 2.4 ppm. Hester et al. (2003) reported a significant
5 increase in NMDA receptor subunit transcripts, along with other neuropeptide genes, in nasal
6 tissue of rats instilled into nostril with 400 mM formaldehyde (Described in Section 4.2.1.2.2.1).
7 Together, these changes may relate to formaldehyde-induced sensory irritation and, perhaps,
8 changes throughout the brain.

9 In regard to inflammatory, immune, and pulmonary effects, transcript and protein
10 changes in rodent tracheal tissue and lung tissue indicate that exposure to 3 to 38 ppm
11 formaldehyde results in genes involved in oxidative stress and cell proliferation and may
12 additionally increase airway ADH3 levels (Lee et al., 2008; Sul et al., 2007; Yi et al., 2007; Im et
13 al., 2006; Yang et al., 2005). Together, these data provide evidence for adverse pulmonary
14 effects that may exacerbate or facilitate asthma.

15 In lung tissue, Yang et al. (2005) identified three proteins up regulated and one protein
16 down regulated following 15 days of exposure to about 28 ppm formaldehyde. None of the
17 proteins corresponded with transcript changes reported by Sul et al. (2007). Interestingly, Sul et
18 al. (2007) reported that only two transcripts were significantly up regulated in the lung in
19 response to 5–10 ppm formaldehyde, while 19 were down regulated. In this regard, it is worth
20 considering that changes in proteins may not relate to their regulation but rather to their overall
21 percent composition in a cell (relative to other protein changes) before and after exposure. In
22 addition to transcript changes, Sul and colleagues (2007) reported DNA damage and lipid
23 peroxidation and noted that the observed down regulation of GR would facilitate oxidative stress,
24 while the down regulation of phospholipase A2 (PLA2) might represent a mechanism for
25 mitigating lipid peroxidation. It is worth noting that an increase in either of these genes could
26 also be argued to support similar conclusions (i.e., that GR is up regulated to increase GSH
27 levels and that PLA2 up regulation explains lipid peroxidation); this highlights the problem with
28 interpreting these data. Nevertheless, these studies indicate adverse effects (e.g., oxidative stress,
29 lipid peroxidation, cell proliferation etc.) in the rodent lung in response to 5–30 ppm
30 formaldehyde.

31 In a hypothesis-driven study by Yi et al. (2007), formaldehyde exposure was shown to
32 increase lung ADH3 levels. Several studies indicate that allergic responses and hyperreactivity
33 are uncoupled and may relate to ADH3 expression and activity. Que et al. (2005) has shown
34 that, in a rodent asthma model, ADH3 knockout mice exhibit similar signs of inflammation but
35 are protected from bronchoconstriction. Lino dos Santos Franco et al. (2006) provided evidence,

1 in rodents, that formaldehyde may induce inflammatory responses (e.g., leukocyte infiltration in
2 the lung) through neurogenic mechanisms but that bronchial tone is mediated by NO. The latter
3 effect is likely to be mediated by S-nitrosoglutathione GSNO and thus influenced by ADH3.
4 Interestingly, the single nucleotide polymorphisms (SNPs) that Wu et al. (2007) reported as
5 linked to other polymorphisms in the promoter region was the one demonstrating protection
6 against asthma. Hedberg et al. (2001) reported that at least one SNP in the promoter region
7 reduced ADH3 expression. Together, these data suggest that reduced ADH3 expression might
8 lower GSNO turnover and bronchial tone, thereby reducing signs of asthma. In this regard, it is
9 conceivable that wheezing and bronchoconstriction are the symptoms that lead to medical
10 intervention and not the inflammation per se. Thus, while ADH3 polymorphisms may not cause
11 asthma, ADH3 polymorphisms may influence hyperresponsivity and the likelihood of asthma
12 diagnosis. This is discussed further in Section 4.6 on susceptible populations. Formaldehyde
13 has been shown to accelerate GSNO breakdown (Staab et al., 2008a; Yi et al., 2007); thus,
14 pulmonary responses to formaldehyde may represent a balance between mechanisms that induce
15 NO (i.e., inflammation) and those that terminate GSNO (i.e., ADH3).

16 In regard to -omics changes in blood samples, the apparent limited distribution of
17 formaldehyde may suggest that these changes are secondary to effects at sites of contact but
18 could also indicate systemic distribution. As noted elsewhere in this report, bradypnea can
19 induce changes in dosimetry as well as changes in core body temperature and blood gases
20 (hypoxia itself induces hypothermia in rodents, and thus the reduction in minute volume and gas
21 changes may both contribute to hypothermia). These physiological responses (hypothermia and
22 hypoxia) surely induce changes in gene expression. Observed gene and protein changes in the
23 blood following formaldehyde exposure could also relate to irritation and inflammation at sites
24 of contact. In this regard, Im et al. (2006) reported changes in cytokines indicative of Th-2
25 responses. Altogether, the authors identified 32 proteins altered in the plasma of rats exposed to
26 formaldehyde. Although no coherent mechanisms are apparent from these changes, the authors
27 posited that they could serve as biomarkers for formaldehyde exposure. The concordance of
28 such changes across species remains to be demonstrated.

29 Li et al. (2007) identified dose-response relationships for six genes in human blood
30 samples that were putatively associated with formaldehyde exposure. Three of these genes are
31 reported to inhibit apoptosis and were posited as supporting in vitro data by Tyihak et al. (2001);
32 however, Li and colleagues (2007) did not report any increase in blood cell count or in Hs 680.Tr
33 cell counts in vitro (i.e., these changes were not phenotypically linked to changes in cell kinetics
34 or hematology). However, these findings are not inconsistent with those of Hester et al. (2003)
35 that indicated no significant increase (or decrease) in nine genes involved in apoptotic pathways.

1 Finally, serum and glucocorticoid-induced protein kinase 1 (SGK1) was elevated in blood
2 samples and was posited to relate to possible inflammatory and immune responses.

3 4 4.4.7. Immunotoxicity

5 Results from studies that evaluated the immunotoxicity of formaldehyde are mixed. For
6 example, most human studies that investigated systemic immune effects by measuring increases
7 in formaldehyde-specific IgE are negative (Doi et al., 2003; Kim et al., 2001; Palczynski et al.,
8 1999; Krakowiak et al., 1998; Wantke et al., 1996; Grammer et al., 1990). Vandenplas et al.
9 (2004) reported a transiently positive increased formaldehyde-specific IgE titer in occupationally
10 exposed workers. In contrast, Thrasher et al. (1990, 1988, 1987) reported positive
11 formaldehyde-specific IgE titers in small (six to eight person) case studies of exposed workers,
12 and Carraro et al. (1997) reported elevated IgE titers in smokers. Grammer et al. (1990) did not
13 report any differences in albumin IgE in formaldehyde-exposed workers compared with controls.
14 In a residential study, Pross et al. (1986) found that formaldehyde insulation in homes had no
15 effect on tested human immunologic parameters.

16 One study suggests that immune system parameters are perturbed by formaldehyde
17 exposure. Lyapina et al. (2004) reported decreased immune resistance in all 29 workers exposed
18 to formaldehyde. This effect was associated with decreased neutrophil respiratory burst activity.
19 A LOAEL of 700 ppb was established.

20 Results from animal studies are mixed as to whether formaldehyde causes
21 immunotoxicity. Leach et al. (1983) reported systemic immunomodulation in F344 rats that was
22 attributed to formaldehyde exposure, but the formaldehyde effects on measures of humoral and
23 cell-mediated immunity were not confirmed in B6C3F1 mice (Dean et al., 1984). Jakab et al.
24 (1992) detected no differences in phagocytic ability of alveolar MPs from mice after
25 formaldehyde exposure. Formaldehyde-exposed rats that were injected with pneumococcus
26 antigen or tetanus toxoid produced antibodies in amounts similar to nonexposed, infected control
27 animals (Holmström et al., 1989b).

28 However, specific immune parameters appear to be affected by formaldehyde exposure.
29 For example, increased host resistance and hydrogen peroxide release from peritoneal MPs were
30 reported and confirmed (Adams et al., 1987; Dean et al., 1984) and suggest a putative role for
31 ROS. Increased host resistance may be mediated by formaldehyde-induced chronic
32 inflammation and respiratory mucosal damage that causes an up regulation in MPs and therefore
33 increases host immunity. Jakab et al. (2002) reported reduced pulmonary bacterial resistance in
34 mice after exposure to formaldehyde, as determined by increased bacterial loading. This result

1 contrasts with Dean et al. (1984) and is attributed to differential exposure regimens and
2 experimental design.

3 4 ***Mode of Action***

5 Circulating immune cells present in the mouth and upper airways, such as intraepithelial
6 lymphocytes, direct a local inflammatory response but may also contribute to systemic responses
7 through secreted cytokines and soluble factors released into the bloodstream (Togias, 1999).

8 Altered host resistance and hydrogen peroxide release from peritoneal MPs were reported
9 and confirmed (Adams et al., 1987; Dean et al., 1984) and suggest a putative role for ROS.
10 Indeed, increased neutrophilic ROS have been associated with formaldehyde-induced dermatitis
11 (Gorski et al., 1992), and, conversely, decreased neutrophil respiratory burst activity has been
12 shown in workers with history of formaldehyde-induced respiratory tract inflammation (Lyapina
13 et al., 2004). Oxidative stress may occur directly as a result of formaldehyde exposure or as a
14 secondary consequence to inflammatory responses.

15 16 4.4.8. Effects on the Nervous System

17 There is considerable evidence that formaldehyde exposure causes adverse effects on the
18 nervous system following inhalation at relatively low exposure levels (see Sections 4.1.1.6 and
19 4.2.6) but little or no information regarding a possible mechanism of action for these effects.
20 Data regarding adverse effects on the nervous system following oral exposure are very limited,
21 reflecting a data gap in this area. Relevant data in animals and humans for several types of
22 neurological endpoints, following inhalation exposure, are summarized below.

23 24 **4.4.8.1. *Irritant Threshold Detection***

25 Humans are exquisitely sensitive to the irritant properties of formaldehyde, as has been
26 discussed previously (see Section 4.1.1.1). Animal data confirm the irritant properties of this
27 compound at very low concentrations (Wood and Coleman, 1995).

28 29 **4.4.8.2. *Behavioral Effects***

30 Limited data in humans, as well as more robust data in animals, provide evidence of
31 behavioral changes following exposure to formaldehyde at levels as low as 0.1 ppm. Studies in
32 animals have found effects that persist for days to weeks after termination of exposure. In spite
33 of significant limitations, the available human data are consistent with the animal findings.

34 Several types of behavior have been evaluated in animals following formaldehyde
35 exposures. The most consistent findings, demonstrated by multiple laboratories and in multiple

1 species, have been changes in motor activity, habituation, and learning/memory task
2 performance. Motor activity and habituation have been evaluated under a variety of exposure
3 conditions, using both rats and mice. Consistent decreases in activity have been seen in adult
4 animals (Malek et al., 2004, 2003 a, b; Usanmaz et al., 2002). Senichenkova (1991) and
5 Sheveleva (1971) also found changes in motor activity in offspring following in utero exposure,
6 including decreased habituation in juvenile rats exposed in utero. Decreased performance in
7 learning and/or memory paradigms have been seen in multiple laboratories, also in both rats and
8 mice (Lu et al., 2008; Malek et al., 2003c; Pitten et al., 2000).

9 Data from controlled human exposures are very limited, but studies have shown
10 decreased performance in addition tasks and reaction time tasks following acute exposures to
11 formaldehyde (Lang et al., 2008; Bach et al., 1990). In contrast, Andersen and Molhave (1983)
12 indicated they found no change in performance on several types of tasks (including addition,
13 multiplication, and card punching) following acute exposure to volunteers, but supporting data
14 were not provided.

15 Data for humans are also available from epidemiology studies of individuals who were
16 occupationally exposed to formaldehyde. A variety of neurobehavioral deficits, including lack
17 of concentration and loss of memory, disturbed sleep, impaired balance and dexterity, and
18 changes in mood, were identified (Kilburn et al., 1987, 1985). However, most of the individuals
19 evaluated in these studies were also occupationally exposed to other solvents, raising questions
20 regarding possible confounding of the results due to multiple exposures. In addition, the
21 formaldehyde exposure information provided in the studies is not sufficient to permit a reliable
22 dose-response assessment for the effects identified. The types of effects seen in humans in the
23 available epidemiology studies are, however, consistent with those seen in available animal
24 studies.

25 In general (and noting the differences in exposure paradigms and types of tasks),
26 behavioral effects in animals and humans appear to occur at similar exposure levels. Animal
27 studies demonstrated LOAELs as low as 100 ppb following acute or repeated exposures (Malek
28 et al., 2003b, c); human controlled exposure studies have found effects in that same range, with
29 LOAELs of approximately 300 ppb following acute exposures (Lang et al., 2008; Bach et al.,
30 1990).

31 32 **4.4.8.3. Neurochemistry, Neuropathology, and Mechanistic Studies**

33 Limited data are available regarding neurochemical and neuropathological sequelae of
34 formaldehyde exposure. Studies from one laboratory (Sorg et al., 2004, 2001) have suggested
35 that behavioral sensitization to formaldehyde is linked to alterations in HPA control of

1 corticosterone and changes in mesolimbic dopamine pathways. Neurochemical changes in
2 response to formaldehyde exposure have also been documented in other laboratories (Fujimaki et
3 al., 2004b; Hayashi et al., 2004). Some of these data appear to be conflicting, and there are no
4 definitive data supporting a specific mechanism for formaldehyde effects on the nervous system
5 at this time. Neuropathological data are also limited, although data from one laboratory
6 (Sarsilmaz et al., 2007; Aslan et al., 2006) suggest a concern for changes in brain structure in
7 neonatal rats following exposure at 6 or 12 ppm. No human data are available that address these
8 endpoints. However, a prospective cohort study of nearly one million people followed for 15
9 years reported strongly significant dose-response associations between death from ALS and
10 exposure to formaldehyde of a known duration, with longer exposures associated with greater
11 risk (Weisskopf et al., 2009). This large, well-designed prospective cohort study strongly
12 supports the causal association of neuropathological effects in humans following long-term
13 formaldehyde exposure.

14 15 **4.4.8.4. Summary**

16 Overall, there is strong evidence that formaldehyde exposure via inhalation may cause
17 adverse effects on nervous system function in experimental animals at relatively low levels of
18 exposure (LOAELs as low as 100 ppb). Although human data regarding neurotoxicity following
19 formaldehyde inhalation are limited, available data provide support that the types of effects seen
20 in humans are similar to those found in animal studies. Evidence from available human
21 controlled inhalation exposure studies indicates that humans may be affected at doses similar to
22 those used in animal studies; however, the human data are extremely limited.

23 There are insufficient data to evaluate the potential for neurotoxicity following oral
24 exposure to formaldehyde. Limited evaluations of brain weight or histopathology in available
25 chronic or subchronic oral studies found no evidence of formaldehyde-induced changes (Til et
26 al., 1989, 1988; Tobe et al., 1989; Johannsen et al., 1986). However, reliable studies examining
27 nervous system function or focused studies of neuropathology following oral exposure to
28 formaldehyde are not available.

29 30 **4.4.8.5. Data Gaps**

31 Major data gaps were found regarding the evaluation of changes in nervous system
32 structure or function following formaldehyde exposure by both the inhalation or oral routes.

33 With respect to inhalation exposure, none of the available human studies resulted in data
34 sufficient to conduct a reliable dose-response assessment for changes in nervous system function.
35 Most of the available animal inhalation studies used short exposure durations (acute or short-

1 term), precluding a reliable evaluation of neurotoxicity following chronic exposure. Available
2 data for neurodevelopmental exposures are also quite limited, consisting of evaluation of
3 neuropathology in only one brain region and functional evaluations focused only on changes in
4 motor activity.

5 Major data gaps also exist regarding neurotoxicity following oral exposure, with no
6 relevant human data and extremely limited animal data. Available oral exposure studies were
7 insufficient to permit a reliable evaluation of the potential for neurotoxicity following oral
8 exposure to formaldehyde.

10 4.4.9. Reproductive and Developmental Toxicity

11 A number of studies have been identified that indicate an effect of formaldehyde
12 exposure on reproductive and developmental outcomes. Human data are described in
13 Section 4.1.1.7, and animal studies are addressed in Section 4.2.7 of this document.

15 **4.4.9.1. *Spontaneous Abortion and Fetal Death***

16 Several epidemiologic studies reported increases in risk of spontaneous abortion
17 following maternal occupational formaldehyde exposure (Taskinen et al., 1999, 1994; John et al.,
18 1994; Seitz and Baron, 1990; Axelsson et al., 1984). While these findings have been questioned
19 (Collins et al., 2001b), upon careful examination, none of the principal biases in epidemiologic
20 studies, including information bias, selection bias, and confounding, appear to be more likely
21 causes of these reported findings than the conclusion that they may reflect an underlying causal
22 process. While each of these occupational studies focused on women who were coexposed to
23 formaldehyde and other chemicals, the occupational groups were quite different and had
24 different sets of coexposures. The woodworkers in the Taskinen et al. (1999) study were
25 potentially coexposed to organic solvents related to painting and lacquering, dusts, and phenols,
26 none of which was shown to be an independent predictor of adverse risk. The cosmetologists
27 studied by John et al. (1994) were coexposed to hair dyes, bleach, alcohol-based disinfectants,
28 and chemicals specific to services, such as fingernail sculpturing, but, in analyses that were
29 specifically adjusted for other work exposures and their potentially confounding effects, the
30 investigators reported an increased risk for the use of formaldehyde-based disinfectants. The
31 laboratory workers studied by Axelsson et al. (1984) were potentially coexposed to a wide range
32 of solvents, but the miscarriage rate was highest among those exposed to formaldehyde. For a
33 potential confounder to entirely explain an observed effect of another exposure, it must be more
34 strongly associated with the adverse outcome. It does not appear that the collective results of

1 formaldehyde exposures associated with increased risk of spontaneous abortion—often in spite
2 of exposures being crudely measured—can be explained by information bias or confounding.

3 Taken together, these findings are consistent with an adverse effect of formaldehyde
4 exposure on the risk of pregnancy loss. The single study with the strongest quantitative
5 assessment of that risk is Taskinen et al. (1999), and the results presented are of sufficient quality
6 to support quantitative risk assessment by using the LOAEL/NOAEL approach.

7 This study was a well-designed population-based case-control study that appears to have
8 been well executed and appropriately analyzed. The study population of Finnish women was
9 well defined and adequately selected to allow for meaningful comparisons of health effects
10 between individuals with different levels of exposure to formaldehyde. The participation rate
11 was 64%, which is low enough to raise a concern about the potential for selection bias.
12 However, the authors noted that selection bias has not influenced the results of other
13 reproductive epidemiology studies reporting results on smoking, irregular menstruation, and
14 earlier miscarriages, which are known to lengthen the time to pregnancy (Bolumar et al., 1996;
15 Sallmén et al., 1995; Baird and Wilcox, 1985). Furthermore, there is no evidence to support
16 conjecture that an individual’s decision to participate in this study would be differential with
17 respect to their workplace formaldehyde exposures while being nondifferential with respect to
18 the other exposures of interest, including organic solvents, wood dust, and phenols. Since the
19 women who chose to participate in this study were not likely to be aware of the specific
20 hypotheses under investigation nor could they have known the formaldehyde exposures that were
21 independently estimated by an industrial hygienist, selection bias is not a likely explanation for
22 the findings of adversity.

23 Data on pregnancy history, including spontaneous abortions, were collected by
24 questionnaire. Spontaneous abortion is the most common adverse outcome of pregnancy (Klein
25 et al., 1989), and retrospective self-report of spontaneous abortion has been found to match well
26 with prospectively collected reproductive histories (Wilcox and Horney, 1984). Many
27 spontaneous abortions, however, are missed with self-reporting, with the magnitude likely
28 exceeding 25%, but only rarely do women self-report false positive events (Wilcox and Horney,
29 1984). The effect of such an undercount is to cause a bias towards the null when the likelihood
30 of undercounting is unrelated to formaldehyde exposure. The implication is that the observed
31 association of increased risk of spontaneous abortion associated with occupational exposure to
32 formaldehyde may be an underestimation of the true risk.

33 The findings by Taskinen et al. (1999) of reduced fertility and increased risk of
34 spontaneous abortion are internally consistent and coherent with other reports of increased risk
35 of pregnancy loss associated with exposure to formaldehyde (John et al., 1994; Taskinen et al.,

1 1994; Seitz and Baron, 1990; Axelsson et al., 1984). Absent evidence of alternative explanation
2 for these findings, it is concluded that exposure to formaldehyde is associated with pregnancy
3 loss and diminished fertility.

4 In animal studies, Sheveleva (1971) noted an increase in preimplantation loss in rats
5 exposed to 0.04 and 0.4 ppm formaldehyde by inhalation on GDs 1–19, and Kitaev et al. (1984)
6 observed evidence of degeneration in harvested embryos on GD 2, following 4 months of
7 maternal inhalation exposure to 0.41 ppm formaldehyde in rats. In a second series of tests
8 reported in Kitaev et al. (1984), female rats were exposed to 0.41 and 1.22 ppm formaldehyde for
9 4 months to test the hypothesis that the embryo degeneration could have been the result of
10 disrupted reproductive hormone levels in the dams. Ovarian weight and blood levels of LH were
11 increased at 0.41 ppm (but not at 1.22 ppm), and significantly increased levels of FSH were
12 observed at 1.22 ppm. Kitaev et al. (1984) proposed that effects at the 0.41 ppm might be related
13 to disruption of the hypothalamic-pituitary axis and that at the higher exposure level (1.22 ppm)
14 frank toxic effects to the embryo were observed. The increased FSH levels at 1.22 ppm may also
15 be indicative of hormonal perturbations induced by formaldehyde exposure that could affect
16 pregnancy maintenance in humans. The finding of treatment-related increased preimplantation
17 loss in rats appears to support the evidence of spontaneous abortion in the epidemiologic data. In
18 addition, a dominant lethal study in rats by Odeigah (1997) identified significant
19 postimplantation loss following pre-mating I.P. formaldehyde exposures to males, suggesting a
20 potential MOA involving germ cell toxicity. Nevertheless, a number of developmental toxicity
21 studies in rats did not report treatment-related embryo lethality following gestation exposures to
22 formaldehyde. These included inhalation studies by Martin (1990), Saillenfait et al. (1989), and
23 Kilburn and Moro (1985), a series of studies by Gofmekler and Bonashevskaya (1969),
24 Gofmekler (1968), and Pushkina et al. (1968), and studies by Senichenkova and Chebotar (1996)
25 and Senichenkova (1991). It is noted, however, that, to the extent that these studies evaluated
26 embryonic or fetal death, the observations were conducted late in gestation and the studies may
27 not have been designed to detect the preimplantation losses as observed in Kitaev et al. (1984)
28 and Sheveleva (1971). Additionally, a number of the reports for these studies did not include
29 sufficient details to engender a high degree of confidence in the reported results. Fetal death was
30 also not observed in oral studies with formaldehyde in beagle dogs (Hurni and Ohder, 1973) and
31 rats (Seidenberg and Becker, 1987).

1 **4.4.9.2. Congenital Malformations**

2 The effect of occupational exposures to formaldehyde on the incidence of congenital
3 malformations was examined by Dulskiene and Gražulevičiene (2005), Taskinen et al. (1994),
4 and Hemminki et al. (1985). Results were mixed.

5 In animal studies, the most frequently observed structural anomaly noted following
6 inhalation exposures to formaldehyde during gestation was a delay in fetal (i.e., 1st stage) testes
7 descent (Senichenkova and Chebotar, 1996; Senichenkova, 1991; Kilburn and Moro, 1985),
8 although similar findings were not reported by Saillenfait et al. (1989) or Martin (1990) in what
9 appear to be well-conducted prenatal developmental toxicity studies. No study in the available
10 database specifically examined the 2nd stage of postnatal testes descent in pups. Thus, there is no
11 evidence to determine if the observed effect represented a developmental delay or if it was
12 related to disruptions in male reproductive tract ontogeny, which is dependent on normal levels
13 of fetal testicular testosterone and on the expression of insulin-like hormone-3 (insl3) in fetal
14 Leydig cells (Klonisch et al., 2004). Senichenkova (1991) observed an increased incidence of
15 other organ anomalies following formaldehyde exposure during gestation; however, the
16 anomalies are not characterized in the report. Alterations on fetal organ weights and/or size were
17 noted in several studies (Kilburn and Moro, 1985; Gofmekler, 1968), but it is difficult to
18 ascertain if these findings represented agenesis, hypoplasia, or evidence of systemic organ
19 toxicity. Histopathologic evaluation of pup tissues following maternal gestational exposures to
20 0.01 and 0.81 ppm formaldehyde was conducted by Gofmekler and Bonashevskaya (1969),
21 revealing reduced glycogen content in the myocardium, the presence of iron in hepatic Kupffer
22 cells, and a positive Schiff reaction in the basement membrane (indicating functional alterations
23 in the renal tubule) at both exposure levels.

24 25 **4.4.9.3. Low Birth Weight and Growth Retardation**

26 A population-based study (Gražulevičiene et al., 1998) reported an association between
27 atmospheric formaldehyde exposure and low birth weight, with an adjusted OR of 1.37 (95% CI:
28 0.90–2.09).

29 A number of inhalation studies in rats identified reduced fetal weight as an adverse
30 outcome of in utero formaldehyde exposure and are supportive of the association noted in
31 humans. In a study that exposed pregnant rats to formaldehyde during GDs 6–20, Saillenfait et
32 al. (1989) observed significantly decreased male and female fetal rat weights (78 and 81% of
33 control values, respectively) at 40 ppm formaldehyde. In a study that exposed the dams from
34 GDs 6–15, Martin (1990) found decreased fetal weights at exposure levels of 5 and 10 ppm. In
35 both studies, observations of reduced or delayed skeletal ossification (i.e., the thoracic vertebrae

1 in Saillenfait et al. [1989] and the pubes and ischial bones in Martin [1990]) were consistent with
2 the fetal weight deficits. Kilburn and Moro (1985) also reported fetal body weight decreases in
3 rats at an inhalation exposure level of 30 ppm. Conversely, increased fetal body weight as
4 compared with controls (generally considered to be nonadverse) was noted by Gofmekler (1968)
5 and Pushkina et al. (1968) at maternal formaldehyde exposure levels of 0.1 and 0.81 ppm
6 administered for approximately 2–3 weeks prior to mating and then throughout gestation.
7 Increased fetal weight was also noted in rats by Senichenkova (1991) and Senichenkova and
8 Chebotar (1996) following maternal exposures to 0.41 ppm formaldehyde throughout gestation.

9 Studies that assessed the effects of oral administration of formaldehyde on development
10 are quite limited. The only oral study identified that found a treatment-related effect on offspring
11 growth was a study using beagle dogs (Hurni and Ohder, 1973). In this study, formaldehyde was
12 administered at doses of 3.1 or 9.3 mg/kg-day in the feed during gestation, and pup weight
13 decrements at postnatal week 8 were 6.3 and 12% in the low- and high-dose groups, respectively.
14

15 **4.4.9.4. *Functional Developmental Outcomes (Developmental Neurotoxicity)***

16 Indications of effects on the developing nervous system were observed in several rodent
17 studies, although no similar epidemiologic findings in children were identified. These studies
18 (Sarsilmaz et al., 2007; Aslan et al., 2006; Weiler and Apfelbach, 1992; Senichenkova, 1991;
19 Sheveleva, 1971) are described in detail in Section 4.2.6. In the studies by Aslan et al. (2006)
20 and Sarsilmaz et al. (2007), neonatal rats were exposed to formaldehyde for 30 days at 6,000 or
21 12,000 ppb. Decreases in the volume of discrete areas of the brain were observed at both
22 exposure levels in both studies, and, additionally, decreased cell numbers were noted in a region
23 of the hippocampus in the Sarsilmaz et al. (2007) study. Weiler and Apfelbach (1992) exposed
24 juvenile rats to 0.25 ppm formaldehyde for 130 days or adult rats to 0.5 ppm formaldehyde for
25 90 days. Olfactory thresholds measured in this study were significantly higher in the rats that
26 had been exposed as juveniles than in those that had been exposed only as adults. Sheveleva
27 (1971) observed alterations in spontaneous mobility in 1- and 2-month-old pups from dams that
28 had been exposed to formaldehyde at 0.04 or 0.4 ppm throughout gestation. In the Senichenkova
29 (1991) study, maternal rats were exposed to 400 ppb formaldehyde during GDs 1–19, and
30 functional observational testing was conducted on the juvenile offspring. Changes in open-field
31 motor activity, exploratory activity, and habituation were observed in the offspring.
32

33 **4.4.9.5. *Male Reproductive Toxicity***

34 A number of laboratory animal studies have reported effects of formaldehyde exposure
35 on male reproductive system endpoints. These effects include decreased testes weight, changes

1 in Leydig cell quantity and quality, degeneration of seminiferous tubules, decreased testosterone
2 levels, alterations in biomarkers of toxicity in the testes, and alterations in sperm measures
3 (Galilapour et al., 2007; Xing et al., 2007; Zhou et al., 2006; Özen et al., 2005, 2002; Sarsilmaz
4 et al., 1999; Odeigah, 1997; Majumder and Kumar, 1995; Chowdhury et al., 1992; Til et al.,
5 1989, 1988; Tobe et al., 1989; Johanssen et al., 1986; Maronpot et al., 1986; Cassidy et al., 1983;
6 Appelman et al., 1982; Guseva, 1972). Following concurrent exposures to formaldehyde in air
7 and drinking water for 6 months, Guseva (1972) found decreases in testicular nucleic acid levels.
8 In a study conducted by Chowdhury et al. (1992), rats were administered I.P. injections of
9 formaldehyde for 30 days, and evidence of decreased testes weight, serum testosterone levels,
10 and Leydig cell quality was observed. Sarsilmaz et al. (1999) followed up on these findings
11 (exposing male rats to formaldehyde via inhalation at 10 and 20 ppm for 4 weeks) and found
12 decreases in Leydig cell quantity and the percentage of cells with normal nuclei. Hypothesizing
13 that the reported decreases in Leydig cell quality may have been the result of oxidative stress and
14 damage, Özen et al. (2002) evaluated biomarkers of such changes and found that testicular zinc
15 and copper levels were decreased and iron levels were increased following exposures of adult
16 male rats to 10 and 20 ppm formaldehyde for 4 or 13 weeks. Additionally, relative testes weight
17 was decreased in a dose- and duration-dependent manner, although this effect had not been
18 observed by Sarsilmaz et al. (1999). Özen et al. (2005) noted decreased serum testosterone
19 levels, decreased seminiferous tubule diameters, and increased levels of heat shock protein in
20 spermatogonia, spermatocytes, and spermatids of rats following 91 days of exposure to 10 ppm
21 formaldehyde. A study by Gosalipour et al. (2007) observed decreased numbers of testicular
22 germ cells, altered spermatogenesis, and reduced seminiferous tubular diameter and epithelial
23 height in rats following 18 weeks of formaldehyde inhalation exposure; the severity of the
24 seminiferous tubule pathology was positively correlated to the number of hours/week of
25 exposure. Zhou et al. (2006) found decreased testis weight, atrophy of seminiferous tubules,
26 edematous interstitial tissue, and alteration of epididymal sperm count, morphology, and motility
27 in rats after 2 weeks of formaldehyde exposure at 8 ppm. Abnormal sperm were also observed in
28 mice by Xing et al. (2007) after 13-weeks of inhalation exposure at 16.9 ppm, and Cassidy et al.
29 (1983) reported sperm abnormalities in rats following a single oral dose of 200 mg/kg.
30 Additionally, Majumder and Kumar (1995) observed significantly reduced sperm count, motility,
31 and viability following 30 days of I.P. injection of 10 mg/kg-day formaldehyde to male rats.
32 Also in this study, the ability of formaldehyde to affect sperm parameters was confirmed with in
33 vitro testing. A study conducted by Odeigah (1997) demonstrated epididymal sperm count and
34 morphology abnormalities following five I.P. doses of ≥ 0.125 mg/kg formaldehyde and

1 additionally identified dominant lethal effects (decreased live embryos and increased dead
2 implants) following mating of treated male rats with untreated females.

3 Although Til et al. (1989) reported low incidences of Leydig cell tumors in
4 formaldehyde-treated rats in a chronic drinking water study, no alterations in testes weight or
5 histopathologic lesions of the testes were observed in subchronic inhalation studies conducted by
6 Appleman et al. (1982) or Maronpot et al. (1986) or in subchronic or chronic oral studies by
7 Johanssen et al. (1986), Til et al. (1988), or Tobe et al. (1989).

8 No epidemiologic studies have identified an association between formaldehyde exposure
9 and alterations in the male reproductive system (e.g., see Ward et al. [1984]).

11 **4.4.9.6. Female Reproductive Toxicity**

12 The available database for the assessment of the effects of formaldehyde exposure on the
13 female reproductive system was limited. In addition to the findings of spontaneous abortions, as
14 described above, Taskinen et al. (1999) examined fecundability in a cohort of healthy female
15 wood-processing industry workers and found that conception was significantly delayed in
16 women who were occupationally exposed to formaldehyde. The FDR, a ratio of average
17 incidence densities of pregnancies for exposed female employees compared with unexposed
18 female employees, was lower in women exposed to mean formaldehyde levels of approximately
19 0.33 ppm (range: 0.15–1.00 ppm) compared with controls (adjusted FDR = 0.64 [95% CI:
20 0.28-0.92]). An FDR <1.0 is indicative of delayed conception, which is an indicator of reduced
21 fertility. The subfertility observed in this study is supportive of the association observed
22 between formaldehyde exposure and spontaneous abortion, since subclinical pregnancy losses
23 are increased in women with compromised fertility (Gray and Wu, 2000; Hakim et al., 1995),
24 and both spontaneous abortion and subfertility can be related to exposure to environmental
25 toxicants (Correa et al., 1996).

26 As described above, formaldehyde exposures to female rats resulted in decreased ovarian
27 weight and altered LH and FSH levels (Kitaev et al., 1984). Maronpot et al. (1986) reported
28 endometrial hypoplasia and lack of ovarian luteal tissue in female mice exposed to 40 ppm
29 formaldehyde for 13 weeks. Additionally, it is noted that, in developmental toxicity studies that
30 included repeated exposures of dams before mating and/or during gestation, reports of adverse
31 pregnancy outcomes were few. Gofmekler (1968) reported an increase in pregnancy duration
32 and decrease in litter size; however, this finding was not observed in other studies.

33 With the exception of spontaneous abortion and increased time to pregnancy, associations
34 of formaldehyde exposure with adverse female reproductive system outcomes were not observed
35 in the available epidemiologic data.

1 **4.4.9.7. Mode of Action**

2 A strong case cannot be made for any one MOA that explains one or more of the
3 reproductive and developmental outcomes observed in formaldehyde epidemiologic or
4 toxicology studies. This is due to a number of issues, including the following:

5 (1) inconsistencies in study findings for the toxicology studies, which may be explained by study
6 quality issues (see detailed descriptions of studies in Sections 4.1 and 4.2); (2) few studies that
7 allow for comparisons because no study was performed with the same study design (e.g., stage of
8 exposure, dose, species, and strain); (3) few mechanistic studies available to test hypothesized
9 MOAs; and (4) a bias that is pervasive in the formaldehyde literature that outcomes observed
10 beyond the POE (the nose) are not expected from inhalation exposure, which is the route of
11 exposure for most of the developmental and reproductive studies. This discussion presents
12 putative MOAs that have been hypothesized by study authors and the studies that support the
13 hypothesized MOAs. The four hypothesized MOAs are not mutually exclusive. They could be
14 acting alone for certain endpoints (in which case the others are not operable) or in concert for
15 certain endpoints.

16 The focus of this discussion is on analyzing possible MOAs for the developmental and
17 reproductive outcomes that were noted most consistently, across toxicology studies and, in some
18 cases, across human and animal studies. These outcomes include developmental delays, fetal
19 loss, and sperm quality and quantity effects.

20 An endocrine-disrupting MOA is supported by some of the reproductive and
21 developmental epidemiology and toxicology studies. For example, the decreases in fetal body
22 weight (Martin, 1990; Saillenfait et al., 1989), delayed ossifications (Senichenkova and
23 Chebotar, 1996; Senichenkova, 1991; Martin, 1990; Saillenfait et al., 1989), and delayed
24 eruption of incisors (Senichenkova, 1991) noted in rats after gestational exposure to
25 formaldehyde are consistent with developmental delays. In turn, developmental delays can result
26 from effects on the hypothalamic-gonadal-pituitary axis in the dam that cause hormonal level
27 changes in the pup; however, hormone levels in pups were not measured. Kilburn and Moro
28 (1985) also observed organ size changes and undescended testes after developmental
29 formaldehyde exposure. These outcomes can also be explained by an endocrine MOA. There
30 are three studies that directly tested for changes in hormones after formaldehyde exposure.
31 Kitaev et al. (1984) observed ovarian weight and serum LH and FSH increases after inhaled
32 formaldehyde in adult female rats. Chowdhury et al. (1992) assessed serum testosterone levels
33 in adult rats after formaldehyde IP exposure and found significant decreased testosterone and
34 testes weights and a decrease in 3- β , Δ -5-hydroxy steroid dehydrogenase in Leydig cells,
35 suggesting that formaldehyde affects steroidogenesis. Özen et al. (2002) also reported

1 significant serum testosterone level decreases as well as decreased mean seminiferous tubule
2 diameters. Furthermore, the steroidogenesis MOA leading to reduced testosterone is also
3 consistent with the sperm quality and quantity decrements observed in the studies by Özen et al.
4 (2002), Sarsilmaz et al. (1999), and Odeigah (1997) studies.

5 In human studies, delayed time to pregnancy and increased incidence of spontaneous
6 abortion (Taskinen et al., 1999), consistent with some study findings from the toxicology
7 literature, could also be explained by an endocrine MOA. Alterations in hormone levels could
8 lead to pregnancy maintenance problems. Extrapolating the Chowdhury et al. (1992) results of
9 the steroidogenesis MOA to females, formaldehyde exposure could affect progesterone levels
10 required for pregnancy. However, progesterone levels were unchanged in the female rat in the
11 one study that assessed progesterone (Kitaev et al., 1984). Consistent with an endocrine
12 mediated MOA, Maronpot et al. (1986) observed endometrial hypoplasia and lack of ovarian
13 luteal tissue in females exposed to formaldehyde.

14 A second hypothesized MOA for some of the developmental and reproductive outcomes
15 is genotoxicity of the gametes. Such an MOA could explain pregnancy loss in humans
16 (Taskinen, et al., 1999) and preimplantation loss in animal studies (Xing et al., 2007; Kitaev et
17 al., 1984; Sheveleva, 1971) and fetal viability (e.g., litter size decreases) after formaldehyde
18 exposure. Consistent with male gamete genotoxicity, Odeigah (1997) and Xing et al. (2007)
19 observed reduced fertile matings and live embryos, and increased dead implants in a dominant
20 lethal study.

21 Oxidative stress/damage is another MOA that is consistent with testicular toxicity, sperm
22 effects, and reduced embryo viability. Özen et al. (2002) investigated the mechanism of
23 oxidative stress being responsible for the testes quality effects by assessing testicular iron,
24 copper, and zinc levels. Zinc and copper levels were reduced in the rat testes, consistent with an
25 oxidative stress MOA. Özen et al. (2002) also reported increased iron levels and decreased zinc
26 levels in the lung, consistent with oxidative stress. Another study (Zhou et al., 2006) that
27 investigated the oxidative stress MOA in the testes observed significant changes in oxidative
28 stress biochemical markers (decreases in SOD, GPX, GSH, and an increase in MDA levels).
29 The authors also assessed the protective effect of coadministration with vitamin E, an
30 antioxidant, on decreased testes weight, biochemical alterations, histopathologic effects, and on
31 sperm count, motility, and morphology. The study of Pushkina et al. (1968) found reduced
32 levels of Vitamin C, another antioxidant, in the fetus and maternal liver after formaldehyde
33 exposure.

1 The MOAs proposed are not mutually exclusive and in fact could interact with one
2 another. For example, an endocrine MOA could lead to oxidative stress, and that oxidative stress
3 could lead to genotoxicity.

4 5 **4.4.9.8. Data Gaps**

6 The inhalation developmental toxicity studies conducted on formaldehyde and described
7 in Section 4.2.7 comprise an adequate assessment of prenatal developmental toxicity for
8 application to inhalation reference concentration determination. The assessments of postnatal
9 developmental toxicity and of reproductive function following inhalation of formaldehyde are
10 less complete. It is notable that, although the database contains some studies that assess various
11 aspects of reproductive organ system toxicity, particularly in males, there is no assessment of
12 multigenerational reproductive function, such as would be evaluated in a two-generation
13 reproductive toxicity study, nor is there an assessment of potential reproductive effects of
14 formaldehyde exposure in human males.

15 Adequate assessments of developmental and reproductive toxicity following oral
16 exposures to formaldehyde have not been conducted.

17 18 **4.5. SYNTHESIS AND EVALUATION OF CARCINOGENICITY**

19 **4.5.1. Cancers of the Respiratory Tract**

20 Epidemiologic studies of formaldehyde-exposed workers provide sufficient evidence of a
21 causal association between formaldehyde exposure and nasopharyngeal cancer (see
22 Section 4.1.2.1.1) as well as nasal and paranasal cancers (see Section 4.1.2.1.2). The
23 epidemiologic evidence of association between formaldehyde exposure and other upper
24 respiratory tract cancers (see Section 4.1.2.1.3) is consistent with, and supportive of, a causal
25 association but insufficient on its own to reach a causal conclusion. However, taken together
26 with the causal evidence of an association between formaldehyde and nasopharyngeal cancer and
27 sinonasal cancer in neighboring tissues of the upper respiratory tract and sites of first contact
28 with inhaled formaldehyde, along with the strongly supportive evidence of association in
29 animals, the evidence is sufficient to conclude that formaldehyde is causally related to cancers of
30 the upper respiratory tract as a group.

31 The observational evidence from epidemiologic studies reporting associations between
32 formaldehyde exposure and increased risk of nasopharyngeal cancer supports a conclusion of a
33 causal association for this specific cancer. However, epidemiologic studies of rare outcomes
34 such as nasopharyngeal cancer, which has an expected incidence of 1 per 100,000 people per
35 year in the United States, do not typically have great statistical power to rule out the null

1 hypothesis (i.e., no association). However, the weight of evidence of the several studies
2 reviewed in Section 4.1.2.1.1 provide an accumulation of consistent observational evidence
3 sufficient to exclude chance as an explanation for the association. Additionally, there is
4 insufficient evidence of consistent confounding or other bias across the studies that were
5 considered; thus, confounding and bias were also ruled out as explanations for the observed
6 association. The lack of a convincing and consistent alternative hypothesis of causation – in
7 spite of repeated examinations – further supports the conclusion that the association between
8 formaldehyde exposure and increased risk of nasopharyngeal cancer is causal.

9 The single strongest cohort study, Hauptmann et al. (2004), shows a statistically
10 significant exposure-response relationship between formaldehyde exposure and upper respiratory
11 tract cancers. Hauptmann et al. (2004) demonstrated significant excess risk of nasopharyngeal
12 cancer in exposed workers based on U.S. population death rates (standardized mortality ratio
13 [SMR] = 2.1; 95% confidence index [CI] 1.05–4.21) in a large cohort of 25,619 industrial
14 workers. In addition to the SMR based on an external comparison population, relative risks
15 (RRs) were presented based on internal comparisons of workers in order to minimize potential
16 selection bias due to the well known healthy worker effect. Statistically significant exposure-
17 response relationships between increased formaldehyde exposure and increased risk of
18 nasopharyngeal cancer were reported for two different metrics of exposure (peak and cumulative
19 exposure). Relative risks for nasopharyngeal cancer were also elevated for increased duration of
20 exposure to formaldehyde and for the average intensity of exposure. These analyses controlled
21 for potential confounders including calendar year, age, sex, race, and pay category. While
22 exposure measurement error is likely to be present in any epidemiologic study, there was no
23 evidence of any differential measurement error that could have produced the observation of a
24 spurious association. Any nondifferential measurement error would likely have attenuated the
25 effect of formaldehyde was smaller than that which would otherwise have been observed in the
26 absence of measurement error.

27 The case-control studies similarly also report associations between formaldehyde
28 exposure and cancer mortality for nasopharyngeal cancer. Although other risk factors for
29 nasopharyngeal cancer (e.g., Epstein-Barr Virus) and the predominant nasopharyngeal cancer
30 histological subtype (SCC versus undifferentiated) vary significantly across the world, case-
31 control studies consistently provide evidence of an association between occupational exposure to
32 formaldehyde and nasopharyngeal cancer (Vaughn et al., 1986a; Vaughn et al., 2000; Roush et
33 al., 1987; Hildesheim et al., 2001; West et al., 1993). In their more recent study, Vaughn et al.
34 (2000) used worker histories to estimate each individual worker’s formaldehyde exposure.
35 Workers with more than 1.10 ppm-years of cumulative exposure were found to be at

1 significantly higher risk of nasopharyngeal cancer, with an odds ratio (OR) of 3.0 (95% CI: 1.3-
2 6.6) (Vaughn et al., 2000). Two different exposure metrics, duration of exposure and cumulative
3 exposure, were positively associated with increased risk of nasopharyngeal cancer, with a
4 significant test for trend ($p = 0.014$ and 0.033 , respectively). The OR also increased in
5 magnitude as the probability of “Ever” having occupational exposure increased, from OR = 1.6
6 among those whose exposure was judged to be “Possible, probable or definite” to OR = 13.3
7 among those with “Definite” exposure (p -trend < 0.001).

8 Nasopharyngeal cancer histological subtype analysis indicates that these associations
9 held for both SCC and epithelial nasopharyngeal cancer, but not for the undifferentiating and
10 nonkeratinizing nasopharyngeal cancer (Vaughn et al., 2000). However, formaldehyde exposure
11 is also associated with risk of nasopharyngeal cancer in Taipei, Taiwan, where greater than 90%
12 of the cases had nonkeratinizing and undifferentiated carcinomas and less than 10% of the cases
13 were diagnosed as having SCCs (Hildesheim et al., 2001). These reported associations were
14 strengthened by considering higher probability of exposure (RR = 2.6; 95% CI: 1.1-6.3), greater
15 intensity of exposure (RR = 2.1; 95% CI: 1-4.2) and EBV seropositive cases (RR = 2.7; 95% CI:
16 1.2-5.9) (Hildesheim et al., 2001). Case-control studies have also linked residential exposure to
17 formaldehyde, specifically for years of residence in mobile homes (Vaughn et al., 1986b) and the
18 use of mosquito coils in the Philippines (West et al., 1993). Independent testing of 6 brands of
19 East Asian mosquito coils evaluated the emission rates of carbonyl compounds in the mosquito
20 smoke and reported that formaldehyde and acetaldehyde had the highest emission rates. Among
21 the three experiments on each of the six brands, the range of formaldehyde concentrations was
22 from $0.87 \mu\text{g}/\text{m}^3$ (1 ppb) to $25 \mu\text{g}/\text{m}^3$ (31 ppb).

23 As a group, other URT sites of direct contact with formaldehyde upon inhalation (i.e.,
24 salivary gland, mouth, nasal cavity and larynx) also showed evidence of a trend in increasing
25 relative risks with increasing average intensity and peak exposure in the Hauptmann et al. (2004)
26 cohort study, although these trends did not reach the level of statistical significance. The results
27 from other cohort studies and case-control studies are mixed (between positive associations and
28 null findings) for associations between formaldehyde exposure and specific cancers of the URT
29 (IARC, 2006). For rare cancers, extremely large cohorts would be needed to have the statistical
30 power to detect an association for tumors defined by individual sites (e.g., mouth, salivary gland,
31 hypopharynx). Results vary in the smaller cohort studies, where a single case may result in an
32 elevated risk but taken together the evidence is considered suggestive (see Section 4.1.2). Case-
33 control studies have been useful to better understand potential associations between
34 formaldehyde exposure and rare cancers of the URT. Luce et al. (2002) evaluated pooled data
35 from 12 case-control studies and demonstrated a statistically significant increased risk between

1 formaldehyde exposure and sinonasal cancer. A case-control study by Gustavsson et al. (1998)
2 suggested an association between formaldehyde exposure and oral squamous cell carcinoma
3 (SCC), esophageal, and laryngeal cancers, with odds ratios (ORs) of 1.28, 1.90, and 1.45,
4 respectively. However, the individual ORs were not statistically significant. Hypopharyngeal
5 cancer was linked with formaldehyde exposure with an OR of 3.78 (95% CI: 1.50-9.49) in
6 another case-control study (Laforest et al., 2000). While the data on site-specific cancers of the
7 URT is somewhat sparse, they are consistent with a carcinogenic hypothesis and in their large
8 cohort study, Hauptmann and colleagues (2004) concluded that in spite of the small numbers of
9 deaths from cancers of the URT, the positive associations with average intensity and peak
10 exposure were consistent with the carcinogenicity of formaldehyde at these sites of first contact.

11

12 **4.5.1.1. Supporting Animal Evidence**

13 Animal studies, primarily rodent bioassays, strongly support the causal relationship
14 between of formaldehyde exposure and URT carcinogenicity. Formaldehyde-induced cancers
15 are primarily seen in the nasal passages (Kerns et al., 1983; Monticello et al., 1996; Tobe et al.,
16 1985; Kamata et al., 1997; Sellakumar et al., 1985), but it should be noted that rodents, unlike
17 humans, are obligate nose breathers and have convoluted nasal turbinates. Chronic animal
18 studies report tumor incidence in a variety of rodent models. Study descriptions are provided
19 above in detail (see Section 4.2.2, Table 4-42).

20 In rodent studies of the respiratory tract, only nasal tumors are considered to be induced
21 by formaldehyde. Repeated exposures to 10-15 ppm formaldehyde result in gross nasal lesions
22 and high incidence of nasal tumors (see Table 4-42, Section 4.2.1). Although increased cell
23 proliferation, squamous metaplasia, dysplasia, and focal necrotic lesions have been noted in the
24 larynx and trachea in some studies, no tumors in these locations have been reported in the rodent
25 studies. The majority of studies were conducted using rats (F344, Wistar, or Sprague-Dawley),
26 and all studies of 18 months or greater in mice and rats show evidence of formaldehyde-induced
27 nasal carcinogenicity. The nasal tumors are primarily SCCs, although papillomas, polypoid
28 adenomas, adenocarcinomas, fibrosarcomas, and esthesioneuroepitheliomas have been reported
29 (Kamata et al., 1997; Monticello et al., 1996; Morgan et al., 1986a, b; Takahashi et al., 1986;
30 Sellakumar et al., 1985; Kerns et al., 1983; Albert et al., 1982). Although hyperplasia, dysplasia,
31 and squamous metaplasia of the respiratory epithelium have been observed beyond the nasal
32 cavity, other respiratory tract tumors have not been reported to be significantly increased by
33 formaldehyde exposure alone.

34 Increased tumor incidence and decreased latency are correlated with increasing
35 formaldehyde exposure concentration. Reviewing data from the only lifelong inhalation study

1 (i.e., until "natural death") with multiple exposure groups, nasal SCCs occurred much earlier in
2 the high-exposure animals. For example, tumors are first noted at 8 and 9 months following
3 exposure for high-exposed (15 ppm) female and male F344 rats versus tumors not arising until
4 24 months in low-exposed rats (2 ppm) (Kerns et al., 1983). In a follow-up study by Monticello
5 et al. (1996), the incidence of SCC in rats exposed at 15 ppm was 47%, with the first tumor noted
6 at 12 months. The incidence of SCC in male rats exposed at 10 ppm was 22%, with the first
7 tumors observed at 18 months after exposure. Moreover, of the 90 rats exposed at 6 ppm for 20
8 months, only one SCC was noted. No SCCs were detected in rats exposed to 0.7 or 2 ppm
9 formaldehyde. These incidence rates are not mortality-adjusted (see Chapter 5, Section 5.3.4)
10 and include animals from each scheduled sacrifice (3, 6, 12, and 18 months). In a lifelong study
11 of male Sprague-Dawley rats exposed to 10 ppm formaldehyde, the cumulative nasal tumor
12 incidence was calculated as a function of time of exposure (Sellakumar et al., 1985). After 2
13 years of exposure, the adjusted probability of nasal carcinoma was greater than 60%.

14 There is some evidence that less-than-lifetime exposure to formaldehyde can induce nasal
15 tumors over an extended observation period. Two studies, both in male Wistar rats, report nasal
16 tumors in response to less-than-lifetime exposures (Woutersen et al., 1989; Feron et al., 1988).
17 A 13-week exposure at 20 ppm followed by an observation period of 30 months (inclusive of
18 exposure) in Wistar rats resulted in six nasal tumors including three nasal SCCs, one cystic SCC
19 of the nasolacrimal duct, one carcinoma in situ and an ameloblastoma, while no tumors were
20 noted in the corresponding air-exposed controls (Feron et al., 1988). A limited number of
21 formaldehyde-related tumors were noted from 4 or 8 weeks of exposure followed by 30 months
22 of observation. Although the tumor incidence of these less-than-lifetime exposures is low, this is
23 consistent with the 2-year bioassays in Wistar rats. Wistar rats are more resilient to
24 formaldehyde-induced nasal toxicity than F344 or SD rats (see Section 4.2.1), and only 1 of 26
25 (4%) Wistar rats exposed at 10 ppm for 28 months developed SCC (Woutersen et al., 1989)
26 versus 22% in F344 rats (Monticello et al., 1996).

27 The specificity of formaldehyde-induced tumors in the nasal passages of rodents is
28 believed, at least in part, to be a function of tissue dose. Computational fluid dynamics (CFD)
29 modeling used to predict formaldehyde tissue flux during inhalation exposures suggests that at
30 comparable concentrations, tissue flux in the nasal passages of rodents is more intense than for
31 nonhuman primates and humans. Modeling predicts a different pattern of formaldehyde flux into
32 URT tissues of rodents compared to humans, where formaldehyde penetrates more deeply into
33 the respiratory tract of primates than rodents even considering nose-only breathing for primates
34 (see Section 3.4). Humans will generally switch to mouth breathing when sensing an irritating
35 smell and during physical exertion, resulting in direct exposures to the mouth and greater tissue

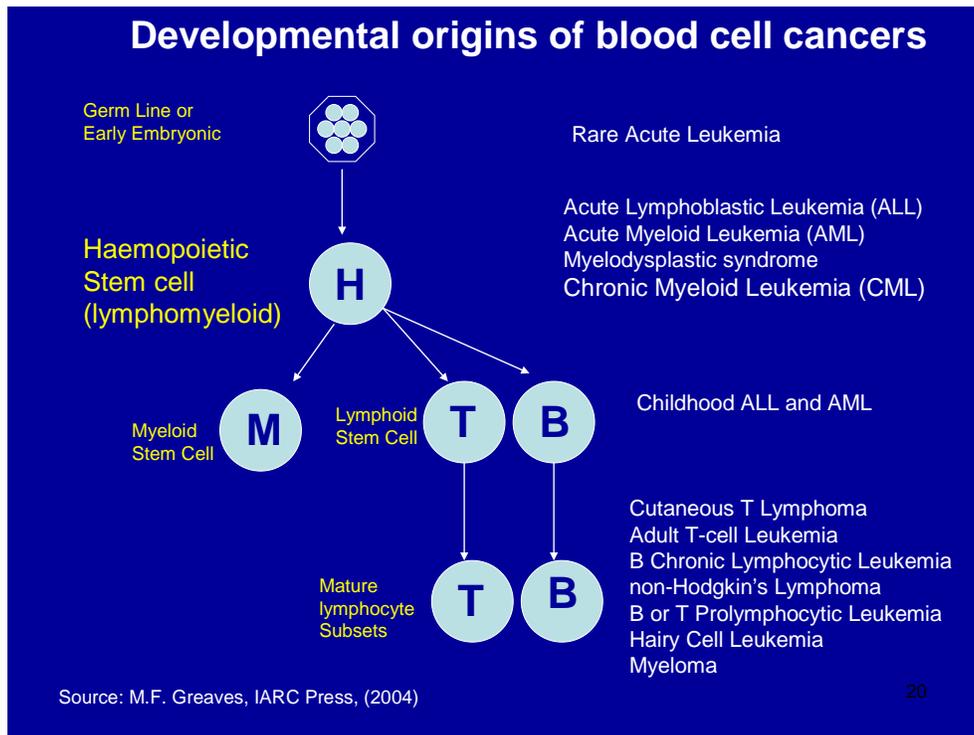
1 flux in tissues beyond the bypassed nasal passages. Therefore, species differences in tissue dose
2 may contribute to formaldehyde-induced tumors in humans beyond the nasal passages, which are
3 not evident in rodent bioassays.

4 4.5.2. Lymphohematopoietic Malignancies

6 4.5.2.1. Background

7 Lymphohematopoietic (LHP) cancers include neoplasms of both lymphoid and myeloid
8 cell origins. Cancers of the immune system are described as leukemia if they primarily involve
9 cells from peripheral blood and bone marrow at diagnosis and lymphomas if they constitute a
10 solid tumor (Robbins, 2004). Some forms of leukemia which present as an immature immune
11 cell phenotype are believed to arise from lymphomyeloid stem cells or progenitor cells normally
12 found in the bone marrow (e.g., acute lymphoblastic leukemia (ALL) and acute myeloid
13 leukemia (AML)) (Greaves, 2004). However, multiple myeloma, lymphomas and some
14 leukemias may arise from mature functional lymphocytes present outside of the bone marrow
15 (Greaves, 2004; see Figure 4-32).

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Figure 4-32. Developmental origins for cancers of the lymphohematopoietic system (Adapted from Greaves (2004)).

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1 **4.5.2.2. All LHP Malignancies**

2 Epidemiologic studies involving formaldehyde-exposed workers provide sufficient
3 evidence of a causal association between formaldehyde exposure and all LHP malignancies (see
4 Section 4.1.2.2.1, summarized in Section 4.1.2.2.1.4). Positive associations between
5 formaldehyde exposure and LHP cancers have been reported for chemical workers (Wong et al.,
6 1983; Bertazzi et al., 1986), embalmers (Walrath and Fraumeni, 1983, Walrath and Fraumeni,
7 1984; Hayes et al., 1990), anatomists and pathologists (Harrington and Shannon 1975; Hall et al.,
8 1991; Levine et al. 1984; Stroup et al., 1986; Matanoski et al., 1989) (see Table 4-90). However,
9 clear associations (in terms of overall standardized mortality ratios [SMRs] or proportional
10 mortality ratios [PMRs]) were not reported in analyses for garment workers, iron-foundry
11 workers, and a large U.S. industrial cohort (Pinkerton et al., 2004; Andjelkovich et al., 1995;
12 Beane-Freeman et al., 2009; Marsh et al., 1996), although associations were observed in some of
13 these studies when exposure-response relationships were considered. Several published meta-
14 analyses are available which more formally assess the strength of association between
15 formaldehyde exposure and mortality from all LHP cancers. Pooled SMRs indicate stronger
16 associations for professional workers (embalmers, anatomists, and pathologists) than industry
17 workers (see Table 4-91). Bosetti et al. (2008) found similar relationships, with a pooled SMR
18 of 1.31 (95% CI: 1.16-1.47) for ‘professionals’ (i.e., embalmers, anatomists and pathologists)
19 versus a pooled estimate of 0.85 (95% CI: 0.74-0.96) for industrial workers. A recent meta-
20 analysis by Zhang et al. (2009) reports a summary relative risk (RR) of 1.25 (95% CI: 1.09-1.43)
21 for both professional and industry workers for all LHP cancers (ICD 9 codes 200-209). These
22 researchers identified 19 cohort study analyses, including cohort study updates. Zhang et al.
23 (2009) used the reported RR from the highest exposure category to increase statistical power and
24 reduce uncertainty regarding confounding or other bias. The criteria for study inclusion and
25 exclusion applied by Zhang et al. (2009) appear to be appropriate and the methodology for using
26 myeloid-specific results where possible also appears to be appropriate. This meta-analysis is
27 supportive of a causal association between formaldehyde and LHP malignancies.

28 The apparent differences by industry/profession may reflect many influences, including
29 exposure potential and demographic characteristics. External analysis (use of the general
30 population for comparison) relies on the assumption that cancer incidence rates are expected to
31 be similar between the general population and the study population in the absence of exposure.
32 The ‘healthy worker effect’ is well known, and there may be differences in the magnitude of this
33 selection bias by industry or profession. For instance, LHP cancer incidence and mortality have
34 many risk factors including socioeconomic status. Therefore, the consistent positive findings in
35 professional workers versus mixed results in industrial workers could be influenced by the
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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 Analysis¹				
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

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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 (<i>n</i> = 1,330)	Employed between 1959–1980 Mortality through 1986	2.01	NR	Bertazzi et al., 1986
Plastic manufacturing (<i>n</i> = 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 (<i>n</i> = 1,132)	Licensed between 1925–1980	1.15	1.32	Walrath and Fraumeni, 1983
Embalmers, CA (<i>n</i> = 1,007)	Licensed between 1925–1980	1.22	1.75 (<i>p</i> < 0.05) 1.24 (<20 years) 2.21 (<i>p</i> < 0.05) (>20 years)	Walrath and Fraumeni, 1984
Embalmers, U.S. (<i>n</i> = 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 (<i>p</i> < 0.05) Nonwhite 2.72 (<i>p</i> < 0.05)	Hayes et al., 1990
Case-Control Studies^a				
American cancer Society Cancer Prevention Study II: (<i>n</i> = 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) (<i>n</i> = 622)	Recruited in 1980–1983	NR	1.0 (0.7–1.4) Low 0.7 (0.2–2.6) High	Blair et al., 1993

^aRelative risk estimate (SMR or OR) presented with 95% confidence intervals, where available.

^bSMR for leukemia for the total group calculated from the published data for lymphatic leukemia (204, myeloid leukemia (205), and other/unspecified (206, 207).

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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}

$$^a \text{SMR} = \frac{\text{Obs}}{\text{Exp}}$$

^b Fischer's exact confidence intervals.

^c See Table 2 of Beane-Freeman et al. (2009).

$$^d \text{RR} = \frac{\text{Deaths}_{\text{Comparison Group}} / \text{Person-Time}_{\text{Comparison Group}}}{\text{Deaths}_{\text{referent Group}} / \text{Person-Time}_{\text{referent Group}}} = \left[\frac{108 - 19}{103 - 14} \right] \times \left[\frac{\text{PT}_{\text{referent Group}}}{\text{PT}_{\text{Comparison Group}}} \right]$$

$$\text{where } \left[\frac{\text{PT}_{\text{referent Group}}}{\text{PT}_{\text{Comparison Group}}} \right] \cong \left[\frac{108^c}{103^c \times 1.37^c} \right] = 0.765$$

$$^e \text{Var}(\log \text{RR}) = \frac{1}{\text{Deaths}_{\text{Comparison Group}}} + \frac{1}{\text{Deaths}_{\text{referent Group}}}$$

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.

1 The only study which has data to inform the effects of either exposure level or the
2 appropriateness of an external comparison group on the association between formaldehyde
3 exposure and all LHP cancer mortality is the National Cancer Institute (NCI) cohort study of
4 industrial workers (Blair et al., 1986; Beane-Freeman et al., 2009), which presents relative rates
5 based on internal comparisons for 3 different exposure metrics. Although SMR analysis with an
6 external comparison group did not indicate increased mortality from all LHP cancers (0.94, CI:
7 0.84-1.06, for the exposed workers), internal analysis using the low-exposed workers as the
8 comparison group demonstrates positive exposure-response relationships for increased mortality
9 from all LHP malignancies cancers and peak exposure across the study periods (1965-2004) (see
10 Figure 4-7 and 4-8) (Beane-Freeman et al., 2009), with a statistically significant trend ($p < 0.05$)
11 for every year since 1977. These results, indicating a positive exposure-response relationship
12 among plant workers, who most likely have similar demographic characteristics, are noteworthy
13 given the apparent lack of association when SMRs for the same cohort are calculated against
14 mortality rates for the general population. The lack of an apparent association with SMRs may
15 be attributable to the healthy worker effect and/or some other difference between the exposed
16 workers and the general population.

17 Although the association between formaldehyde exposure and all LHP cancer mortality
18 in industrial and professional cohorts is mixed, the strength of the internal analysis of the NCI
19 cohort, in the absence of positive SMRs compared to the general population, suggests that SMR
20 analyses may not be the most appropriate methodology for assessing LHP cancer mortality.
21 Given the potential for demographic differences between an industrial workforce and the general
22 population, the results of the internal analysis of the NCI industrial cohort provide a higher
23 quality analysis—and therefore should be given significantly more weight than SMR analyses of
24 industrial workers that could not distinguish their findings from the null. Given the consistency
25 and strength of the positive associations for all LHP cancers cancer mortality in professional
26 cohorts (embalmers, anatomists and pathologists) taken together with the strong positive results
27 of the NCI cohort, human epidemiologic evidence are sufficient to conclude that there is a causal
28 association between formaldehyde exposure and mortality from all LHP malignancies (as a
29 group).

31 **4.5.2.3. All Leukemia**

32 Epidemiologic studies involving formaldehyde-exposed workers provide sufficient
33 evidence of a causal association between formaldehyde exposure and all leukemia as a group
34 (see Section 4.1.2.2.1, summarized in Section 4.1.2.2.1.4). Like the analysis of all LHP cancers,
35 an analysis of all leukemia combines diseases which differ significantly in cell of origin and

1 etiology, including acute and chronic forms of both myeloid and lymphatic leukemia. This class
2 also includes other leukemia (e.g., erythraemia) and a general category of ‘other and unspecified
3 leukemia’ (ICD-8 207). Regardless, there is some utility in evaluating the all leukemia mortality
4 data because many studies provided results for this grouping. Also, the diagnosis of leukemia
5 versus solid LHP tumors is fairly distinct thereby limiting misclassification of the endpoint.

6 Although results are mixed across the studies (see Table 4-90), an association between
7 formaldehyde exposure and leukemia mortality is supported by cohort analyses of embalmers,
8 pathologists and anatomists (Hayes et al., 1990; Walrath and Fraumeni, 1983; Walrath and
9 Fraumeni 1984; Hall et al., 1991; Levine et al., 1984; Stroup et al., 1986; Matanoski et al., 1989).
10 Formaldehyde exposure and formaldehyde-related occupation are associated with leukemia
11 diagnosis in a case-control study (RR = 5.79 (95% CI: 1.44-23.25), but not formaldehyde
12 exposure alone (RR = 0.96; 95% CI: 0.54-1.71) (Stellman et al., 1998) (see Section 4.1.2.2.1 for
13 complete study summaries).

14 In contrast, SMR analyses of the industrial cohorts do not indicate a similar association
15 (Coggon et al., 2000; Beane-Freeman et al., 2009, Pinkerton et al., 2004). Although the SMR
16 analysis provided for the NCI cohort does not indicate a positive association for all leukemia
17 using an external reference group (Beane-Freeman et al., 2009), the SMR for exposed versus
18 unexposed workers within the cohort suggests all leukemia is elevated 2.1-fold with this internal
19 comparison (95% CI: 0.99-4.56)⁶. A positive exposure-response relationship further strengthens
20 the association of formaldehyde exposure to leukemia mortality (Beane-Freeman et al., 2009).
21 Where the referent group is defined as ‘low exposed’ individuals, leukemia is elevated in the
22 highest peak exposure category (RR = 1.42; 95% CI: 0.92-2.18) compared to both the referent
23 group and the unexposed category (RR = 0.59; 95% CI: 0.25-1.36), and there is a statistically
24 significant trend across all groups ($p = 0.02$). Categorical analysis for the average intensity and
25 cumulative exposure metrics suggests greater mortality in the high-exposure groups versus the
26 ‘low exposed’ individuals (RR = 1.10 [95% CI: 0.68-1.78] and 1.11 [0.7-1.74]. respectively), but
27 analysis of individual results across the exposure-response range indicates cumulative exposure
28 is a better predictor ($p = 0.08$ for trend across all exposed and unexposed.)

29 Several meta-analyses have been conducted for formaldehyde exposure and leukemia
30 which indicate a positive association (see Section 4.1.2.2.1.3). Collins et al. (2004) report an
31 overall RR for 18 available studies of 1.1 (CI: 1.0-1.2), suggesting an association of leukemia
32 with formaldehyde exposure. This association was stronger for both pathologists/anatomists

$${}^6 \text{Var} \left(\ln \left(\frac{SMR_{Exposed}}{SMR_{Non\ exposed}} \right) \right) = \frac{1}{Obs_{Exposed}} + \frac{1}{Obs_{Non\ exposed}}$$

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1 (1.4; CI: 1.0-1.9) and embalmers (RR = 1.6; 1.2-2.0), but there was no association when
2 considering only industrial workers (RR = 0.9; 0.8-1.0). Study design also impacted the
3 apparent strength of association, with stronger associations seen in case-control studies
4 (RR = 2.4; 0.9-6.5) versus cohort studies (RR = 1.0; 0.9-1.2). Bosetti et al. (2008) reported an
5 association between formaldehyde exposure and leukemia mortality with a pooled RR of 1.39
6 (95% CI: 1.15-1.68) for 8 groups of professional workers. In the same analysis, the pooled RR
7 for the 4 industrial cohorts was 0.90 (0.75-1.07). Zhang et al. (2009) reported a pooled RR of
8 1.54 (95% CI: 1.18-2.00) for all cohorts identified in their meta-analysis, although this pooled
9 RR should be considered with some caution, as myeloid leukemia alone was included in the
10 analysis where available (Zhang et al., 2009).

11 While the epidemiologic evidence for a causal association between formaldehyde and all
12 leukemia as a group is not as strong as for all LHP as a group, the repeated identification of an
13 association in multiple meta-analyses taken together with the clear causal association between
14 myeloid leukemia demonstrated by Hauptmann et al. (2009) and the consistent evidence reported
15 by Beane-Freeman et al. (2009) are sufficient to conclude that there is a causal association
16 between formaldehyde exposure and mortality from all leukemia as a group (see
17 Section 4.1.2.2.1.4).

18

19 **4.5.2.4. Subtype Analysis**

20 Given the associations discussed above between formaldehyde exposure and both all
21 LHP cancers and all leukemia, further analysis is needed to examine if the observed increase in
22 all LHP cancers is primarily a reflection of increased leukemia, or if other types of LHP cancers
23 may be elevated as well. Although analysis of mortality data by subtype may provide a better
24 understanding of the specific disease associations, there are potential pitfalls as well. Chief
25 among these concerns are the potential for disease misclassification (especially in studies with
26 older mortality data) and lack of statistical power as the number of observed cases is reduced by
27 considering subtypes. Case control studies by design address specific diseases and are well-
28 suited for subtype analysis, but often provide little exposure information. The following analysis
29 will draw from the available data to examine which forms of LHP malignancies may be
30 associated with formaldehyde exposure.

31 There has been speculation that the association between formaldehyde exposure and
32 increases in all LHP cancers and all leukemia are driven by increased myeloid leukemia (Pyatt et
33 al., 2008; Heck and Casanova, 2004; Golden et al., 2006). If this were the case, then mortality
34 from LHP cancers other than myeloid should not be elevated, once the excess mortality from
35 myeloid leukemia is accounted for. Only 2 studies provide the data to evaluate this hypothesis—

1 both conducted by the NCI (Hayes et al., 1990; Beane-Freeman et al., 2009). From the
2 published data, crude mortality statistics can be calculated for alternative disease groupings (see
3 Table 4-91). In the NCI embalmer study (Hayes et al., 1990), only myeloid leukemia was
4 statistically elevated in the subtype analysis. For the NCI industrial cohort (Beane-Freeman et
5 al., 2009), elevations were also seen for Hodgkin’s lymphoma relative to the referent group. In
6 both cases, the association between formaldehyde exposure and LHP malignancies remains when
7 myeloid leukemia is dropped from the analysis. Further, similar associations are found when all
8 leukemia and myeloproliferative diseases are dropped from the analysis and only solid tumors of
9 lymphoid origin are included (lymphosarcoma and reticulosarcoma, Hodgkin’s lymphoma, non-
10 Hodgkin’s lymphoma and multiple myeloma). These reanalyses illustrate the need for a more
11 careful subtype analysis to assess the potential for associations between formaldehyde exposure
12 and various forms of LHP cancers.

13

14 **4.5.2.5. Myeloid Leukemia**

15 The associations between myeloid leukemia and formaldehyde exposure are positive and
16 consistent (see Table 4-92, Section 4.1.2.2.4 for summary and evaluation of studies). Of the six
17 studies which formally assess myeloid leukemia mortality by SMR analysis, five are positive,
18 including cohorts of both professional and industrial workers although only two reach statistical
19 significance (Pinkerton et al., 2003; Stroup et al., 1986; Hayes et al., 1990; Walrath and
20 Fraumeni, 1984; Walrath and Fraumeni, 1983). Myeloid leukemia mortality was not increased
21 in the NCI industrial worker cohort by SMR analysis, but, there is evidence in the internal
22 analyses that myeloid leukemia is elevated by peak exposure (1.78 (0.97–3.64), and when
23 exposed are compared to unexposed within the cohort (1.38 (0.65–2.97)). The strongest
24 association is seen in the recent update of US embalmers (11.2 (1.3–95.6 for ever exposed).
25 Hauptmann et al. (2009) provide several alternative exposure metrics which all show positive
26 associations (number of embalmings, average formaldehyde intensity, cumulative formaldehyde
27 exposure) (see Section 4.1.2.2.1 for study details).

28 Further subtype analysis to distinguish between acute and chronic myeloid leukemia is
29 problematic. Although Walrath and Fraumeni (1983, 1984) note that AML is prominent in their
30 analyses of New York and California licensed embalmers; they do not provide PMR (actually
31 SMR) analyses for CML. Walrath and Fraumeni (1983 and 1984) report leukemia cell types—
32 for both studies the majority of myeloid leukemia are acute (5/6 and 4/6, respectively, for New
33 York State and California embalmers). However, SMRs cannot be calculated for AML versus
34 CML in this paper, as comparison rates are not available from the 1920's through the 1960's—
35 the timeframe with the majority of deaths. The authors do contrast the observed rate of AML in
36 the cohort to the background rate for AML in white men in the 1970s—but given the potential

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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 et 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

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^aRelative risk estimate (SMR, or OR) presented with 95% confidence intervals, where available.

^bLeukemia SMR 1.5 (0.7-2.7) {5 of 10 deaths due to myeloid}; Chronic Myeloid Leukemia (CML) SMR of 8.8.

9 misclassification of late stage CML as AML, especially historically, this may not be an
10 appropriate comparison. Therefore, although these studies support an association between
11 formaldehyde exposure and myeloid leukemia in general (Walrath and Fraumeni, 1983; 1984),
12 the reported AML and CML subtype information does not allow a satisfactory subtype analysis
13 for myeloid leukemia.

14 Several studies do present a formal subtype analysis within myeloid leukemia. Similar
15 SMRs are reported for AML 1.34 (0.61–2.54) and CML (1.39(0.38–3.56)) in garment workers
16 (Pinkerton et al., 2003). The initial NCI study of US embalmers indicates a slightly stronger
17 association of formaldehyde exposure to CML (1.84 (0.79–3.62)) compared to AML (1.52

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1 (0.85–2.52) (Hayes et al., 1990.) Stroup et al. (1986) report that all of the myeloid
2 leukemia observed in anatomists was CML with an SMR of 8.8. Finally, a population-based
3 case-control study of white men in Iowa and Minnesota provides positive SMRs for CML based
4 on estimated formaldehyde exposure (Low: 1.3 (0.6–3.1); High: 2.9 (0.3–24.5)), but no
5 association for AML (Low exposed: 0.9 (0.5–1.6)) (Blair et al., 1993). Therefore, although few
6 cases exist for further subtype analysis, the available data indicate either no differences in SMRs
7 for acute myeloid leukemia (AML) versus chronic myeloid leukemia (CML) (Hayes et al., 1990;
8 Pinkerton et al., 2003) or suggest CML is more prominent (Blair et al., 2000; Stroup et al., 1986).

9 The meta-analysis by Zhang et al. (2009) evaluated the studies of formaldehyde exposure
10 and myeloid leukemia available at the time including Hauptmann et al. (2003), Pinkerton et al.,
11 2003; Hayes et al., 1990; Stroup et al., 1986; Walrath and Fraumeni, 1984, 1983. While the
12 findings of Hauptmann et al. (2003) on the NCI cohort have been recently updated by those of
13 Beane-Freeman et al. (2009) who updated the cohort, the Zhang et al. (2009) analysis provides the
14 only formal meta-analysis specific to myeloid leukemia. Zhang et al. (2009) reported a
15 statistically significant summary RR of 1.90 (95% CI: 1.31–2.76).

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

28 Given the consistency of the positive associations for formaldehyde with myeloid
29 leukemia cancer mortality across five of the six studies (Hauptmann et al., 2009; Pinkerton et al.,
30 2003; Hayes et al., 1990; Stroup et al., 1986; Walrath and Fraumeni, 1984, 1983; but not
31 Beane-Freeman et al., 2009), the statistically significant meta-analysis by Zhang et al. (2009) and
32 the convincing results from Hauptmann et al. (2009), the human epidemiologic evidence is
33 sufficient to conclude that there is a causal association between formaldehyde exposure and
34 mortality from myeloid leukemia (also see Section 4.1.2.2.4 for further evaluation).

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1 **4.5.2.6. Solid Tumors of Lymphoid Origin**

2 Multiple myeloma, Hodgkin’s lymphoma, non-Hodgkin’s lymphoma, lymphosarcoma,
3 reticulosarcoma, and other lymphomas may all be derived from immune cells outside of the bone
4 marrow compartment, in peripheral blood, in the gut and respiratory mucosa and immune tissues
5 at the POE (e.g., lymph nodes, mucosa-associated lymphoid tissue (MALT), gut-associated
6 lymphoid tissue (GALT) (Greaves, 2004). The only meta-analysis to specifically address
7 lymphoid malignancies found evidence for increased lymphoma (Hodgkin’s lymphoma (pooled
8 RR = 1.23; 95% CI: 0.67-2.29) and multiple myeloma (1.31; 1.02-1.67), but not for non-
9 Hodgkin’s lymphoma (1.08; 0.86-1.35) (Zhang et al., 2009). As seen in Table 4-93 below,
10 individual study results are mixed for these lymphoid cell-line malignancies, as they are for all
11 LHP cancers and all leukemia above. Although these tumors are from mature lymphocytes,
12 there is still variability in the etiology, natural history and risk factors for the many subtypes
13 which are included in these categories.

14
15 **4.5.2.6.1. Hodgkin’s lymphoma.**

16 The only meta-analysis to specifically address Hodgkin’s lymphoma was conducted by
17 Zhang et al. (2009) and included eight studies (Anjelkovich et al., 1995; Coggon et al., 2003;
18 Harrington and Shannon, 1975; Hauptmann et al., 2003; Hayes et al., 1990; Pinkerton et al.,
19 2004; Walrath and Fraumeni, 1983, 1984; and Wong, 1983). Zhang et al. (2009) reported a
20 summary RR = 1.23 (95% CI: 0.67-2.29). This elevated, but nonstatistically significant finding
21 is consistent with the large variance on reported results among the individual studies as well as
22 the wide confidence intervals of the results which were based on small numbers of cases—even
23 from the large cohort studies. Six of the eight studies observed three or fewer deaths from
24 Hodgkin’s lymphoma. Coggon et al. (2003) reported 6 deaths from Hodgkin’s lymphoma
25 against 8.5 expected for an SMT = 0.70 (95% CI: 0.26-1.53) and Hauptmann et al. (2003)
26 reported 21 observed deaths with 20 deaths among the exposed workers who has an SMR = 1.26
27 (95% CI: 0.81-1.95). However, the Beane-Freeman et al. (2009) update of the Hauptmann et al.
28 (2003) study had the largest number of observed cases ($n = 27$) and was not included in the
29 Zhang et al. (2009) meta-analysis. In fact, the Beane-Freeman et al. (2009) study describes more
30 deaths from Hodgkin’s lymphoma than all the other studies in Zhang et al. (2009) combined.
31 Excluding the Hauptmann et al. (2003) results from the list of studies in the meta-analysis leaves
32 19 cases.

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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-Freeman 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

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^aRelative risk estimate (SMR or OR) presented with 95% confidence intervals, where available.

^bNR is not reported.

^c“—” no cases observed.

^dLymphosarcoma and reticulosarcoma only.

^e“other lymphoma.”

^fFormaldehyde exposure in a wood-related occupation. RR for wood-related occupation alone was not elevated 0.97 (0.55–1.73).

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1 There is evidence for an exposure-response relationship for Hodgkin’s lymphoma in the
2 NCI industrial cohort among exposed workers (Beane-Freeman et al., 2009). Clear exposure
3 response relationships for Hodgkin’s lymphoma are defined with all three metrics of exposure,
4 peak average intensity and cumulative exposure ($p = 0.01$, $p = 0.05$ and $p = 0.08$ respectively for
5 mortality through 2004). These associations have been evident from first follow-up through the
6 current publication, and statistically significant for the majority of the follow-up period
7 demonstrating that this is a strong and consistent finding in the NCI cohort (see Figures 4-3 and
8 4-4) (Beane-Freeman et al., 2009).

9 As the majority of the studies reported specific data on Hodgkin’s lymphoma report on
10 just three or fewer cases, the best epidemiologic evidence is obtained from the most recent
11 evaluation of the NCI cohort by Beane-Freeman et al. (2009). This cohort study, on its own,
12 reported on more deaths from Hodgkin’s lymphoma than the remainder of the epidemiologic
13 literature. Hodgkin’s lymphoma was both shown to be at increased risk associated with peak
14 exposure concentrations. Peak exposures in the highest exposure category were associated with
15 a significant increase in Hodgkin’s lymphoma deaths comparing death rates among workers with
16 peaks of ≥ 4 ppm to those with >0 to 2.0 ppm (RR = 3.96, 95% CI: 1.31-12.02). Across the three
17 categories of peak exposure, there was a statistically significant exposure-response trend
18 ($p = 0.01$). The exposure-response trend including the never-exposed workers was also
19 statistically significant ($p = 0.004$). The RR was also elevated for average intensity of
20 formaldehyde exposure with RR = 2.48 (95% CI: 0.84-7.32) and there were significant tests for
21 trend among only the exposed workers ($p = 0.05$) and all workers ($p = 0.03$). Similarly, there
22 were nearly significant tests for trend with cumulative exposure among only the exposed workers
23 ($p = 0.08$) and all workers ($p = 0.06$).

24 The majority of the studies reporting on Hodgkin’s lymphoma did not have sufficient
25 statistical power describe any potential association with formaldehyde as the numbers of
26 observed and expected cases were small and the resulting effects estimates were imprecise. As the
27 Beane-Freeman et al. (2009) study reported on the largest number of cases and was the
28 individual study with the most detailed and objectively ascertained exposure assessment and
29 demonstrated significant exposure-response gradients, it is judged that this epidemiologic
30 evidence is supportive of an association between formaldehyde and Hodgkin’s lymphoma (also
31 see Section 4.1.2.2.4 for further evaluation).

32 33 **4.5.2.6.2. Non-Hodgkin’s lymphoma.**

34 The only meta-analysis to specifically address non-Hodgkin’s lymphoma was conducted
35 by Zhang et al. (2009) and included eleven studies. Zhang et al. (2009) reported a summary

1 RR = 1.08 (95% CI: 0.86-1.35). Hauptmann et al. (2009) did not specifically report on non-
2 Hodgkin's lymphoma. Beane-Freeman et al. (2009) did report on 106 deaths from non-
3 Hodgkin's lymphoma but did not identify any significant association in their categorical analyses
4 or in their tests for trend for either peak exposure, average intensity of exposure or for
5 cumulative exposure.

6 Wang et al. (2009) assessed the effect of formaldehyde exposure on the risk of non-
7 Hodgkin's lymphoma in a population-based case-control study. Semiquantitative exposure
8 metrics included average exposure intensity and average exposure probability which were
9 evaluated individually and together. Analyses use unconditional logistic regression and
10 controlled for age, family history of hematopoietic cancers, alcohol consumption, and race. For
11 the low average exposure intensity category the OR = 1.4 (95% CI: 1.0-1.8), while for the
12 Medium-High category the OR = 1.2 (95% CI: 0.8-1.7). For the Low average exposure
13 probability category the OR = 1.3 (95% CI: 1.0-1.7), while for the Medium-High category the
14 OR = 1.4 (95% CI: 0.9-2.3). The investigators also examined the risk of non-Hodgkin's
15 lymphoma among major subtypes. The risk of follicular lymphoma and chronic lymphocytic
16 leukemia/Small lymphocytic lymphoma was slightly elevated but the risk of diffuse large B-cell
17 lymphoma was OR = 1.9 (95% CI: 1.3-2.6) for ever having been exposed to formaldehyde. For
18 Low average intensity exposure, the risk was OR = 2.1 (95% CI: 1.3-2.6) while for Medium-
19 High average intensity exposure, the risk was OR = 1.5 (95% CI: 0.9-2.4). Even so, an
20 exposure-response relationship was demonstrated using the continuous parameterization of
21 average intensity rather than the categorical ($p = 0.03$). Likewise, an exposure-response
22 relationship was demonstrated using the continuous parameterization of average probability of
23 exposure rather than the categorical ($p = 0.01$).

24 The findings of Wang et al. (2009) provide some support for an association between
25 formaldehyde exposure and non-Hodgkin's lymphoma. It should be noted that a population-
26 based case-control study, where incidence rather than mortality defines the case—may be more
27 appropriate for cancers with relatively low mortality (i.e., CCL, large B-cell lymphoma, Small
28 lymphocytic lymphoma).

29 Aside from the semiquantitative study by Wang et al. (2009), non-Hodgkin's lymphoma
30 does not appear to be associated with formaldehyde exposure. There is not sufficient evidence of
31 a causal association between formaldehyde exposure and non-Hodgkin's lymphoma (also see
32 Section 4.1.2.2.4 for further evaluation).

33

1 **4.5.2.6.3. Multiple myeloma.**

2 The only meta-analysis to specifically address Hodgkin's lymphoma was conducted by
3 Zhang et al. (2009) and included nine studies (Boffetta et al., 1989; Coggon et al., 2003; Dell and
4 Teta, 1995; Edling et al., 1987; Hauptmann et al., 2003; Hayes et al., 1990; Heineman et al.,
5 1982; Pottern et al., 1992; Stellman et al., 1998). Zhang et al. (2009) reported a summary
6 RR = 1.31 (95% CI: 1.02-1.67). This statistically significant finding is consistent with the
7 findings of Beane-Freeman et al. (2009) who reported that peak exposures in the highest
8 exposure category were associated with a significant increase in multiple myeloma deaths
9 comparing death rates among workers with peaks of ≥ 4 ppm to those with >0 to 2.0 ppm
10 (RR = 2.04, 95% CI: 1.01-4.12). Across the three categories of peak exposure, there was also
11 some evidence of an exposure-response trend ($p = 0.08$); however, there was no evidence of an
12 exposure-response trend including the never-exposed workers. The association of multiple
13 myeloma with formaldehyde exposure was also shown throughout the cohort experience (see
14 Figure 4-3 and 4-4) which adds strength to this finding.

15 The epidemiologic evidence for a causal association between formaldehyde and all
16 multiple myeloma as described by the statistically significant increased risk identified in the
17 meta-analysis of Zhang et al. (2009) and the most recently updated analysis of the NCI cohort by
18 Beane-Freeman et al. (2009) are considered to be supportive of an association between
19 formaldehyde exposure and mortality from multiple myeloma (also see Section 4.1.2.2.4 for
20 further evaluation).

21
22 **4.5.2.7. Supporting Evidence from Animal Bio-Assays for Formaldehyde-Induced**
23 **Lymphohematopoietic Malignancies**

24 Chronic animal studies provide limited supporting evidence for formaldehyde-induced
25 leukemia and lymphoma. Although the majority of chronic animal bioassays do not report either
26 leukemia or lymphoma, many studies focused primarily on the respiratory tract and did not
27 provide routine examination of other tissues, limiting the detection of leukemia and lymphoma
28 (Horten et al., 1963; Holmström et al., 1989; Wouterson et al., 1989; Appleman et al., 1988;
29 Monticello et al., 1996; Dalbey, 1982). Kamata et al. (1986) did examine additional tissues, but
30 there were only 5 animals at each sacrifice. Drinking water studies were similarly limited, where
31 Takahashi et al. (1986) only examined tissues from the stomach and intestines, and the study by
32 Tobe et al. (1989) only examined 20 Wistar rats per sex per exposure group including interim
33 sacrifices. Therefore, few studies have adequately evaluated the carcinogenic potential of
34 formaldehyde with respect to leukemia and lymphoma. [Complete study descriptions for all
35 experiments evaluated are found in Section 4.2.2.]

1 The two-year drinking water study by Til et al. (1989) in male and female Wistar rats, did
2 have adequate examination of tissues outside of the portal of entry, but found no increase in
3 leukemia or lymphoma, with only 4 tumor-bearing animals in all treatment groups sacrificed at
4 24 months ($n = 200$). Sellakumar et al. (1985) conducted a lifelong inhalation study in male
5 Sprague-Dawley rats exposed at 15 ppm formaldehyde. Some tissues outside of the respiratory
6 tract were routinely examined (liver, kidneys, and testes), including any organ exhibiting gross
7 pathology. However, spleen, thymus, and lymph nodes were not routinely examined, limiting
8 detection of leukemia and lymphoma, especially smaller lesions. Although Sellakumar et al.
9 (1985) was a lifelong study, there was a high mortality rate at 2 years (>80% from the figure),
10 again limiting the power of this study to detect late-in-life malignancies. Although, this study
11 did not indicate formaldehyde-induced lymphoma or leukemia, the limitations of the study
12 design should be acknowledged when interpreting these results.

13 The largest and most comprehensive cancer bioassay from formaldehyde inhalation
14 exposures is the study conducted at the Battelle Columbus Laboratory (1981). Although the
15 summary reports of this study do not discuss leukemia or lymphoma rates (Swenberg et al.,
16 1980; Kerns et al., 1983), mouse lymphoma and rat leukemia were selected by the study
17 pathologist and biostatistician for analysis and presented in the final laboratory report (Battelle
18 Columbus Laboratories, 1981).

19 Unadjusted leukemia incidence in male and female rats was similar between control and
20 formaldehyde exposed rats (9% and 6% in female rats; 9% and 4% in male rats respectively:
21 $p > 0.05$) (Battelle Columbus Laboratories, 1981). However, both male and female rats at the
22 highest exposure (15 ppm) exhibited significant early deaths due to nasal lesions (see
23 Figure 4-33). Additionally, the unadjusted leukemia rates included animals from all scheduled
24 sacrifices, with early time points representing 30% of the experimental animals (6, 12 and 18
25 months). Statistical analysis performed by Battelle (Tyrone extension of the Cox Test), which
26 accounted for time to lesion and survivorship rates, indicated a significant increase leukemia for
27 female rats but not male rats exposed at 15 ppm ($p = 0.0003$ and $p = 0.6891$ respectively)
28 (Battelle Columbus Laboratories, 1981). Since only gross pathology was performed on tissues
29 outside of the respiratory tract of mid-dose animals, trend analysis was not performed (Battelle
30 Columbus Laboratories, 1981). As the first leukemia in unexposed rats was noted at 21 months,
31 the early deaths prior to that time in formaldehyde-exposed rats, reduced the number of animals
32 in which the leukemia could have been observed. Leukemia incidence in Fischer 344 rats
33 surviving at least 21 months are shown in Table 4-94. Although the histopathology was not as
34 rigorous in the mid-dose animals, leukemia was reported in these animals at a similar or greater
35 incidence than in control animals.

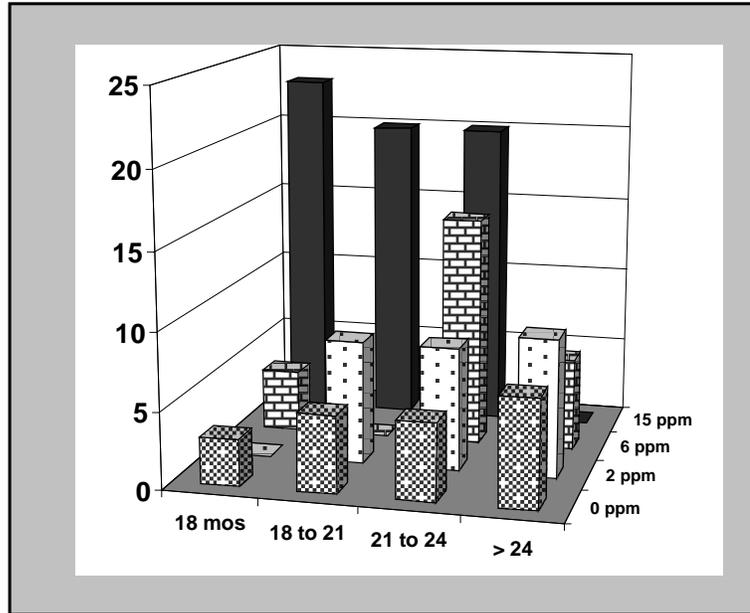


Figure 4-33. Unscheduled deaths in female F344 rats exposed to formaldehyde for 24 months.

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%)

^aLeukemia incidence is given as the number of tumor bearing animals for the total number of animals examined for each dose group.

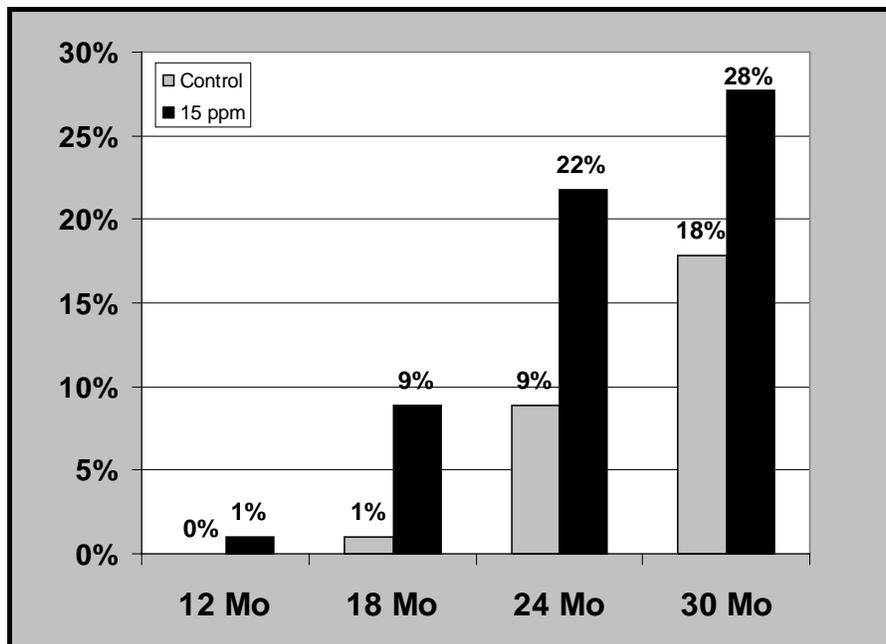
Source: Data extracted from the Final Report by Battelle Columbus Laboratories, 1981

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

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1 observed in male mice due to infighting and are not further considered here (Battelle Columbus
2 Laboratories, 1981). Statistical analysis performed by Battelle, which accounted for time to
3 lesion and survivorship rates, suggest an increase in lymphoma in female mice ($p = 0.06$, Tyrone
4 extension of the Cox Test) (Battelle Columbus Laboratories, 1981). However, this analysis
5 included animals from the 6 month sacrifice where tissues outside of the respiratory tract were
6 not examined. When these animals are removed from the analysis, the cumulative incidence of
7 lymphoma in formaldehyde-exposed female B6C3F1 mice is (28%) versus controls (22%)
8 ($p < 0.05$) (see Figure 4-34). Results for the mid-dose groups are not shown here as tissues
9 outside of the respiratory tract were not routinely examined (e.g., spleen, liver, thymus, and
10 lymph nodes).

11



12

13

14 **Figure 4-34. Cumulative incidence of tumor bearing animals for lymphoma**
15 **in female B6C3F1 mice exposed to formaldehyde for 24 months ($p < 0.05$).**

16

17 Note: Mice from the 6-month interim sacrifice are not included since only nasal
18 passages were examined.

19

20 Source: Data extracted from the Final Report by Battelle Columbus Laboratories
21 (1981).

22

23

1 **Summary**

2 There have been numerous chronic animal bioassays assessing lesions from inhalation
3 exposure to formaldehyde: however, few have adequate tissue pathology or study design (study
4 size and duration) to assess leukemia and lymphoma incidence. Of the available studies, the
5 study conducted at Battelle Columbus Laboratories (1981) provides the only evidence of
6 formaldehyde-induced leukemia or lymphoma. Although there were significant early deaths in
7 some of the exposure groups, formaldehyde exposure slightly increased leukemia incidence in
8 female but not male rats (Battelle Columbus Laboratories, 1981). Lymphoma incidence was also
9 elevated in female B6C3F1 mice exposed at 15 ppm versus control mice (see Figure 4-6).

10 Although one chronic inhalation study which examined tissues outside of the respiratory
11 tract did not find increased leukemia in Wistar rats (Sellakumar et al., 1985), this study did not
12 have the comprehensive histopathology, study size, or duration as the study conducted at Battelle
13 Columbus Laboratories (1981). Thus, the negative results in the Sellakumar et al. (1985) study
14 do not contradict the positive findings in Fisher 344 Fisher female rats or B6C3F1 mice.
15 Therefore, the available evidence for increased Fisher 344 female rat leukemia and mouse
16 lymphoma from the Battelle study do provide limited support for the plausibility of
17 formaldehyde-induced leukemia and lymphoma.

18
19 4.5.3. Carcinogenic Mode(s) of Action

20 The EPA 2005 *Guidelines for Carcinogen risk Assessment* recommend a mode of action
21 (MOA) analysis when data are available for evaluation. The purpose of this MOA analysis is to
22 determine if sufficient data exist to adequately inform the exposure-response relationship for
23 cancer below the range of observed data in either human or animal studies. Since the majority of
24 the data supporting the carcinogenicity of formaldehyde comes from animal bio-assays and
25 epidemiological studies of workers, EPA must extrapolate from the observed risk of cancer
26 mortality/incidence in those studies to levels considered protective of human health for lifelong
27 environmental exposures. In this context, the EPA *Cancer Guidelines* provide a framework to
28 review MOA information for relevant data to establish an MOA informing appropriate low-dose
29 extrapolation.

30 The supporting data for the MOA evaluation of formaldehyde are complex, and presented
31 across multiple sections of a large document; therefore, this section includes a brief summary of
32 the biological actions of formaldehyde and key mechanistic data which are believed to be
33 relevant to the MOA evaluation (see Section 4.5.3.1). This information is not intended as a
34 stand-alone description of the evidence for a particular mechanism, but is intended to highlight
35 the major supporting arguments and direct the reader to text providing more detailed discussion.

1 The summary of data discussed below combines what is known about the human cancer
2 of concern (nasopharyngeal cancer, sinonasal cancer, leukemia and other lymphohematopoietic
3 cancers) with the potential formaldehyde-specific mechanisms of action to postulate
4 carcinogenic modes of action for each cancer or group of cancers (see Sections 4.5.3.2 and
5 4.5.3.2). The resulting evaluation provides multiple possible MOAs for formaldehyde-induced
6 cancers where some key mechanistic events may be commonly at work in different tissues, and
7 some key events may be more relevant to a specific tissue/cancer type. Each of these MOAs is
8 evaluated with respect to its relevance to human cancer, and the overall weight of evidence for
9 its relevance to formaldehyde-related human cancer.

10 Overall, multiple MOAs considered relevant to humans are presented for each cancer
11 type. Although some MOAs may have a greater level of supporting evidence, this reflects in part
12 how well a particular mechanism or key event may have been studied. For example, there are a
13 large number of studies across many testing systems, and levels of biological organization to
14 support the mutagenicity of formaldehyde. In contrast other likely MOAs, such as viral
15 reactivation, have little direct mechanistic evidence, but the available evidence is supportive.

16 The MOAs considered most relevant to upper respiratory tract cancers (e.g.
17 nasopharyngeal cancer and sinonasal cancer) are: (1) direct mutagenicity; (2) inhibition of DNA
18 repair mechanisms; (3) formaldehyde-induced cell proliferation; (4) cytotoxicity-induced cell
19 proliferation; (5) tumor promotion activity; and (6) localized immunosuppression/viral
20 reactivation (see Section 4.5.3.2). The majority of these MOAs would apply equally to immune
21 cells present at the site of first contact and may also contribute to those lymphohematopoietic
22 cancers which arise from peripheral immune cells (e.g., Hodgkin's lymphoma, multiple
23 myeloma and some forms of leukemia). Additional MOAs are considered, specifically for
24 formaldehyde-induced leukemia are: (1) damage of a circulating hematopoietic stem cell or
25 progenitor cell at the site of first contact; and (2) bone marrow toxicity (see Section 4.5.3.3).

26 In summary—no single MOA is singled out as the best explanation for cancer resulting
27 from formaldehyde exposure. Only one MOA—cytotoxicity induced cell proliferation—
28 suggests an exposure threshold below which the MOA would not be active. However this MOA
29 is the least applicable to humans and other MOAs are considered operative at exposures below
30 exposures associated with cytotoxicity-induced cell proliferation. Therefore, multiple MOAs are
31 considered supported by formaldehyde-specific mechanistic information which provide
32 biological plausibility for the cancers observed in formaldehyde exposed populations.

33

1 **4.5.3.1. Mechanistic Data for Formaldehyde**

2 **4.5.3.1.1. DNA reactivity/genotoxicity/mutagenicity.**

3 An agent's genotoxic potential and ability to induce mutations is a key consideration in
4 assessing a carcinogenic MOA, as cancer results from a series of genetic and epigenetic
5 alterations affecting genes that control cell growth, division, and differentiation (Hanahan and
6 Weinberg, 2000; Vogelstein et al., 1988; Kinzler and Vogelstein, 2002). The EPA *Cancer*
7 *Guidelines* suggest several lines of evidence which are key to evaluating a mutagenic MOA:
8 (1) Is the chemical under study DNA-reactive and/or has the ability to bind to DNA; (2) Does the
9 chemical generate positive results in in vitro mutagenic test systems (specifically gene mutations
10 and chromosomal aberrations); (3) Does the chemical induce manifestations of genetic damage
11 in in vivo tests (specifically gene mutations and chromosomal aberrations), and (4) Does the
12 chemical have properties and structure-activity relationships (SAR) similar to known mutagens
13 (U.S. EPA, 2005). As reviewed in Section 4.3 above, there is adequate evidence for
14 formaldehyde-induced genotoxicity and mutagenicity for consideration of these key events in
15 formaldehyde's carcinogenic MOA.

16 Formaldehyde, as a reactive chemical, forms DPX or DPC, DNA adducts and DDX or
17 DDC and may act to form adducts between other chemicals and DNA (Brutlag et al., 1969;
18 Donecke, 1978; Ohba et al., 1979; Fennel, 1999; Casanova-Schmitz and Heck, 1983, 1984; Heck
19 and Casanova, 1987; Casanova et al., 1989). The high reactivity of formaldehyde results in little
20 specificity indicating that a range of adducts and crosslinks might be expected. Formaldehyde
21 induces a variety of genotoxic and mutagenic events when tested both in vitro and in vivo
22 systems including DNA-protein crosslinks (DPC or DPX), point mutations, DNA single strand
23 breaks (SSB) and chromosomal aberrations (CAs) (see Section 4.3).

24 Numerous studies have shown that formaldehyde induces genotoxic and mutagenic
25 effects under a variety of experimental conditions (see Section 4.3 for a detailed discussion, also
26 reviewed by IARC 2006; Ma and Harris 1988; Auerbach et al., 1977). As discussed,
27 formaldehyde is known to directly react with DNA forming DPC and DNA adducts. A dose-
28 response in DPX formation has been described at exposure levels of 2-30 ppm formaldehyde in
29 rodent tissues (Casanova-Schmitz and Heck, 1983). Dose-dependent increases were also
30 observed for SSBs in peripheral blood lymphocytes and livers of rats (Im et al., 2006), and lung
31 epithelial cells of rats exposed to formaldehyde by inhalation (Sul et al., 2007), and for MN and
32 comet assay parameters in workers occupationally exposed to formaldehyde (Yu et al., 2005)
33 have also been reported. All these studies suggest that formaldehyde can induce dose-dependent
34 increase in the genotoxicity in both animals and humans. The DPX induced by formaldehyde are
35 bulky adducts which may induce distortion of the DNA helix and are likely to induce mutations.

1 Mutations may occur during repair of formaldehyde-induced DNA damage, or as a result of
2 replication errors during mitogenesis. Additionally, there is some evidence that DNA single
3 strand breaks (SSB) may be induced directly by formaldehyde reactivity (Grafstrom et al., 1984).
4 Clastogenic effects including increased micronuclei (MN), chromosomal aberrations (CAs) and
5 sister chromatid exchanges (SCEs) are also reported in a range of in vitro study systems.

6 Formaldehyde caused a concentration-dependent increase in mutations at the *tk* locus of
7 human lymphoblastoid cells (Craft et al., 1987) and clastogenicity (e.g., MN) in human cell lines
8 deficient in either DNA nucleotide excision repair (NER) or DDC repair systems even though
9 there is no change seen in DPC induction or removal between these cell lines (Speit et al., 2000).
10 These data suggest that alteration of DNA repair, not DPC removal, contributes to
11 formaldehyde-induced clastogenicity. Since DPC repair involves proteolytic removal of proteins
12 from the DNA, it has been hypothesized that single peptides or small peptide chains cross-linked
13 to the DNA as in the case of DPC are critical to formaldehyde-induced mutations.

14 Formaldehyde-induced MN and CAs are associated to concentration-dependent
15 mutagenic effects in L5178Y mouse lymphoma cells (Speit and Merk, 2002). Detailed analysis
16 of both spontaneous and formaldehyde-induced lesions indicate that recombination or deletion of
17 DNA from the thymidine kinase (*tk*) locus was primarily responsible for the loss of heterogeneity
18 leading to the observed mutant phenotype. Therefore, it is believed that formaldehyde is
19 mutagenic by a clastogenic mechanism, rather than through point mutations in the L5178Y
20 mouse lymphoma cell system. This finding is consistent with Craft et al. (1987) who
21 demonstrated formaldehyde-induced mutagenicity in the *tk* locus of TK6 human lymphoblastoid
22 cells, while Grafström et al. (1984) demonstrated increased SSBs in formaldehyde-exposed
23 human cell lines. The elegant series of experiments by Speit and Merk provide the possible links
24 between DPC, clastogenicity and locus-specific mutations firmly demonstrating formaldehyde-
25 induced mutations in the in vitro mouse lymphoma testing system.

26 Formaldehyde is genotoxic at the portal of entry (POE) in animal studies, resulting in
27 increased DPC formation in the nasal mucosa as discussed above. However, there are no animal
28 studies which directly examine the mutagenicity in nasal or respiratory epithelial cells in the
29 early stages of exposure. It is likely that the mutations are seen in advanced stage of tissue
30 transformation with formaldehyde exposure. With weak positive results in pulmonary lavage
31 cells (Dallas et al., 1992) and clastogenicity demonstrated in gastro-intestinal epithelial cells of
32 rats (Migliore et al., 1989), below exposure levels which trigger regenerative cell proliferation,
33 the existing evidence, although thin, supports clastogenic effects of formaldehyde.

34 Clastogenic effects are consistently reported in humans exposed to formaldehyde in the
35 industrial workplace or during anatomy or mortuary classes (see Section 4.3 for a full

1 discussion). Increased micronuclei have been reported in nasal epithelial cells from industry
2 workers (Ballarin et al., 1992; Ye et al., 2005), buccal epithelial cells from anatomy and
3 mortuary science students and/or staff (Kitaeva et al., 1996; Titenko-Holland et al., 1996; Burgaz
4 et al., 2001, 2002) compared to corresponding controls. Comparisons of micronuclei in nasal
5 and buccal cells of anatomy students before and after classes where they are exposed to
6 formaldehyde indicate an exposure-related increase in clastogenicity (Ying et al., 1997). An
7 examination of exfoliated buccal and nasal cells in mortuary students indicates greater increases
8 in centromere-negative micronuclei, suggesting the effects are due to chromosome breakage or
9 clastogenicity rather than aneuploidy (Titenko-Holland et al., 1996). Micronuclei were also
10 increased in a dose-dependent manner in buccal cells as well as peripheral blood lymphocytes
11 (PBLs) in mortuary students during the course of an embalming class; however, SCEs were
12 reduced in postexposure samples (Suruda et al., 1993). Buccal, oral, and nasal epithelial cells
13 present at the portal of entry may be directly exposed to formaldehyde and thus reports of
14 clastogenic effects are consistent with direct interaction of formaldehyde at the POE.
15 Mutagenicity in the form of *p53* mutations has been observed in the SCCs of chronically-
16 exposed rats, however, it is uncertain about the stage at which the mutations are induced (Recio
17 et al., 1992). There is some supporting evidence for the mutagenicity of formaldehyde in human
18 populations. Shaham et al. (2003) reported an increase in mutant *p53* protein in the peripheral
19 blood lymphocytes of individuals with mean formaldehyde exposure duration of 16 years.
20 Additionally there was is a significant association between mutant *p53* protein and DPC in this
21 study suggesting a relationship between the formaldehyde's genotoxic effects. More recently,
22 Zhang et al. (2010a) have reported aneuploidy in circulating hematopoietic stem cells in
23 formaldehyde exposed workers with increases in both monosomy 7 and trisomy 8.

24 In summary, there are several lines of evidence supporting mutagenic effects of
25 formaldehyde exposure:

- 26
- 27 1) Formaldehyde directly interacts with DNA generating DPC, DNA adducts, and DDC
- 28 2) DPC in tissues at the POE exhibit a dose-response relationship to formaldehyde
- 29 exposure,
- 30 3) Formaldehyde-induced DPC are associated with formaldehyde-induced MN and CAs,
- 31 4) Mutations induced by formaldehyde due to small deletions and rearrangements in DNA
- 32 in various experimental systems are consistent with formaldehyde's observed clastogenic
- 33 effects (MN and CAs),
- 34 5) Formaldehyde-induced mutations and clastogenic effects occur at levels below where
- 35 significant cytotoxicity is detected, and

- 1 6) Formaldehyde exposure has been correlated to similar increased MN and CAs in human
2 buccal and oral cells corresponding to sites where formaldehyde-induced tumors arise.

3
4 **4.5.3.1.2. *Inhibition of DNA repair.***

5 Studies indicate that formaldehyde exposure may inhibit DNA repair mechanisms
6 directly (see Section 4.3.1.5). Grafström (1985) first documented formaldehyde effects on DNA
7 repair mechanisms, reporting that formaldehyde treatment of human bronchial fibroblasts in vitro
8 inhibited repair of O⁶-methyl-guanine adducts induced by *N*-methyl-Nitrosurea (NMU).
9 Inhibition of DNA repair in human keratinocytes and fibroblasts cultured at 10 µM
10 formaldehyde affected repair of DNA single strand breaks from ultraviolet light but was specific
11 to UVB and UVC, not impacting repair of single strand breaks from UVA (Emri et al., 2004).

12 To determine if formaldehyde may have similar effects in exposed humans, Hayes et al.,
13 (1997) assessed the activity O⁶-alkylguanine-DNA alkyltransferase (AGT) an enzyme critical in
14 repairing DNA damage induced by alkylating agents in formaldehyde-exposed mortuary students
15 previously shown to have increased micronuclei in both buccal cells and peripheral lymphocytes
16 (Suruda et al., 1993). AGT activity was lower in mortuary students with prior embalming
17 exposures versus students with no prior exposure ($p = 0.08$). Seventeen of 23 students had lower
18 AGT activity after the 9 week course ($p < 0.05$) with a larger proportion of naïve students
19 demonstrating decreased activity (7 of 8) versus previously exposed students (10 of 15).
20 Although detailed exposure measurements were taken for each student, the changes in AGT
21 activity were not correlated to cumulative exposure (ppm-hrs).

22
23 **4.5.3.1.3. *Protein to protein cross-links.***

24 Formaldehyde is a reactive molecule that is likely to interact with both low molecular
25 weight cellular components (e.g., reduced glutathione [GSH]) as well as high molecular weight
26 cellular components. Unlike nuclear DNA, which has additional membrane barriers to exposure
27 (i.e., nucleus), extracellular and intracellular proteins, are obvious primary targets for interacting
28 with formaldehyde. Formaldehyde is a well-known cross-linking agent that is used in the
29 fixation of tissues, inactivation of toxins and viruses (e.g., preparation of vaccines), and study of
30 protein-protein interactions (Metz et al., 2006). Using several identical synthetic polypeptides
31 differing on one amino acid, Metz et al. (2004) have shown that formaldehyde initially reacts
32 with the primary amino and thiol groups of amino acids forming unstable methylol adducts,
33 which later are partially dehydrated forming labile Schiff bases that are capable of forming
34 crosslinks with other amino acid residues, such as arginine, asparagine, glutamine, histidine,
35 tryptophan, and tyrosine through methylene bridges, but not between two primary amino groups.
36 The same group (Metz et al., 2006) has also shown that formaldehyde forms seven

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1 intramolecular crosslinks in proteins with defined structure, such as insulin, involving arginine,
2 tyrosine and lysine and the *N*-terminus of insulin was converted to a imidazolidinone adducts
3 similar to that observed with the synthetic peptide (Metz et al., 2004). (Figure 3-1 provides a
4 general reaction scheme for formaldehyde-mediated modifications of amino acids.)
5

6 **4.5.3.1.4. Break-down of the mucociliary apparatus.**

7 The mucociliary apparatus of the upper respiratory tract is the first line of defense against
8 airborne toxicants. Comprised of a thick mucus layer (epiphase), hydrophase and ciliated
9 epithelium, the mucociliary apparatus may entrain, neutralize and remove particulates and
10 airborne chemicals from inspired air (see Figure 4-7). Formaldehyde reacts with the components
11 of the mucous layer (proteins, glycoprotein, and lipids), crosslinking proteins. Formaldehyde
12 exposure induces slowing of the mucous flow, stiffing and breaking up of the mucous layer and
13 eventual mucostasis where gaps have been observed exposing the underlying hydrophase and
14 epithelium. Although ciliary beat first increases in response to formaldehyde exposure, perhaps
15 to compensate for reduced flow of the epiphase, ciliastasis ensues with both higher levels of
16 exposure and increased duration of exposure. Altered ciliary beat has been noted in as little as
17 15 minutes of exposure (1.25 ppm) with functional deficits in the mucociliary apparatus at 30
18 minutes. Altered ciliary beat has been reported at the lowest concentration tested (0.5ppm) for a
19 single 6 hour exposure. Severity of effects increase with both duration and level of exposure
20 (see Section 4.2.1.2.1).
21

22 **4.5.3.1.5. Induced cell proliferation.**

23 There are several reports apparently demonstrating formaldehyde-induced proliferation in
24 cells below cytotoxic levels of exposure. This phenomenon has been reported from studies
25 involving both in vitro and in vivo exposures. Tyihak et al. (2001) demonstrated significantly
26 increased cell proliferation in both HT-29 human colon carcinoma and human umbilical vein
27 endothelial cell (HUVEC) lines treated with 0.1mM (the lowest dose) formaldehyde compared to
28 untreated controls ($p < 0.0001$). This effect was quantified as both an increase in cell number
29 over time (see Figure 4-35), and an increase in the percentage of cells undergoing mitosis at each
30 time point. The authors also report a significant ($p < 0.01$) inhibition of apoptosis in
31 formaldehyde-treated cells as compared to untreated cells (data not shown here). In a novel
32 system using xenotransplanted human tracheobronchial epithelial cells, formaldehyde was shown
33 to induce increased cell proliferation at doses below those required for a “massive toxic effect”
34 (Ura et al., 1989).

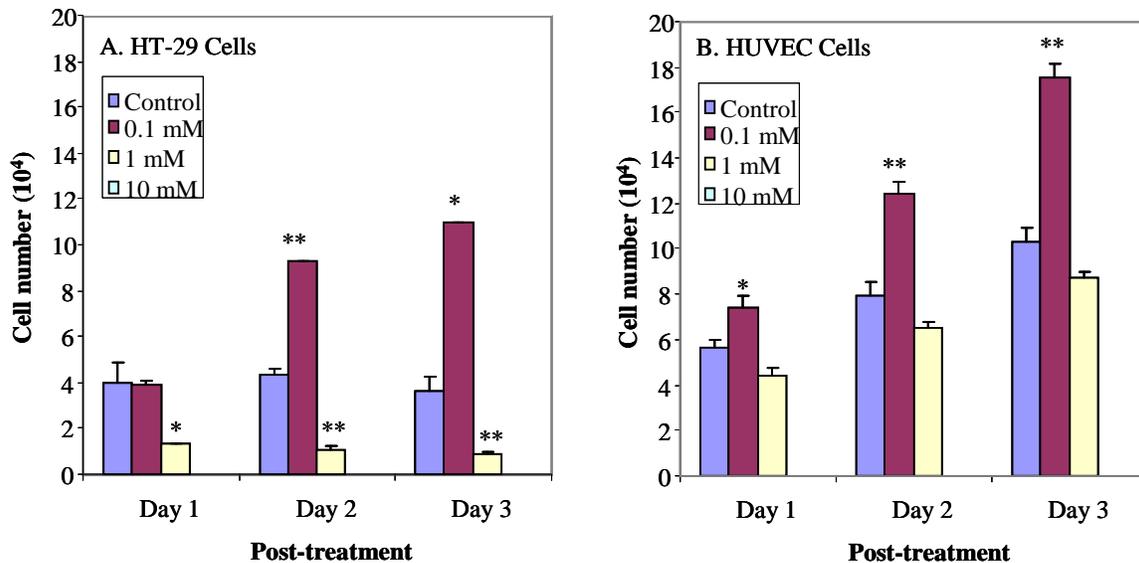


Figure 4-35. Effect of various doses of formaldehyde on cell number in (A) HT-29 human colon carcinoma cells and in (B) human umbilical vein epithelial cells (HUVEC).

Values are average of three samples \pm 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

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1 investigators using a similar exposure regimen, reported no increase in cell proliferation after
2 repeated 8-hour exposures at 1 or 2 ppm formaldehyde for 3 days or 4 weeks. Swenberg et al.
3 (1986) demonstrated a transient increase in cell proliferation after a single 8-hour exposure to 0.5
4 or 2 ppm formaldehyde in male F344 rats but no increases after 3 days or repeated 8-hour
5 exposures. The authors suggest that adaptive responses of the nasal mucosa contribute to the
6 transient nature of formaldehyde-induced cell proliferation. After a series of acute studies at
7 various formaldehyde concentrations, Swenberg and coworkers concluded that, in addition to
8 cell proliferation being concentration-, dose- and time-dependent, the response varies by species
9 and by location of exposure in the nose (Swenberg et al., 1983, Swenberg et al., 1986).

10 Other methods of quantifying cell proliferation in the nasal mucosa have demonstrated
11 formaldehyde-induced cell proliferation at similar low exposure concentrations. For example,
12 Roemer et al. (1992) measured cell proliferation by flow-cytometry in epithelial cells harvested
13 from the nose and trachea of male Sprague-Dawley rats exposed to 2 ppm formaldehyde for 6
14 hours/day for 1 or 3 days and found increased cell proliferation after the 1-day exposure. These
15 increases were transient and were not evident after 3 days of exposure. Cassee and Feron (1994)
16 identified proliferating cells by staining for the presence of proliferating cell nuclear antigen
17 (PCNA). Formaldehyde exposure at 3.6 ppm for 6 consecutive periods of 12 hours (8-hour
18 exposures followed by 4-hour-periods of nonexposure) over three days, qualitatively increased
19 the expression of PCNA in respiratory epithelium at levels 2 and 3 of the nose in albino male
20 Wistar rats (Cassee and Feron, 1994). Hyperplasia, squamous metaplasia and frank necrosis
21 were also reported for these tissues.

22 Monticello et al. (1990, 1991, 1996) conducted in vivo cell proliferation studies in which
23 they exposed F344 rats for short durations (1, 4, 9 and 42 days) as well as much longer durations
24 (13, 26, 52 and 78 weeks) to exposure concentrations of 0, 0.7, 2.0, 6.0, 10.0, and 15.0 ppm.
25 These data are unique in that they also included low exposure concentrations. The authors
26 reported statistically significant increases in cell proliferation only at 6.0 ppm and higher
27 exposure concentrations in the short duration study and only at 10.0 ppm and higher
28 concentrations in the longer duration study. These data have undergone considerable statistical
29 analysis in several papers as well as in this document. Conolly et al. (2002, 2003) and Gaylor
30 and Conolly (2004) interpreted these data, when combined, as indicating a nonmonotonic
31 behavior at low dose. In other words, formaldehyde was judged to result in a reduction in cell
32 proliferation at low dose in comparison to baseline rates, with increased proliferation effect
33 kicking in only at exposures that were cytotoxic. However, as shown in Appendix C and in
34 Subramaniam et al. (2008), Crump et al. (2008), analysis of the individual animal data shows
35 considerable uncertainty and variability, both quantitative and qualitative, in the interpretation of

1 these cell proliferation data. (For example, even the control data vary over an order of
2 magnitude in some cases. See Figures 5-22 and 5-23 in Appendix C.) These analyses (which
3 were based on the replicate animal data used in the above studies) considered regional
4 formaldehyde dose to the tissue (flux), nasal site and duration of exposure, as well as the number
5 of cells at a given site. The overall conclusion in Section 5.3.3 (and detailed in Appendix C) is
6 that the cell proliferation dose-response at low dose could be reasonably described by both
7 monotonic (with and without a threshold) and nonmonotonic curves.

8 Only one study, by Monticello et al. (1989), quantified cell proliferation in primates after
9 formaldehyde exposure; this study, reported an 18-fold increase in cell proliferation in the nasal
10 epithelium (respiratory and transitional), larynx, trachea and carina of male Rhesus monkeys
11 exposed to 6 ppm formaldehyde compared to controls (see Section 4.2.1 for detailed study
12 description). The authors also noted that increased cell proliferation was seen in locations with
13 minimal histological changes, indicating proliferation may be a more sensitive predictor of
14 adverse health effects of formaldehyde exposure.

15 16 **4.5.3.1.6. Cytotoxicity and resulting regenerative cell proliferation.**

17 The toxic and cytotoxic effects of formaldehyde exposure at the POE are well
18 documented after both inhalation and oral exposures (see Section 4.2.1). The nature and
19 progression of tissue injury has been best documented in rodent inhalation assays. Early effects
20 on the nasal mucosa include altered ciliary beat and mucus flow, hyperplasia and metaplasia of
21 nasal epithelium (Morgan et al., 1986a, b; Monteiro-Riviere and Popp, 1986; Maronpot et al.,
22 1986; Rusch et al., 1983; Monticello et al., 1986). These first changes may be considered
23 adaptive responses. Squamous epithelium may thicken and transitional epithelium may change
24 to squamous epithelium as evidenced by squamous hyperplasia, squamous metaplasia and
25 thickening of the epithelium in these anterior portions of the nose. Tissue damage may be
26 transient at lower formaldehyde exposures as these changes serve to protect tissue from
27 formaldehyde's reactivity. However, higher formaldehyde concentrations can overwhelm these
28 adaptive responses and result in gross tissue damage. Frank necrosis and focal erosions have
29 been reported in time- and concentration-dependent manner in rodent bioassays.

30 Both adaptive changes and cytotoxicity are associated with cell proliferation. However,
31 where adaptive changes are successful, e.g., prevent continued toxic insult to the tissue, cell
32 proliferation is transient. Exposure regimens where the adaptive changes are not adequate to
33 protect the tissue, would result in continued cytotoxicity and cell death. Sustained damage to the
34 epithelium would result in sustained cell proliferation to compensate for cell death. A series of
35 rodent bioassays present convincing evidence that chronic inhalation exposures 6 hours a day,

1 5 days a week at 6, 10, and 15 ppm formaldehyde doses do result in sustained damage to the
2 nasal epithelium, sustained cell proliferation and tumor development (Kerns et al., 1983, Morgan
3 et al., 1986; Monticello et al., 1990, 1991, 1996). Work by Monticello and coworkers
4 demonstrate that chronic repeated inhalation exposures at 6, 10, or 15 ppm formaldehyde result
5 in sustained cell proliferation at the lateral meatus, mid-septum and maxilloturbinates of rat nasal
6 passages (Monticello et al., 1991, 1996).

7 8 **4.5.3.1.7. Evidence for promotion.**

9 There is some evidence, although mixed, that formaldehyde may promote tumor
10 development by other carcinogens, and known initiating agents by various routes of exposure.
11 Formaldehyde exposure in drinking water (0.5% formalin) increased glandular stomach
12 adenocarcinomas in male Wistar rats after initiation with 100 mg/L, *N*-methyl-
13 *N'*-nitrosoguanidine (MNNG), compared to MNNG-only-treated rats (Takahashi et al., 1986). In
14 white noninbred rats, inhalation exposures (3, 30, or 300 µg/m³ formaldehyde 7hr/day,
15 5 days/week for 1 year) increased tumor multiplicity per animal and decreased latency of
16 benzo[a]pyrene induced tumors in white noninbred rats (Yanysheva et al., 1998.) Similarly,
17 formaldehyde skin application decreased tumor latency, in 7,12-dimethylbenz(a)anthracene
18 (DMBA) initiated hairless Oslo mice (Iversen, 1986). Although formaldehyde exposure also
19 increased the tumor multiplicity in Syrian golden hamsters where diethylnitrosamine (DEN)
20 (0.25mg I.P.) was the tumor initiator, positive results were only reported for the exposure
21 regimen where hamsters were exposed to formaldehyde via inhalation 48 hours prior to DEN
22 injection, and then one a week thereafter for life. However, formaldehyde did not increase the
23 number of tumors per tumor bearing animals when only administered beginning one week after
24 all DEN injections. In contrast, bladder cancer was not enhanced by intravesical instillation of
25 0.5ml of 0.3% formalin, one week after instillation of *N*-methyl-*N*-nitrosourea (MNU) in male
26 Fisher rats (Homma et al., 1986).

27 The observed promotion activity of formaldehyde has been tested in several systems, by
28 different routes of exposure. By several routes of exposure, formaldehyde enhanced tumor
29 development at a site where formaldehyde did not induce tumors alone, without the initiating
30 agent (Takahashi et al., 1986, Yanysheva et al., 1998; Iversen, 1986). Promotion activity in
31 these studies was evidenced by increased in tumor bearing animals (oral route), increase in
32 tumors per animal (inhalation routes) and decreased tumor latency compared to those animals
33 only exposed to the initiating agent (inhalation route) (Takahashi et al., 1986, Yanysheva et al.,
34 1998; Iversen, 1986). Although these experiments do not indicate how formaldehyde acts as a

1 promoter in these systems, it is possible formaldehyde-induced mutation, increased cell
2 proliferation or other toxic action could enhance tumor development from another agent.

4 **4.5.3.1.8. Localized immunosuppression.**

5 Formaldehyde exposure has induced localized immune suppression in experimental
6 animals (Dean et al., 1984) and in exposed workers (Lyapina et al., 2004). Repeated inhalation
7 exposures in rodents depopulated the URT and pulmonary tissues of resident macrophages,
8 resulting in a transient decrease in POE host defenses (Admas et al., 1987). After cessation of
9 exposure, the mononuclear phagocyte (MP) populations were replenished and there was a
10 subsequent increase in host defense representing both increased MP numbers and increased
11 bacteriocidal activity of the MPs. These data suggest that peak exposures of formaldehyde may
12 present localized immunosuppression for components of the mononuclear phagocyte system
13 (MPS) in tissues at the site of first contact.

14 A number of studies have evaluated the ability of formaldehyde to induce systemic
15 immunotoxic effects in humans (Ohtani et al., 2004a, b; Erdei et al., 2003; Thrasher et al., 1990,
16 1987; Pross et al., 1987). Some studies have reported altered innate immune responses
17 associated with formaldehyde exposure (Erdei et al., 2003), while others have noted adaptive
18 immune response suppression associated with formaldehyde exposure (Thrasher et al., 1990,
19 1987) and changes associated with alterations to a predominant T—lymphocyte helper 2 (Th2)
20 pattern (Ohtani et al., 2004a, b). In contrast, Pross et al. (1987) did not observe formaldehyde-
21 associated changes in systemic immune function.

22 Numerous studies have reported increased respiratory tract infections in formaldehyde
23 exposed individuals both in occupational and residential environments (Lyapina et al., 2004;
24 Krzyzanowski et al., 1990; Holness and Nethercott, 1989). Incidences of physician-diagnosed
25 chronic bronchitis were more prevalent in children (under age 15) living in homes with higher
26 formaldehyde (>60 ppb) readings in the kitchen ($p < 0.001$) but this effect was more pronounced
27 ($p < 0.001$) in children simultaneously exposed to environmental tobacco smoke (Kryzanowski
28 et al., 1990). The prevalence of chronic cough was also increased in adults living in homes with
29 measurable levels of formaldehyde, but data were not shown. Holness and Nethercott (1989)
30 assessed chronic bronchitis in 87 funeral workers, where the average formaldehyde exposure was
31 reported at 0.38 ± 0.19 ppm. Chronic bronchitis was observed in 20 funeral workers ($n = 87$)
32 exposed to formaldehyde compared with 3 cases of chronic bronchitis in nonexposed referent
33 controls ($n = 38$). A statistically significant association of self-reported chronic bronchitis and
34 decreased resistance to URT infection was reported in formaldehyde exposed workers compared
35 with controls ($p = 0.02$) (Lyapina et al., 2004). Of the workers, 41% had a history of chronic

1 respiratory infection and frequent long-lasting infectious inflammatory relapses (Group 1a).
2 Another group (group 1b) consisted of 17 exposed workers, 12 of whom had no history of
3 recurrent viral infections of the URT. There was a statistically significant association of
4 frequency and duration of inflammatory relapses between groups 1a and 1b.

5 Lyapina et al. (2004) also reported effects of formaldehyde exposure on neutrophil
6 respiratory burst activity (NRBA), the capacity of polymorphonuclear leukocytes to produce
7 reactive oxygen radicals in response to chemical or microbial stimuli using flow cytometry. A
8 suite of hematological tests and flow cytometric analysis for respiratory burst activity were
9 performed. Although no significant difference was observed in the spontaneous and stimulated
10 NRBA (median percentage of oxidizing cells) between the 29 exposed workers with URT
11 inflammation and the healthy controls (0.83 vs. 1.35, respectively), a separate comparison of the
12 NRBA of 12 workers with chronic, repeating URT infections and 17 workers with short,
13 infrequent episodes of URT inflammations was significant (0.45 vs. 1.00, $p = 0.037$). When the
14 NRBA of the group with chronic URT infections ($n = 12$) was separately compared with that of
15 the healthy controls ($n = 21$), the results were also significant (0.45 vs. 1.35, $p = 0.012$).
16 Individuals with chronic URT infections have reduced NRBA that could be due to formaldehyde
17 exposure. Neutrophils respond to tissue damage or local invasion of microorganisms and act to
18 phagocytize foreign cells. If neutrophilic activity is hampered or altered by formaldehyde
19 exposure, then the ability to fight infection will be diminished, leading to prolonged infection.
20 However, no dose-response pattern of formaldehyde exposure could be determined from this
21 study.

22 23 **4.5.3.1.8. Potential for systemic transport of formaldehyde.**

24 In aqueous solution formaldehyde exists in equilibrium with its hydrated form
25 methanediol (CH_2OH_2) ($K_d = 5.5 \times 10^{-4}$). The equilibrium favors methanediol at physiological
26 temperature and pH (>99.9%) and is readily reversible. In biological systems, as free
27 formaldehyde is removed from aqueous solution through binding with serum proteins and
28 cellular components, the equilibrium is reestablished by dehydration of methanediol to free
29 formaldehyde. The reversible nature of this hydration reaction describes how a pool of free
30 formaldehyde may be sustained in biological systems.

31 There is strong and consistent evidence in biological testing systems in vitro that treating
32 cells with formaldehyde in an aqueous media results in significant cytotoxicity, cell proliferation,
33 clastogenic effects and clear evidence of mutational events (see Section 4.3). Similarly, animal
34 bioassays where formaldehyde is administered in drinking water report portal of entry toxicity
35 including hyperplasia, increased cell proliferation, focal lesions, and tumors (see Section 4.2.1).

1 It should be noted that URT tissues are covered by an aqueous mucous layer, through which
2 formaldehyde must pass to react the cellular components of the URT. It has been postulated that
3 formaldehyde transports through this mucous layer and the underlying tissues as methanediol
4 (Georgieva et al., 2003).

5 The dynamic equilibrium between the hydrated and unhydrated forms of formaldehyde in
6 biological systems is well understood. Since the hydration reaction favors methanediol, it is
7 expected that exogenous formaldehyde which reaches the blood will primarily exist as
8 methanediol and is subject to physiological elimination. As free, unhydrated formaldehyde
9 continues to react with serum proteins and cellular components, the blood levels of methanediol
10 are expected to reduce as it is dehydrated to maintain equilibrium. Although some attempts to
11 measure significant changes in free formaldehyde levels in blood after inhalation exposure have
12 not been successful, the half-life in blood has been measured after i.v. injection at approximately
13 2 minutes (McMartin et al., 1979). Additionally, the detection of antibodies to formaldehyde-
14 hemoglobin adducts and formaldehyde-albumin adducts in exposures workers, smokers and
15 laboratory animals exposed via inhalation provides direct evidence that formaldehyde is able to
16 react with serum albumin and hemoglobin in biological systems (Thrasher et al., 1990, Grammer
17 et al., 1990, 1993; Dykewicz et al., 1991; Varro et al., 1997; Li et al., 2007). These data support
18 the hypothesis that exogenous formaldehyde may reach and transport through the blood. If so,
19 formaldehyde (or methanediol) may reach sites distal to the portal of entry.
20

21 **4.5.3.2. Mode of Action Evaluation for Upper Respiratory Tract Cancer (Nasopharyngeal** 22 **Cancer, Sino-nasal)**

23 From the above discussion, it can be seen that numerous mechanisms of action for
24 formaldehyde-induced cancer can be reasonably supported based on various known biological
25 actions of formaldehyde (e.g., mutation, cell proliferation, cytotoxicity, and regenerative cell
26 proliferation). Additionally, alternative actions, such as immunosuppression or viral
27 reactivation, are possible, although less data exist to evaluate these MOAs. Rather than a single
28 MOA, it is plausible that a combination of these factors contribute to cancer incidence in an
29 exposed population. Considering multiple factors may help to better understand the biological
30 and mechanistic basis for the increases in cancer incidence observed in exposed human
31 populations. Unlike animal bioassays, human epidemiological studies may reflect not only the
32 effects of the agent of concern but also numerous other risk factors (e.g., viral status, diet,
33 smoking, etc.). Additionally, human studies may be impacted by biological human variability
34 across individuals, cancer biology (subtypes), wide variability in exposure regimens in human
35 populations, etc. Therefore, if the purposes of exploring the carcinogenic MOA of an agent are
36 to better understand the relevance of a given carcinogen to human populations and to inform the

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1 exposure-response analysis, then discussions of MOAs which recognize the interaction of an
2 agent with human variability and various risk factors is an appropriate analysis. For each of the
3 below postulated MOAs, the detailed supporting evidence for the referenced key events are
4 provided above in Section 4.5.3.1 (e.g., mutation, cell proliferation, cytotoxicity, and
5 regenerative cell proliferation), and is not repeated here in detail each time a key event is
6 proposed as part of a postulated MOA.

7
8 **a) Direct mutagenicity of formaldehyde in cells at the site of first contact:** Mutations,
9 the permanent heritable changes in the genome of the cell, are a primary mechanism for
10 the activation of oncogenes or the inactivation of tumor suppressor genes. Mutagenicity
11 is the most widely recognized determinant of chemical-induced carcinogenicity, and it is
12 difficult to set aside the relevance of direct formaldehyde-induced mutations from its
13 demonstrated carcinogenicity. Formaldehyde-induced mutation in mucosal cells of the
14 URT, throat and buccal cavity may serve to initiate cells, or provide subsequent
15 mutagenic events to already initiated cells. Since the mucosal cells have proliferative
16 capacity, and cell proliferation is a normal tissue function, mutations may be fixed and
17 passed to daughter cells due to baseline cell proliferation of the tissue.

18 **Relevance to humans:** This MOA is relevant to humans. The well-documented DNA
19 reactivity (e.g., DPC and DNA adducts) and clastogenicity of formaldehyde in the URT
20 of laboratory animals is a direct effect of formaldehyde on tissues of first contact. As this
21 is a direct acting agent—no distribution or metabolism is required for the genotoxic
22 action—there is little expected species variability. As discussed in Chapter 3, there are
23 species differences in flux of formaldehyde into the respiratory mucosal tissues, but this
24 introduces species differences in dosimetry—not mechanism. Finally, the clastogenic
25 effects in nasal and buccal epithelial cells in formaldehyde- exposed workers confirms
26 the direct genotoxic effects of formaldehyde at the first site of contact in humans.

27 **b) Decrease in DNA repair function within cells at the site of first contact:** A decrease in
28 DNA repair capacity in these tissues by formaldehyde may increase total mutations over
29 time due to either endogenous or exogenous sources of mutation. Although there are
30 only a few studies which have explored the potential for formaldehyde to reduce DNA
31 repair capacity, the evidence is positive, both in vitro testing systems, and in one study of
32 occupationally exposed humans (Grafstrom, 1985; Hayes et al., 1997).

33 **Relevance to humans:** This MOA is considered relevant to humans. The general
34 population is exposed to various carcinogens, many with mutagenic potential, at sites of
35 first contact including; air pollution, tobacco products, nitrosamines and viruses.
36 Additionally, there are endogenous sources of DNA damage and mutagenicity in humans
37 (e.g., lipid peroxidation, oxidative stress). The demonstration of reduced DNA repair
38 activity (O6-alkylguanine-DNA alkyltransferase activity) in formaldehyde-exposed
39 mortuary students suggests this toxic action of formaldehyde is possible in humans.

40 **c) Formaldehyde-induced cell proliferation:** Formaldehyde-induced cell proliferation in
41 the oral and respiratory mucosa may be considered a key event in conjunction with the
42 genotoxic effects, and induced mutational events observed with formaldehyde exposure.

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1 This MOA is intended to describe events which occur below exposure levels which
2 induce cell death and mucosal lesions. Therefore this MOA is comprised of two key
3 events:

- 4 a. Formaldehyde-induced genotoxicity or mutation
- 5 b. Formaldehyde-induced cell proliferation

6
7 DNA replication during cell proliferation may serve to translate DNA damage or a
8 formaldehyde-related DNA lesion into a permanent change in the sequence of nucleic
9 acids during replication of the DNA—e.g. ‘fix’ a mutation from DNA damage.
10 Additionally formaldehyde-induced cell proliferation may provide an opportunity for
11 initiated cells to proliferate, increasing the potential for additional mutation events and
12 transformation. The increased cell proliferation observed in the mucosal tissues in direct
13 contact with formaldehyde during inhalation exposures may serve to amplify the risk of
14 cell transformation from mutation alone. Researchers have noted that increased cell
15 proliferation may be transient in some locations as adaptive responses compensate
16 (Swenberg, 1983). However, evidence in both monkeys and rodents indicate that
17 increased cell proliferation in repeated exposures across time do result in sustained cell
18 proliferation. Data in Rhesus monkeys indicates increased cell proliferation is observed
19 beyond the nasal cavities to the larynx, trachea and carina (first tracheal branching)
20 (Monticello et al., 1989). Additionally, the authors note that cell proliferation is a more
21 sensitive indicator of effects on the epithelium, observed even when minimal histological
22 changes were present.

23 **Human Relevance:** Both formaldehyde-induced mutation and cell proliferation are
24 direct effects on the oral and nasal mucosa, well documented in rodent models with
25 supporting evidence in human epidemiological studies. Therefore both key events are
26 relevant to humans. As noted above, there are species differences in localized flux of
27 formaldehyde into the tissues of the oral and respiratory tract based on structural
28 differences in the airways, as well as breathing patterns. Although these differences may
29 effects the dosimetry of the formaldehyde absorption into the tissues, this only influences
30 the magnitude of response at any given location. Data from exposed Rhesus monkeys
31 which documents formaldehyde-induced cell proliferation in tissues beyond the nasal
32 cavity, and tissues with minimal histological changes supports a role for cell-proliferation
33 in the observed cancers in humans, which occur beyond the nasal cavities, and in tissues
34 without formaldehyde-related focal lesions.

- 35 **d) Cytotoxicity-induced cell proliferation (CICP):** Cell death followed by compensatory
36 cell proliferation is a reasonable MOA for agent-induced cancer. It should be noted that
37 the exposure conditions which result in CICP in rodents is known to result in significant
38 DNA reactivity and genotoxicity. Therefore, formaldehyde-induced mutations cannot be
39 excluded from this MOA. The animal bioassays support the carcinogenic potential of
40 formaldehyde in this context (Kerns et al., 1983; Selkemer et al., 1983; Monticello et al.,
41 1986). The majority of squamous cell carcinomas (SCCs) seen in formaldehyde-exposed
42 rats have been localized to the lateral meatus and mid-septum in the nasal passages
43 (Morgan et al., 1986; Monticello et al., 1996), while polyploid adenomas have

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1 predominantly been reported at the maxilloturbinates (Morgan et al., 1986; Monticello et
2 al., 1996). Morgan et al. (1986) speculated that the maxilloturbinate was less susceptible
3 to SCC due to metabolic differences. However, Monticello et al. (1996) later suggested
4 that the smaller population of cells available at the maxilloturbinate accounted for fewer
5 SCCs observed at that site. Regardless, for those locations where SCCs do arise in rats
6 chronically exposed to formaldehyde, a clear temporal relationship can be demonstrated
7 for dose regimens capable of producing sustained epithelial damage and sustained cell
8 proliferation to eventual tumor development. Conversely, tumors are not observed in
9 these rodent models at those sites in the nasal passages without sustained cell
10 proliferation.

11 **Relevance to humans:** Human exposure to formaldehyde would most likely involve
12 chronic exposures to indoor levels of formaldehyde, and episodic exposures in the
13 environment or from an occupational exposure (see review in Chapter 2). An exposure
14 scenario parallel to that used in chronic rodent bioassays is unlikely (e.g. 2-15 ppm
15 6–8 hours/day, 5 days/week for 10-30 months). Exposure conditions are difficult to
16 assess especially in retrospective studies. However, only the most extreme industrial
17 work conditions would result in human exposures similar to those that produce sustained
18 compensatory cell proliferation in animal studies (i.e. 6-15 ppm 6 hours/day, 5 days per
19 week). Gross tissue lesions as reported in rodents from repeated chronic exposures at
20 6 and 10 ppm formaldehyde have not been reported from workplace exposure, and only
21 minor histopathological changes have been noted (Boysen et al., 1990; Holmström and
22 Wilhelmsson et al., 1989). It is possible that workers were episodically exposed to
23 formaldehyde levels which resulted in cell death and focal or gross lesions requiring cell
24 proliferation for tissue remodeling or repair. However, it is unexpected that these
25 conditions would be relevant to human environmental exposures. Therefore, although
26 regenerative cell proliferation is retained as a reasonable MOA for formaldehyde
27 carcinogenicity in experimental animals, it is unclear whether it is relevant to the
28 extrapolation of health risks to formaldehyde exposures in the general environment.

- 29 e) **Promotion:** Several animal studies indicate that formaldehyde exposure may promote
30 tumor formation due to other carcinogenic or initiating agents. There are positive data by
31 several routes of exposure (oral, dermal and inhalation) and promotion has been reported
32 as an increase in tumor bearing animals, an increase in tumors multiplicity or a decrease
33 in tumor latency with formaldehyde exposure in conjunction with the initiating agent
34 compared to tumors from the initiating agent along, or formaldehyde alone. The specific
35 key events which may explain this promotion effect are unknown but may include several
36 of the mechanisms discussed as potential MOAs for formaldehyde: mutagenicity,
37 mitogenesis, cocarcinogenicity, immunosuppression. Promotion is considered here as a
38 separate MOA, since these activities are noted for experimental conditions and tumor
39 sites where formaldehyde did not induce tumors in the absence of the initiating agent.

40 **Relevance to humans:** Although the human epidemiologic literature doesn't address
41 issues of tumor promotion, the nature of the cancers of concern indicate that chemical
42 promotion may be relevant to cancer incidence for these sites. Many of the risk factors
43 for nasopharyngeal cancer and other mouth and oral and URT cancers include direct
44 mutagens (e.g., smoking, dietary nitrosamines) where a promoting agent would be
45 expected to increase cancer incidence with these other risk factors. Additionally, the well

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1 known viral risk factors for cancers of the mouth and URT also suggest a role for
2 promoting agents to human cancer incidence. Although only tangential evidence, this
3 does suggest that the promoting activity of a chemical agent, would be relevant to the
4 agent's carcinogenicity at these sites. Therefore, the potential for formaldehyde to act as
5 a promoter with other initiators—is considered relevant to formaldehyde's carcinogenic
6 MOA.

7 **f) Increased URT infections/viral reactivation:** Inhalation exposure to formaldehyde has
8 been shown to decrease the defenses of the body against infection through two
9 mechanisms: (1) damage to the protective mucous barrier and function of the mucociliary
10 apparatus; and (2) localized immunosuppression. These effects have been demonstrated
11 in both exposed humans and controlled animal experiments. Additionally, increased
12 respiratory tract infections are associated with formaldehyde exposure in several
13 populations. Common viral agents (e.g., Epstein barr virus) are known risk factors for
14 nasopharyngeal cancer, sinonasal cancers, and other URT cancers. Although direct
15 evidence does support increased URT infections due to formaldehyde exposure, and URT
16 infections are considered risk factors for URT cancers, direct evidence for formaldehyde-
17 related infections leading to cancer is lacking. There is however one epidemiological
18 study which finds the association between formaldehyde and nasopharyngeal cancer is
19 strengthened in Epstein barr virus sero-positive cases versus sero-negative cases. These
20 data suggest a possible role for formaldehyde in infection, viral reactivation, or
21 cocarcinogenicity with a viral agent.

22 **Relevance to humans:** The potential role of increased URT infections and
23 immunosuppression at the portal of entry is considered to be relevant to humans. Data in
24 humans are available to support both key events in this MOA. Additionally,
25 epidemiological studies are conducted in human populations where individuals may be
26 exposed to various viral agents across the study period. Therefore, toxic actions by
27 formaldehyde which may increase URT infections, or viral-reactivation at the site of first
28 contact, could be acting in conjunction with viral agents to contribute, in part, to observed
29 associations between formaldehyde exposure and increased URT cancer.

30 31 *Summary and integration of key events*

32 Each of the hypothesized MOAs discussed above to better understand the carcinogenic
33 potential of formaldehyde is supported by formaldehyde-specific evidence, either in animal
34 studies, human studies, or both. For those key events studied in animal models such as cell
35 proliferation, genotoxicity, degradation of the mucociliary apparatus and C1CP, supporting
36 evidence is available in more than one species, multiple strains (e.g., rats) and has been reported
37 by multiple researchers. Therefore the overall database supporting these key events in laboratory
38 studies, and their corresponding MOAs is fairly large. In contrast, some key events relevant to
39 humans, but less studied in animal models may have a small supporting database (e.g., increased
40 respiratory tract infections). These alternative MOAs are retained as potentially relevant to the
41 carcinogenic action of formaldehyde as the intent of this discussion is to identify modes of action

1 will may contribute to the observation of increased upper respiratory tract cancers in exposed
2 human populations. It is noted that additional study is needed to better understand the range of
3 effects formaldehyde may have at sites of first contact in humans.

4 The MOAs which include genotoxicity, mutation, decreased DNA repair, increased cell
5 proliferation and C1CP are interrelated. Conditions which provide both a source of cell
6 proliferation and increased mutation would be expected to increase neoplastic transformation.
7 Formaldehyde acts on the target tissue, the respiratory epithelium, to induce each of these events.
8 However, these key events operate across different exposure ranges and present different
9 exposure response relationships. For example, formaldehyde-induced mutations would be
10 expected across the exposure range, where any incremental increase in genotoxicity and
11 formaldehyde-related mutation would contribute to background levels, with the potential to
12 increase cancer risk incrementally. In contrast, focal and gross lesions to the respiratory mucosa
13 due to cytotoxicity are not observed unless exposure concentrations are sufficient to provide
14 localized tissue doses (flux) required to result in cell death and related compensatory cell
15 proliferation. Since tissue dose (flux) is dependent on not only exposure concentration but also
16 duration of exposure and location in the respiratory tract (see Section 3.4), and varies by species,
17 correlation of exposure concentrations to tissue responses directly are complex. Exposure
18 response relationships for the key events (cell proliferation, genotoxicity, degradation of the
19 mucociliary apparatus and C1CP) are reported by exposure concentration, not tissue flux, which
20 would be a more biologically relevant measure.

21 Although the tissue dose-response relationships for formaldehyde induced mutation,
22 mitogenesis and cytotoxicity are different, the effects at the tissue level cannot be easily
23 disaggregated. At any given exposure concentration, target cells in the respiratory tract will
24 experience different effective tissue concentrations of formaldehyde. Measurement of cell
25 proliferation, DNA protein crosslinks, or genotoxicity may require examining a population of
26 cells which would have been subject to different flux rates of formaldehyde (see Chapter 3).
27 Similarly, when evaluating the tumor dose response, cells within the target tissue will represent a
28 range of target tissue formaldehyde concentrations. Therefore, an integrated MOA scheme is
29 hypothesized where key events may influence the observed tumor response differentially across
30 the exposure response range (see Figure 4-36). This schematic illustrates the potential for
31 genotoxicity and formaldehyde-induced mutation to occur where tissue dose (flux of
32 formaldehyde into the tissue is minimal). Where tissue dose is increased, formaldehyde-induced
33 cell proliferation is observed in addition to genotoxicity. As tissue dose increases and
34 formaldehyde effects on the respiratory mucosa are more severe, gross pathology including focal
35 and gross lesions due to cell death are noted. Therefore, several of the MOAs presented above

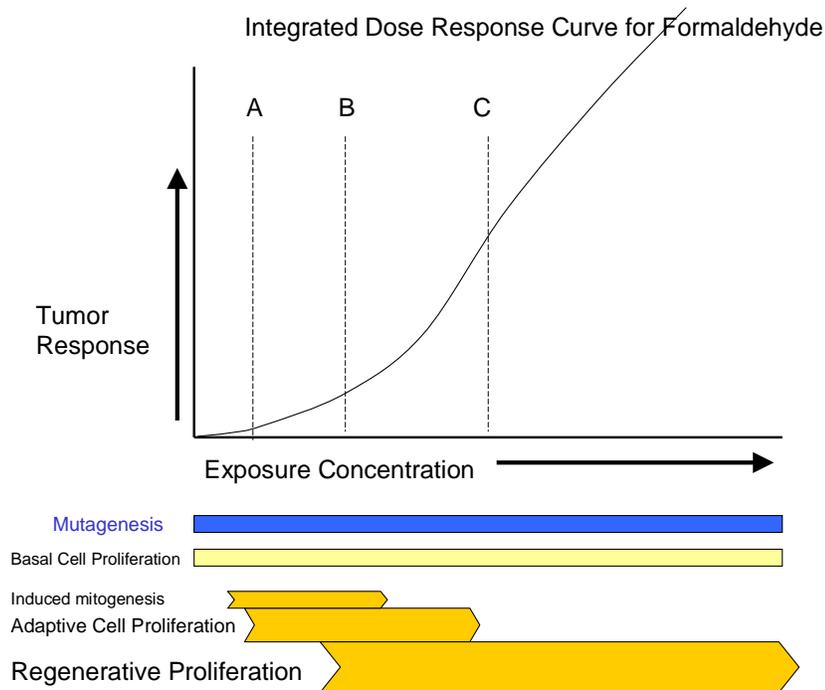


Figure 4-36. Integrated MOA scheme for respiratory tract tumors.

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

1 code 207). Therefore, this MOA evaluation will focus on mechanisms which may impact all
2 forms of leukemia (e.g., bone marrow toxicity) or those specific to myeloid leukemia. The
3 mechanistic data supporting the key events in this analysis are presented in Section 4.5.3.1. For
4 each of the below postulated MOAs, the detailed supporting evidence for the referenced key
5 events are provided above in Section 4.5.3.1 (e.g., mutation, cell proliferation, cytotoxicity, and
6 regenerative cell proliferation), and is not repeated here in detail each time a key event is
7 proposed as part of a postulated MOA.

8
9 **a) Direct effects of formaldehyde on a circulating stem cell or progenitor cell present at**
10 **the portal of entry:** Hematopoietic stem cells do circulate throughout the body and can
11 be harvested from peripheral blood. Formaldehyde exhibits a range of toxic effects at the
12 site of first contact including genotoxic effects believed to be mediated by direct DNA
13 reactivity (see Section 4.3). Formaldehyde is known to directly react with blood
14 components in formaldehyde exposed humans and animals resulting in both hemoglobin
15 and albumin adducts (Thrasher et al., 1990, Grammer et al., 1990; Grammer et al., 1993;
16 Dykewicz et al., 1991; Varro et al., 1997; Li et al., 2007). Therefore, it has been
17 hypothesized that formaldehyde could react with DNA in circulating hematopoietic stem
18 cells (Zhang et al., 2009) resulting in heritable mutations which may contribute to
19 leukemia incidence. Recently, Zhang et al. (2010a) have tested the hypothesis that
20 exogenous formaldehyde may damage circulating stem cells. Clastogenic effects were
21 found in circulating hematopoietic stem cells cultured from formaldehyde exposed
22 workers. The reported aneuploidy was demonstrated as significant increases in both
23 monosomy 7 and trisomy 8. These specific chromosomal changes are consistent with
24 those reported for agent-induced myeloid leukemia (Zhang et al., 2010a).

25 **Relevance to Humans:** This hypothesized MOA is considered relevant to humans.
26 Supporting evidence is found in humans for formaldehyde direct reactivity with blood
27 proteins (e.g., albumin and hemoglobin) as well as clastogenic effects in circulating
28 hematopoietic stem cells in formaldehyde exposed workers.

29 **b) Bone marrow toxicity:** Direct bone marrow toxicity is the most studied leukemogenic
30 action for an endogenous agent (e.g., benzene, ionizing radiation). It is believed that an
31 agent which exerts its toxicity on the bone marrow, resulting in translocations and
32 heritable mutations in hematopoietic stem cells may cause leukemia. It has been
33 hypothesized that formaldehyde may transport to the bone marrow in its hydrated form
34 (methandiol) and react with cellular proteins, and DNA causing direct effects on
35 components of the bone marrow. A study by Ward et al. (1983) has shown that mice
36 given a single dose of formaldehyde, or methanol by gavage were able to develop
37 cytogenetic changes (SCEs, CAs and aneuploidy) in bone marrow cells suggesting that
38 formaldehyde can reach to bone marrow and induce toxicity. Pancytopenia (a reduction
39 in blood borne cells formed in the bone marrow) is a symptom of direct bone marrow
40 toxicity and is observed with other leukemogenic agents (e.g., benzene, ionizing
41 radiation). A recent review of 8 published studies of formaldehyde exposed workers in
42 China by Tang et al. (2009) indicates 7 of the studies provide evidence of reduced white
43 blood cell counts, platelet levels and hemoglobin levels associated with formaldehyde

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1 exposure. A study of occupationally exposed nurses provided a correlation between
2 decreased white blood cell counts and formaldehyde exposure (Kuo et al., 1997). A
3 recent study by Zhang et al. (2010a) provides the best evidence for bone marrow toxicity,
4 where they report not only a reduction in white blood cell counts, but reductions in cell
5 counts of all the blood cells, as well as increased mean cell volume. Although these
6 reductions did not meet the clinical definition of pancytopenia (when averaged across the
7 study population), reduction of all blood borne cells formed in the bone marrow is
8 consistent with the bone marrow toxicity associated with pancytopenia seen with other
9 leukemogens (Zhang et al., 2010b).

10 **Relevance to Humans:** This hypothesized MOA is considered relevant to humans.
11 Supporting evidence is found in humans for bone marrow toxicity in formaldehyde
12 exposed workers.

13 14 **4.5.3.3.2. MOA evaluation for Lymphomas (e.g., Hodgkin’s lymphoma, Multiple myeloma).**

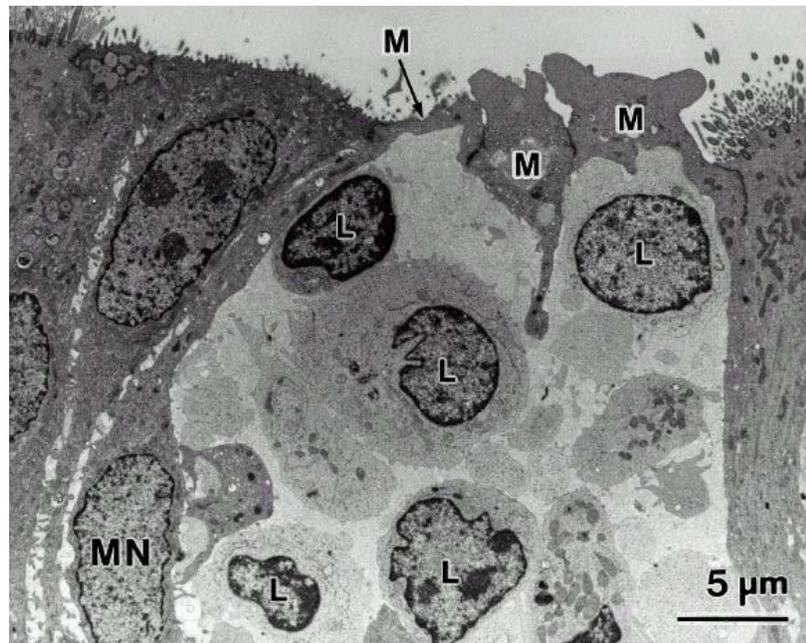
15 The general MOA for formaldehyde is based on direct chemical reactivity and toxic
16 effects at the portal of entry (POE). Formaldehyde is directly and indirectly genotoxic, and
17 reacts with cellular proteins and DNA in cells which it comes into contact. Additionally,
18 immunosuppression, viral reactivation and promotion effects are relevant to lymphoma and
19 related malignancies. Therefore, the key events for the adult cell lymphoid cancers would
20 include these actions. For each of the below postulated MOAs, the detailed supporting evidence
21 for the referenced key events are provided above in Section 4.5.3.1 (e.g., mutation, cell
22 proliferation, cytotoxicity, and regenerative cell proliferation), and is not repeated here in detail
23 each time a key event is proposed as part of a postulated MOA.

24 Lymphoid tumors (e.g., lymphocytic leukemia, B-cell lymphoma, mantle cell lymphoma
25 [a rare form on non-Hodgkin’s lymphoma] and myeloma) may arise from cells present at the
26 portal of entry (POE) (see Figure 4-32). The location and function of mature lymphocytes
27 contribute to their vulnerability to transformation by agents at the POE. Therefore, a brief
28 summary of the immuno-biology of these cells is provided in order to provide context for the
29 MOA evaluation:

30
31 **Location:** Lymphocytes are present in the oral and respiratory tract epithelium, as well as
32 in cell aggregates and tertiary immune structures (e.g., germinal centers) in the mucosal
33 tissues (Zuercher and Cebra, 2002; Zuercher et al., 2002; Wu et al., 1997; Kupper et al.,
34 1990). These mucosa-associated lymphoid tissues (MALT) provide the opportunity for
35 formaldehyde to directly interact with components of the immune system present at the
36 POE (Wu et al., 1997, Claeys et al., 1996, Park et al., 2003; Fujimura, 2000).
37 Intraepithelial lymphocytes are present in the pseudostratified epithelium of the
38 nasopharyngeal passages and there are aggregates of immune cells and germinal cells
39 present in these tissues. Crypts containing mature lymphocytes exist at the surface of the
40 nasal epithelium (Fujimura, 2000). Microfold cells or M-cells form the crypts, where the

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1 lymphocytes are covered by a thin membrane (see Figure 4-37). Functionally, these
2 lymphocytes identify and process foreign antigens at the POE (Fujimura, 2000).
3 Therefore the mature lymphocytes within these crypts, exposed to exogenous agents, are
4 involved in active immune responses to foreign antigens.



6
7
8 **Figure 4-37. Location of intraepithelial lymphocytes along side epithelial**
9 **cells in the human adenoid.**

10
11 Source Fujimura et al., 2000

12
13
14 **Clonal expansion:** Mature lymphocytes (both B and T-cells) clonally expand their
15 populations in response to an exogenous antigen stimulation when a humoral immune
16 response is stimulated. Therefore cell proliferation is a normal function of these mature
17 lymphocytes and occurs every time there is an infection. Cell proliferation of mature B
18 and T-cells, responsive to a particular antigen, occurs in active germinal centers
19 (including those within the respiratory tract). Cells may be exposed to exogenous agents
20 during the immune response, or cells responding in the germinal center may have
21 previously been in the epithelium or M-cell crypt.

22 **Somatic hypermutation:** Normal immune function includes the process of somatic
23 hypermutation where B-cells undergo DNA rearrangement of the variable region genes to
24 produce novel antibodies specific to a given antigen. This process is key to adaptive
25 immunity and demonstrated by the basic principles of immuno-biology which underlie
26 vaccination theory. Gene sequencing of adult B-cell lymphomas and leukemias indicate
27 that the chromosomal regions involved in somatic hypermutation correspond to known
28 oncogenes in these cancers. The vulnerability of these processes is evidenced by the

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1 observation that approximately 90-95% of adult lymphomas and leukemias are of B-cell
2 origin (Gordon et al., 2003). Formaldehyde-induced protein-protein crosslinking could
3 disrupt cellular processes including somatic hypermutation and cell mitosis, resulting in
4 agent-induced translations similar to those found in spontaneous B-cell malignancies.

5 **Postulated MOAs for lymphomas (e.g., Hodgkin's lymphoma, multiple myeloma):** For
6 each of the below postulated MOAs, the detailed supporting evidence for the referenced
7 key events are provided above in Section 4.5.3.1 (e.g., mutation, cell proliferation,
8 cytotoxicity, and regenerative cell proliferation), and is not repeated here in detail each
9 time a key event is proposed as part of a postulated MOA.

- 10 **a) Direct or indirect formaldehyde-induced mutations in cells at the site of first**
11 **contact:** Immune cells including intraepithelial lymphocytes, and cells in MALT are
12 collocated with the epithelial cells from which URT cancers arise (see Figure 4-37).
13 Therefore direct and indirect mutagenic potential of formaldehyde is equally applicable
14 to components of the immune system present in these tissues. Mutations, the permanent
15 heritable changes in the genome of the cell, are a primary mechanism for the activation of
16 oncogenes or the inactivation of tumor suppressor genes. Mutagenicity is the most
17 widely recognized determinant of chemical-induced carcinogenicity, and it is difficult to
18 set aside the relevance of direct formaldehyde-induced mutations from its demonstrated
19 carcinogenicity. Formaldehyde-induced mutation in immune cells present at the site of
20 first contact, may provide initiated cells or subsequent mutagenic events to already
21 initiated cells. The competence of the immune system relies on the proliferation of
22 peripheral blood lymphocytes in response to immune challenge (e.g. infection).
23 Additionally, heritable changes to the variable gene regions in B-cells generated during
24 somatic hypermutation are essential to adaptive immunity (e.g., immunization)
25 demonstrating that permanent heritable changes in the DNA of peripheral B-cells are
26 passed to daughter cells and retained in the body for decades. Any agent-induced
27 mutations would be similarly propagated and retained with the potential to contribute to
28 the transformation of mature lymphocytes.

29 **Relevance to humans:** This MOA is relevant to humans. The well-documented DNA
30 reactivity (e.g., DPC and DNA adducts) and clastogenicity of formaldehyde at the POE in
31 laboratory animals is a direct effect of formaldehyde on tissues of first contact, and these
32 mechanisms are considered relevant to humans. As with epithelial cells, clastogenic
33 effects in peripheral lymphocytes are documented in formaldehyde-exposed students and
34 workers, confirming the genotoxic effects of formaldehyde in immune cells, from which
35 lymphomas and related hematopoietic diseases may arise (see Section 4.5.2,
36 Figure 4-32).

- 37 **b) Formaldehyde-induced protein-protein crosslinks may disrupt somatic-**
38 **hypermutation:** Although not as well studied as DNA-protein crosslinks, formaldehyde
39 also forms crosslinks between amino acids on proteins (see Section 4.5.3.1.3 for details).
40 Specific oncogenes for malignancies which arise from mature B-cells are linked to errors
41 in the process of somatic hyper-mutation (Greaves et al., 2004). If formaldehyde creates
42 protein crosslinks in competent B-cells which affects the process of DNA rearrangement,
43 formaldehyde may generate translocations and related oncogenes similar to those
44 observed in spontaneous B-cell malignancies.

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1 **Relevance to humans:** This hypothesis has not been tested in either exposed human or
2 animal test systems. However, the link between somatic-hypermutation and B-cell
3 oncogenes is well established and perturbation of this process by an exogenous agent is a
4 reasonable extension of the current understanding of the etiology of B-cell malignancies.

- 5 c) **Increased URT infections/viral reactivation:** Inhalation exposure to formaldehyde has
6 been shown to decrease the defenses of the body against infection through two
7 mechanisms: (1) damage to the protective mucous barrier and function of the mucociliary
8 apparatus in the nasal passages; and (2) localized immunosuppression (see
9 Section 4.5.3.1). These effects have been demonstrated in both exposed humans and
10 controlled animal experiments. Additionally, increased respiratory tract infections are
11 associated with formaldehyde exposure in several populations. Common viral agents
12 (e.g., Epstein barr virus) are known risk factors for malignancies which arise from mature
13 lymphocytes. Thus, increased URT infections or viral reactivation due to formaldehyde
14 exposure may influence the incidence of these cancers.

15 **Relevance to humans:** The potential role of increased URT infections and
16 immunosuppression at the portal of entry is considered to be relevant to humans. Data in
17 humans are available to support both key events in this MOA. Additionally, coexposure
18 to infectious agents (including viruses) would be expected in participants in an
19 epidemiological study, suggesting an MOA which acted in conjunction with infectious
20 agents may be relevant to agent-induced cancer. Therefore, immunotoxic actions by
21 formaldehyde which may increase URT infections, or viral-reactivation at the site of first
22 contact, could be acting in conjunction with viral agents to contribute, in part, to observed
23 associations between formaldehyde exposure and increased lymphoma and related
24 diseases.

25
26 **4.5.3.3.3. Summary and evaluation of hypothesized MOA(s) for Lymphohematopoietic**
27 **Malignancies.**

28 The well-documented direct toxic action of formaldehyde on cells at the site of first
29 contact is a general effect based on the reactivity of formaldehyde with cellular components
30 (e.g., proteins and DNA) (see Section 4.5.3.1). As a general effect, it is reasonable that these
31 toxic effects would be relevant to all cells which come into contact with formaldehyde. The
32 current debate regarding the biological plausibility of formaldehyde-induced
33 lymphohematopoietic malignancies centers around a perspective that the diseases within this
34 general grouping are systemic cancers arising only out of bone marrow toxicity (Heck et al.,
35 2006, Pyatt et al., 2008) and that it is implausible for formaldehyde to induce bone marrow
36 toxicity. The above MOA evaluation expands the current debate by considering the impact of
37 POE toxicity on elements of the immune system and cancers might arise from these cells (see
38 Section 4.5.3.3.2) and by presenting data which support the observation that formaldehyde is
39 associated with bone marrow toxicity and damage to circulating stem cells in exposed humans
40 (see Section 4.5.3.3.1).

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1 As significant increases in free formaldehyde in peripheral blood from exogenous
2 exposure has not been detected (Heck et al., 1987), it has been hypothesized that formaldehyde
3 does not transport and therefore cannot exert toxic effects outside of the tissues at the site of first
4 contact (Heck et al., 2006, Pyatt et al., 2008). In contrast to this hypothesis, at least one study by
5 Ward et al. (1983) in rats has shown that orally administered formaldehyde was able to induce
6 bone marrow genotoxicity, and also effects are seen in formaldehyde-exposed humans, which
7 indicate systemic effects on the hematopoietic system including reduced white blood cell counts,
8 clastogenic effects in peripheral blood lymphocytes and aneuploidy in circulating stem cells
9 (Tang et al., 2009, Zhang et al., 2010a; Section 4.5.3.1). These observed effects in humans are
10 consistent with agent-induced bone marrow toxicity and are observed with other well-studied
11 exogenous leukemogens (e.g., benzene and ionizing radiation). It is unknown if formaldehyde is
12 distributed systemically to exert its effects directly on cells in the bone marrow or if damage to
13 circulating stem cells or progenitor cells would be sufficient to result in the observed effects in
14 humans (Zhang et al., 2010b). Additional research is needed to better determine the potential for
15 systemic transport of formaldehyde considering both detection of its hydrated form (methylene
16 glycol) as well as formaldehyde protein adducts (e.g., formaldehyde-GSH, formaldehyde-Hb and
17 formaldehyde-albumin). Similarly the results of Zhang et al. (2010a) need to be extended
18 (analysis for additional chromosomal aberrations) and repeated. Although further evidence is
19 needed to better understand the hypothesized mechanisms for formaldehyde-induced effects on
20 hematopoietic stem cells, the observed hematologic effects in humans cannot be set aside.
21 Therefore, however unlikely, the current data support the biological plausibility of formaldehyde
22 effects on the hematopoietic system.

23 24 4.5.4. Hazard Characterization for Formaldehyde Carcinogenicity

25 ***Formaldehyde is Carcinogenic to Humans by the Inhalation Route of Exposure***

26 Human epidemiological evidence is sufficient to conclude a causal association between
27 formaldehyde exposure and nasopharyngeal cancer, nasal and paranasal cancer, all leukemias,
28 myeloid leukemia and lymphohematopoietic cancers as a group. Epidemiological evidence is
29 also strongly supportive of, but in itself not sufficient for, a conclusion of causal association for
30 other upper-respiratory tract cancers, Hodgkins lymphoma, or multiple myeloma. Animal
31 bioassays consistently demonstrate formaldehyde-induced nasal cancers in rodents which
32 provide strong support for the observed upper respiratory tract cancers in humans. Limited
33 evidence from animal bioassays is available to support the conclusion from human
34 epidemiologic data that formaldehyde causes some types of lymphohematopoietic cancers.

1 The epidemiologic evidence is sufficient to characterize the association between
2 formaldehyde exposure and nasopharyngeal cancer as causal in humans, based on the results
3 from a large and well-followed longitudinal cohort study of industrial workers and several case-
4 control studies (Hauptmann et al., 2004; Vaughan et al., 2000; West et al., 1993; Vaughan et al.,
5 1986b). The epidemiologic evidence of association between formaldehyde exposure and other
6 upper respiratory tract cancers (see Section 4.1.2.1.3) is consistent with, and supportive of, a
7 causal association but insufficient on its own to reach a causal conclusion. Case-control studies
8 have demonstrated associations between formaldehyde exposure and rare cancers of the URT.
9 Luce et al. (2002) evaluated pooled data from 12 case-control studies and demonstrated a
10 statistically significant increased risk between formaldehyde exposure and sinonasal cancer.
11 Hypopharyngeal cancer was linked with formaldehyde exposure with an OR of 3.78 (95% CI:
12 1.50–9.49) in another case-control study (Laforest et al., 2000). Hauptmann and colleagues
13 (2004) concluded that in spite of the small numbers of deaths from cancers of the URT, the
14 positive associations with average intensity and peak exposure were consistent with the
15 carcinogenicity of formaldehyde at these sites of first contact. The finding that formaldehyde
16 inhalation causes nasal squamous cell carcinoma in animals (see Section 4.2.1.2) further supports
17 a causal association of formaldehyde exposure and increased risk of upper respiratory tract
18 cancer in humans. Both humans and animals developed tumors within the upper respiratory
19 tract, the POE site expected to receive direct exposure to formaldehyde.

20 Also, overall, there is a consistent association between formaldehyde exposure and
21 various forms of lymphohematopoietic (LHP) cancers, with all leukemias, myeloid leukemia
22 specifically, Hodgkin’s lymphoma and multiple myeloma demonstrating the greatest strength
23 and consistency of results. Where exposure-response data exist, exposure-response trends have
24 been seen for all LHP malignancies, all leukemia, myeloid leukemia and Hodgkin’s lymphoma
25 (Pinkerton et al., 2004; Beane-Freeman et al., 2009). Taken together, the data demonstrate a
26 consistent association, across various worker populations, with the expected temporal association
27 to exposure and defined exposure–response relationships in two different worker cohorts. The
28 strongest associations tend to be with myeloid leukemia and Hodgkin’s lymphoma. The criterion
29 of reasonable biological plausibility is easily met for the majority of the diseases which
30 contribute to an observation of all LHP cancers, specifically the cancers derived from mature
31 lymphocytes. Although it is largely unknown how inhalation exposure to formaldehyde would
32 influence bone-marrow derived malignancies, new evidence supports formaldehyde-induced
33 bone marrow toxicity as well as damage to circulating stem cells which may be of importance to
34 formaldehyde’s leukemic potential (Zhang et al., 2010, Tang et al., 2009). Limited support for
35 the potential for formaldehyde-induced LHP cancer is found when considering animal bioassays,

1 where formaldehyde exposure influenced leukemia and lymphoma incidence in female animals
2 of two species (rats and mice) in a long-term bioassay (Battelle Laboratories, 1981).

4 4.6. SUSCEPTIBLE POPULATIONS

5 “Susceptible subpopulations” is used here to refer to factors, such as life stage, genetics,
6 health status, etc., that may predispose individuals to greater response to an exposure. This
7 greater response could be achieved either through differences in exposure to the chemical or
8 differences in underlying toxicokinetic (TK) and toxicodynamic (TD) differences between the
9 susceptible and other populations. For example, life stages may include the developing
10 individual before and after birth up to maturity (e.g., preconception, embryo, fetus, young child,
11 adolescent), adults, or aging individuals. Another susceptibility factor is genetics. Specifically,
12 susceptible subpopulations may also include people with specific genetic polymorphisms that
13 render them more vulnerable to a specific agent or people with specific diseases or pre-existing
14 conditions (e.g., asthmatics). The term may also refer to gender differences, lifestyle choices, or
15 nutritional state (U.S. EPA, 2002, Section 4.3.2.3).

16 A discussion of a comprehensive list of all possible susceptibility factors affecting
17 exposure and response to formaldehyde, or any chemical, is not possible. Therefore, the
18 discussion of susceptibility factors focuses on (1) factors hypothesized to be of importance to
19 formaldehyde; and (2) factors for which there are available formaldehyde data. A partial list of
20 these factors includes gender, genetic polymorphisms, preexisting disease status, nutritional
21 status, diet, and previous or concurrent exposures to other chemicals. Qualitatively, the presence
22 of multiple susceptibility factors will increase the variability that is seen in a population response
23 to formaldehyde toxicity.

25 4.6.1. Life Stages

26 Individuals at different life stages are physiologically, anatomically, and biochemically
27 different. Examples include physiological changes that occur through the lifespan (Selevan et
28 al., 2000). They may also have distinctive exposure pathways (i.e., transplacental, breast milk
29 ingestion), and exhibit differences in behavior (U.S. EPA, 2006b; NRC, 1993). Early life stages
30 (i.e., during development, prior to mature adulthood) and the later life stages (i.e., aging) differ
31 greatly from mature adulthood in body composition, organ function, and many other
32 physiological parameters that can impact the TK and/or TD of chemicals and their metabolites
33 (ILSI, 2003). This section presents and evaluates the pertinent published literature available to
34 assess whether and how individuals of differing life stages may respond differently to
35 formaldehyde.

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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

1 breathing scenarios, both of which appear likely to happen more regularly in children,
2 formaldehyde dose to the lung will be substantial.⁷

3 The toxicokinetic characteristics of formaldehyde are described in Chapter 3, with
4 absorption and distribution studies discussed in Sections 3.2 and 3.3. Studies to assess
5 differential absorption or distribution of formaldehyde in early life stages have not been
6 performed and represent a significant data gap. The metabolism of formaldehyde is described in
7 Section 3.4. Expression of the enzymes that metabolize formaldehyde (ALDH2 and FALDH,
8 and specifically ADH3) is known to be developmentally regulated and thus may alter the TK of
9 formaldehyde in early life stages. ADH3 is ubiquitously expressed and is present in the human
10 fetus, neonate, and 1- to 10-year-old children (Hines and McCarver, 2002; Estonius et al., 1996).
11 During early development in rodents, when neurulation first begins and forms collections of
12 somites along the neural tube, ADH3 activities are significantly lower (at 8–10 and 11–13
13 somite stages) and suggest a decreased ability to detoxify formaldehyde in the early embryo
14 (Harris et al., 2003). ADH mRNA expression levels appear to be age related, with decreased
15 expression of ADH common in premature neonates and infants up to 5 months old. Thereafter,
16 ADH expression increases and is dependent on body weight (Ginsberg et al., 2004). Benedetti et
17 al. (2007) reported that decreased ADH expression persisted until age 2 to 5 years. Westerlund
18 et al. (2005) tracked the ontogeny of ADH3 specifically and reported that ADH3 expression was
19 ubiquitous in mouse and rat embryos and was the only ADH enzyme to be consistently localized
20 to brain tissue, suggesting a housekeeping function. Thus, neonates and very young children
21 may have a decreased ability to metabolize formaldehyde due to differential expression of ADH3
22 in development compared that of with adults; however, activity levels of this enzyme and
23 alternate pathways specific to children are not available in the literature.

24 25 **4.6.1.1.2. *Life-stage exposure and adverse health outcomes.***

26 In general, exposure to toxic agents during early development (i.e, preconception,
27 prenatal stages, or postnatal development) may affect organ development and may also lead to
28 increased disease susceptibility later in life. Following early life stage exposure to
29 formaldehyde, a number of adverse health outcomes have been observed, including alterations in
30 the respiratory, reproductive, and neurological systems. For example, the developing respiratory
31 tract may be more vulnerable to insult compared with an adult respiratory tract, and thus,
32 increase the severity of response. The potential for reproductive and developmental toxicity of
33 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.

1 studies), while effects on the nervous system are discussed in Sections 4.1.1.6 and 4.2.6 (human
2 and animal studies, respectively). The specific case of formaldehyde exposure and pulmonary
3 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
4 summary of identified effects of formaldehyde that may indicate susceptibility during particular
5 life stages is provided below.

6 7 **4.6.1.1.2.1. Preconception.**

8 Exposure prior to conception may damage reproductive organs and/or germ cells that
9 could affect reproduction and/or damage the genetic makeup of the offspring. Effects on
10 reproduction are discussed in Sections 4.1.1.7 and 4.2.7. In summary, an epidemiological study
11 (Taskinen et al., 1999) reported significantly delayed conception among female workers exposed
12 to formaldehyde at average daily ambient formaldehyde levels; these effects could be consistent
13 with adverse effects on either preconceptional and/or gestational exposure. One animal study
14 (Maronpot et al., 1986) reported endometrial hypoplasia and lack of ovarian luteal tissue in
15 female mice exposed for 13 weeks to 40 ppm formaldehyde via inhalation, suggesting the
16 potential for treatment-related alterations to the female reproductive system. Since the exposure
17 was to the adult, the findings suggest that preconceptional formaldehyde exposure caused female
18 reproductive system effects that in turn could affect pregnancy. In the rodent study of Kitaev et
19 al. (1984), a three-fold increase in embryo degeneration on gestational days 2–3 was observed
20 after formaldehyde exposure to the dams during premating. Since the exposure was to the adult
21 in these three studies, the findings suggest that preconceptional formaldehyde exposure caused
22 female reproductive system effects and/or affected the gametes.

23 24 **4.6.1.1.2.2. Prenatal.**

25 A population-based study (Gražulevičiene et al., 1998) found an association between
26 atmospheric formaldehyde exposure and low birth weight, yielding an adjusted OR of 1.37 (95%
27 CI: 0.90–2.09). Three studies (Dulskiene and Gražulevičiene, 2005; Taskinen et al., 1994;
28 Hemminki et al., 1985) that examined the effect of occupational exposures on the incidence of
29 congenital malformation produced mixed results.

30 Results from Taskinen et al. (1999) support associations between formaldehyde exposure,
31 subfertility, and spontaneous abortion. Subfertility and spontaneous abortion are biologically
32 linked (subclinical pregnancy losses are increased among women with fertility problems) (Gray
33 and Wu, 2000; Hakim et al., 1995), and both subfertility and spontaneous abortion may be
34 related to sensitivity to environmental agents (Correa et al., 1996).

1 Two experimental animal studies (Martin, 1990; Saillenfait et al., 1989) evaluated a
2 standard battery of developmental endpoints following inhalation exposure on GDs 6–10, but
3 effects were minimal. Similarly, Chernoff and Kavlock (1982), Marks et al. (1980), and Hurni
4 and Ohder (1973) reported minimal reproductive or developmental effects in rodents in studies
5 in which dams were exposed orally during early gestation. When formaldehyde was
6 administered via inhalation throughout gestation in female rats, some developmental effects,
7 including increased pup weight and decreases in lung and liver weight in newborns, were
8 reported at 0.01 and 0.4 ppm (Senichenkova and Chebotar, 1996; Senichenkova, 1991; Kitaev et
9 al., 1984; Gofmekler and Bonashevskaya, 1969; Gofmekler, 1968; Pushkina et al., 1968). Two
10 studies also reported changes in motor activity in offspring of dams exposed via inhalation to 0.4
11 ppm formaldehyde during gestation (Senichenkova, 1991; Sheveleva, 1971).

12 13 **4.6.1.1.2.3. Postnatal.**

14 Following early life stage exposure to formaldehyde, a number of adverse postnatal
15 outcomes are possible, including effects on the developing and adult respiratory, reproductive,
16 and neurological systems. The potential for increased risk of childhood cancer is also discussed
17 below.

18
19 **4.6.1.1.2.3.1. Respiratory toxicity.** Formaldehyde is known to induce changes in
20 pulmonary function and cause pulmonary irritation in human studies (Rumchev et al., 2002;
21 Garrett et al., 1999; Krzyzanowski et al., 1990; Holmström et al., 1989; Holmström and
22 Wilhelmsson, 1988; Ritchie and Lehnen, 1987) and animal studies (Ohtsuka et al., 2003, 1997;
23 Riedel et al., 1996; Swiecichowski et al., 1993; Lee et al., 1984). Exposure to formaldehyde in
24 early life can cause damage to the lungs and permanently influence airway function, resulting in
25 increased vulnerability to toxicants later in life. Thus, young children may demonstrate
26 increased susceptibility to formaldehyde-related health effects. Krzyzanowski et al. (1990)
27 reported an association between physician-diagnosed asthma and chronic bronchitis in children
28 who lived in homes with formaldehyde levels that were higher than 60 ppb, after controlling for
29 socioeconomic status and ethnicity. Rumchev et al. (2002) reported a statistically significant
30 increased risk of asthma with increased residential concentrations of formaldehyde. Garrett et al.
31 (1999) found an increased association between bedroom concentration of formaldehyde and
32 increased risk of atopy in children. These studies suggest that formaldehyde exposure may
33 exacerbate responses in sensitive airways, particularly in children. Exacerbation of response has
34 also been noted in asthmatic adults and will be discussed below.

1 Another child-specific concern is that respiratory irritation may have greater impact on
2 lung function in children due to their smaller lung size; this is true even if the lung development
3 is normal. Irritation is commonly accompanied by inflammation, which can have a greater
4 impact on children’s airways because they are narrower than adult airways. Thus, less
5 inflammation is required for significant airway obstruction in children than in adults.
6

7 **4.6.1.1.2.3.2. *Developmental neurotoxicity.*** In neonatal exposure paradigms, changes in
8 brain structure (Sarsilmaz et al., 2007; Aslan et al., 2006), and brain chemistry (Songur et al.,
9 2008) were seen in young rats following inhalation exposures (6,000 or 12,000 ppb, 5 days per
10 week from postnatal day 0-30). In addition, Weiler and Apfelbach (1992) found juvenile
11 animals to be more sensitive to formaldehyde-induced changes in olfactory thresholds when
12 compared with adult animals (shifts in olfactory thresholds were greater when exposure [at
13 250 ppb] was initiated at PND 30 than at adult ages). These studies are consistent with the
14 hypothesis that early life exposure to formaldehyde can lead to long-lasting neurological effects.
15 Exposure levels in these studies (250–6,000 ppb) were in the same range as those producing the
16 behavioral effects in adults (as low as 100 ppb), but provide limited information regarding
17 relative sensitivity as no NOAELs were identified, and (with the exception of Weiler and
18 Apfelbach), similar parameters were not measured in adult animals using the same exposure
19 paradigms.
20

21 **4.6.1.2. *Later Life Stages***

22 In general, older adults may be at risk for increased susceptibility to exposure to
23 environmental chemicals by virtue of their slower metabolism and increased incidence of altered
24 health status (Benedetti et al., 2007; Ginsberg et al., 2005; U.S. EPA, 2005a). Additionally,
25 adverse effects of earlier exposure to some toxicants may be observed in older adults as a result
26 of latency in expression of the effect (Olsen et al., 1997; Sweeney et al., 1986). No studies have
27 examined the differential effects of formaldehyde exposure for elderly adults (>65 years old) as
28 compared to other age groups.
29

30 **4.6.1.3. *Conclusions on Life-Stage Susceptibility***

31 In summary, timing both of the exposure and of the assessment of health outcomes may
32 be important for understanding the relative risk of adverse effects from formaldehyde exposure
33 during different life stages. There are known developmental differences in kinetics across life
34 stages, including differences in enzymes involved in formaldehyde metabolism, but the
35 contribution of these differences to formaldehyde-related health effects is unknown. Similarly,

1 information regarding life-stage differences in respiratory physiology raises possible concern
2 regarding increased exposure to children, but studies for formaldehyde are not available.
3 Available data do support an increased risk for adverse effects on lung function in children. The
4 overall body of evidence shows some support, although minimal, for susceptibility in
5 reproductive or developmental endpoints associated with exposure to formaldehyde. Some
6 studies observed altered development of the nervous system following formaldehyde exposure
7 during early life. Older adults may be at risk for increased susceptibility to formaldehyde
8 because of slower metabolism and clearance rates. Elderly adults have an increased probability
9 of having both altered health status and altered metabolism, which could impact their ability to
10 process and recover from an adverse effect. The available data are consistent with some life-
11 stage susceptibility differences for formaldehyde at the level of TD or TK differences, the results
12 are nonetheless inconclusive due to the number of data gaps.

14 4.6.2. Health/Disease Status

15 The factor for which we have the greatest evidence is pre-existing disease, and
16 specifically asthma. Numerous studies have assessed the potential for increased susceptibility to
17 formaldehyde in asthmatics. Formaldehyde does not induce airway hyperreactivity directly
18 (Sheppard et al., 1984) and has not been shown to increase airway hyperreactivity in either
19 asthmatics or nonasthmatics (Pazdrak et al., 1993; Harving et al., 1991; Kulle et al., 1987).
20 Significantly decreased forced expiratory volume (FEV₁) measurements were reported among
21 asthmatics in two studies (Casset et al., 2006; Green et al., 1987), while others did not find any
22 significant change in FEV₁ following formaldehyde exposure (Ezratty et al., 2007; Frigas et al.,
23 1984).

24 A few available case reports of bronchial asthma do suggest direct respiratory tract
25 sensitization to formaldehyde gas (Lemiere et al., 1995; Burge et al., 1985; Hendrick et al., 1982;
26 Hendrick and Lane, 1977, 1975). All cases displayed marked changes in FEV₁ or pulmonary
27 airflow rate in response to acute challenges with formaldehyde gas at exposure levels <3 ppm.
28 Formaldehyde-induced IgE production has been reported in some studies (Vandenplas et al.,
29 2004; Wantke et al., 1996a).

30 There is a large quantity of human data providing evidence of an association between
31 formaldehyde exposure and increased incidence of asthma or exacerbation of asthmatic
32 responses in compromised individuals. For example, Krzyzanowski et al. (1990) reported an
33 association between physician-diagnosed asthma and chronic bronchitis in children who lived in
34 homes with formaldehyde levels that were higher than 60 ppb, after controlling for
35 socioeconomic status and ethnicity. Rumchev et al. (2002) reported a statistically significant

1 increased risk of asthma with increased residential concentrations of formaldehyde. Garrett et al.
2 (1999) found an increased association between bedroom concentration of formaldehyde and risk
3 of atopy in children. These studies suggest that formaldehyde exposure may exacerbate
4 responses in sensitive airways, particularly in children. Exacerbation of response has also been
5 noted in adults. Kriebel et al. (1993) reported a greater decrease in peak expiratory flow (PEF)
6 in asthmatic medical students (7.3% decrement) compared with nonasthmatic medical students
7 (2% decrement) after 2 weeks exposure to formaldehyde (average concentration 0.73 ppm) in an
8 anatomy lab. This effect does not appear to be immunogenic in nature (Fujimaki et al., 2004a;
9 Lee et al., 1984).

10 Several animal studies document increased airway resistance and bronchial constriction
11 following inhalation exposure to formaldehyde (Nielson et al., 1999; Swiecichowski et al., 1993;
12 Biagini et al., 1989; Amdur et al., 1960). Sadakane et al. (2002) demonstrated that formaldehyde
13 exposure exacerbated sensitization and challenge with a common dust mite allergen (Der f) as
14 measured by increased eosinophil infiltration into the interstitium around the bronchi and
15 bronchioles as well as goblet cell proliferation in the bronchial epithelium; they suggested that
16 formaldehyde exposure may aggravate eosinophilic infiltration and goblet cell proliferation that
17 accompanies allergic responses. The MOA underlying this response is unknown. These
18 decrements may occur indirectly in response to formaldehyde and may be mediated via
19 neurogenic potentiation (Sadakane et al., 2002; Riedel et al., 1996; Tarkowski and Gorski, 1995).
20 In particular, Tarkowski and Gorski (1995) suggest that formaldehyde may increase permeability
21 of respiratory epithelium and destruction of immunologic barriers. Thus, the respiratory tract
22 may become vulnerable to inhaled allergens after formaldehyde exposure (Tarkowski and
23 Gorski, 1995).

24 In summary, the data indicate that formaldehyde exposure can aggravate a type I
25 hypersensitivity response and that this hypersensitivity may in turn increase the susceptibility to
26 formaldehyde exposure in these individuals. Formaldehyde exposure may predetermine an
27 asthmatic phenotype or may induce new incidences of asthma via indirect mechanisms, though
28 definitive evidence and a proposed mechanism remain to be determined. Individuals that exhibit
29 chemically induced sensitivity and are exposed acutely or chronically to formaldehyde in
30 residential and occupational settings might exhibit adverse responses at lower concentrations of
31 formaldehyde than the average healthy person.

32 33 4.6.3. Nutritional Status

34 Limited available data indicate that certain types of malnutrition may increase
35 susceptibility to formaldehyde exposure. Senichenkova and Chebotar (1996) reported increased

1 fetal anomalies in fetuses from iron-deficient pregnant mice after formaldehyde exposure
2 compared with anemic mice that had not been exposed to formaldehyde. Forced iron reduction
3 (induced by addition of bipyridyl treatment in pregnant mice) *in utero* increased the overall
4 incidence of fetal anomalies when paired with formaldehyde exposure (Senichenkova and
5 Chebotar, 1996). The findings are difficult to evaluate due to poor reporting and have not been
6 substantiated by other laboratories.

8 4.6.4. Gender Differences

9 Males and females can differ greatly in body composition, organ function, and many
10 other physiological parameters that may influence the toxicokinetics of chemicals and their
11 metabolites in the body (Gochfeld, 2007; Gandhi et al., 2004).

12 The human epidemiology data set does not support any specific gender susceptibilities
13 for noncancer effects due to formaldehyde exposure. In general, data suggest that nonpregnant
14 women, on a per kg body weight basis, may have slightly lower air intake than men, which
15 would suggest that women may be less susceptible to inhaled pollutants like formaldehyde than
16 men, but this has not been investigated in the available formaldehyde literature.

17 A few isolated reports have investigated potential gender differences in development of
18 nasal pharyngeal carcinomas following exposure to formaldehyde. One case-control study
19 identified a higher OR for sinonasal adenocarcinomas in women (OR = 6.2 [95% CI: 2.2–19.7])
20 compared with the OR observed in men (OR = 3.0 [95% CI: 1.5–5.7]) following exposure to
21 formaldehyde (Luce et al., 2002). However, the overall body of evidence remains scant.

22 There are a few reports concerning differential formaldehyde-induced effects on the male
23 and female reproductive systems. Özen et al. (2002), Sarsilmaz et al. (1999), and Woutersen et
24 al. (1987) reported reduced Leydig cell numbers in adult male rats exposed by inhalation. In
25 female mice, inhalation exposure to formaldehyde resulted in endometrial hypoplasia and lack of
26 ovarian luteal tissue (Maronpot et al., 1986). The clinical significance of these effects in humans
27 is unknown, and due to limited data it is unclear whether the female or male reproductive system
28 is more susceptible to perturbation by formaldehyde.

30 4.6.5. Genetic Differences

31 There are some data for polymorphisms in humans that affect formaldehyde TK. As
32 discussed in Section 3.4, the primary metabolizing enzymes of formaldehyde are ALDH2 and
33 ADH3, with the latter enzyme considered more relevant to low exposures. Polymorphisms in
34 ALDH2 have been shown to have implications in human risk assessment, specifically in regard
35 to acetaldehyde metabolism (Ginsberg et al., 2002). Teng et al. (2001) demonstrated the

1 importance of ALDH2 for formaldehyde metabolism in rat hepatocytes at fairly high
2 formaldehyde concentrations (2.5 mM and greater). Cheng et al. (2008) investigated the
3 relationship between occupational exposure to formaldehyde and genetic polymorphisms of
4 ALDH2 and CYP2E1. There was a positive relationship between the concentration of formic
5 acid in the urine and ALDH2 genotypes ($\chi^2 = 9.241, p < 0.05$). Urinary formic acid
6 concentration may be affected by formaldehyde exposure concentration and ALDH2 genotype
7 (Cheng et al., 2008) for individuals that have high exposure levels. Thus, although ALDH2 may
8 not be involved in formaldehyde metabolism if exposure levels are low, polymorphisms of this
9 enzyme may lead to differences in response at higher exposure levels.

10 Wu et al. (2007) looked for and identified two SNPs in ADH3 among a population of
11 Mexican asthmatic children 4 to 17 years of age. Carrying one or two copies of the minor allele
12 for one SNP resulted in a decreased RR of asthma (RR = 0.66–0.77). For the second SNP,
13 homozygotes for the minor allele had an RR of 1.6 for asthma. The functional characteristics of
14 these SNPs are unknown. Studies evaluating whether any of the polymorphisms affect the
15 expression, regulation, stability, or activity of the enzyme *in vivo* are lacking; therefore, the
16 relative susceptibility of individuals with different polymorphisms cannot be characterized at this
17 time.

18 One study (Hedberg et al., 2001) identified three polymorphisms in human ADH3
19 involving four base-pair substitutions in the promoter region of which one (C→T) showed
20 reduced activity (~50–70% of control). Hedberg et al. (2001) reported differences in allele
21 frequencies among Chinese, Spanish, and Swedish groups, consisting of Asian-Caucasian
22 differences and ethnic subgroups among Caucasians. Their results suggest that a small
23 percentage of Caucasians may have decreased ADH3 expression and thus, be more susceptible to
24 formaldehyde exposure. Additional studies to validate these findings have not been performed.

25 The relative activity level of these enzymes may also impact the metabolism of
26 formaldehyde. In pharmacokinetic studies, deletion of ADH3 increased the sensitivity of mice to
27 formaldehyde (Deltour et al., 1999) and was deleterious to yeast (Achkor et al., 2003). These
28 results suggest that deficiencies in ADH3 may confer an increased susceptibility to formaldehyde
29 toxicity (Teng et al., 2001). The importance of properly functioning enzymes also suggests that
30 genetic differences in ADH3 or ALDH2 may affect the response to formaldehyde exposure.
31 However, comparable human data are not available.

32 Race/ethnicity may be a surrogate for genetic differences but racial or ethnic groups may
33 also reflect socioeconomic, and/or cultural factors that are distinct from genetics. Possible ethnic
34 differences may be related to genetic polymorphisms of enzymes ALDH2 and ADH3, relevant
35 for formaldehyde metabolism. ALDH2 variants, present primarily in East Asians, are known to

1 have protective effects against alcoholism but were not found in the people of Indo-Trinidadian
2 descent (Moore et al., 2007) or in American Indians or Alaska natives (Ehlers, 2007). However,
3 there is no direct evidence to associate these variants to differential susceptibility to
4 formaldehyde exposure, nor is there direct evidence to associate these ethnic groups specifically
5 with differential susceptibility to formaldehyde. Further, no studies have specifically assessed
6 ethnic variability in responses to formaldehyde.

7 There are complex pathways through which genetic polymorphisms in ADH3 can
8 potentially affect differential susceptibility to formaldehyde. Firstly, ADH3 is central to the
9 metabolism of formaldehyde. However, ADH3 itself may indirectly contribute to the adverse
10 effects of formaldehyde on pulmonary physiology (Thompson et al., 2009; Staab et al., 2008a, b;
11 Thompson and Grafström, 2008). Exposure to formaldehyde is itself thought to alter the activity
12 of ADH3 resulting in the perturbation of critical metabolic pathways. ADH3 participates in the
13 oxidation of retinol and long-chain primary alcohols, as well as the reduction of S-
14 nitrosoglutathione (GSNO). The activity of ADH3 toward some of these substrates has been
15 shown to be significantly increased in the presence of formaldehyde. ADH3 has recently also
16 been shown to contribute to NO signaling through its dual role in metabolizing GSNO, an
17 endogenous bronchodilator and reservoir of NO (Staab et al., 2008a; Hess et al., 2005; Jensen et
18 al. 1998). Through its regulatory function on GSNO, ADH3 may thus play a central role in
19 regulating bronchial tone allergen-induced hyperresponsiveness (Gerard, 2005; Que et al., 2005).
20 As concluded by California Environmental Protection Agency (CalEPA) (2008), “the
21 dysregulation of NO by formaldehyde [in this manner] helps to explain the variety and
22 variability in the toxic manifestations following formaldehyde inhalation.”
23

24 4.6.6. Coexposures

25 **4.6.6.1. Cumulative Risk**

26 When considering health risks, it is important to consider the impact of coexposures to
27 other agents that may interact with the chemical under evaluation. Coexposure to other
28 pollutants, particularly those that produce some of the same metabolites and similar health
29 effects as formaldehyde, is likely to occur in both occupational and nonoccupational settings.

30 Due to effects on metabolic enzymes (inducing and/or inhibition) as well as direct effects
31 on organ system function, coexposures may alter the way in which formaldehyde is metabolized
32 and cleared from the body. Inhibition or induction of the enzymes responsible for metabolism of
33 chemicals may alter susceptibility to toxicity (Lash and Parker, 2001; IARC, 1995; U.S. EPA,
34 1985a). Smokers may be at increased risk for effects of formaldehyde exposure, because
35 formaldehyde is one of the components of cigarette smoke and is likely to heighten the point-of-

1 entry effect when combined with occupational or residential exposures to inhaled formaldehyde.
2 However, no evidence is available to evaluate the potential aggregate effects.

3 4 **4.6.6.2. Aggregate Exposure**

5 In addition, multiple routes of exposure to a single agent may increase the cumulative
6 risk by increasing the overall body burden of the chemical. A human aggregate exposure model
7 developed by McKone and Daniels (1991) incorporated likely exposures from air, water, and soil
8 media through inhalation, ingestion, and dermal contact. The authors hypothesized that the
9 aggregate exposure could be age-dependent but did not present any data for persons of differing
10 life stages. The role of multiple exposures on different genders, genetic susceptibility, or altered
11 health and nutrition status has not been investigated. The available database regarding the
12 potential for multiple routes of exposure (or aggregate exposure) formaldehyde is limited.

13 Guseva (1972) specifically assessed the reproductive and developmental effects caused
14 by coexposure to formaldehyde via both inhalation (0.25 mg/m³) and ingestion (0.01 mg/L)
15 routes in male rats. The authors reported reduced nucleic acid levels in testes to 88 and 92% of
16 controls, which suggests a possible toxic gonadotropic effect. The ability of male rats (receiving
17 combined exposure to formaldehyde at a low concentration level for a long period of time) to
18 reproduce was preserved since all the cohabited females were impregnated. The number and
19 weight of the fetuses and newborn rat pups in the experimental coexposure groups did not differ
20 substantially from those figures observed in the control group. No developmental defects or
21 anomalies were observed in the offspring for up to 1 month postnatally. Thus, at low exposures,
22 the reproductive effects due to combined ingestion and inhalation exposure are unknown.

23 24 **4.6.7. Uncertainties of Database**

25 There is a need to better characterize the implications of formaldehyde exposures to
26 susceptible populations. A number of areas where the database is currently insufficient are
27 identified below.

28 29 **4.6.7.1. Uncertainties of Exposure**

30 Although information exists on early life exposure to formaldehyde, a number of
31 uncertainties regarding children's susceptibility remain. First, inhalation is believed to be of
32 most concern for formaldehyde, since formaldehyde vapors are released from insulation or from
33 ambient sources of formaldehyde, including secondary production from other pollutants involved
34 in photo-oxidant reactions. Any additional pathways of exposure for children have not been
35 characterized. Since formaldehyde is nearly ubiquitous in the environment, it is difficult to

1 quantify the total exposure. Second, children have different respiratory, metabolic, and activity
2 rates compared with healthy adults, potentially influencing ADME and target tissue exposure to
3 formaldehyde. However, studies to identify the specific changes in absorption of formaldehyde
4 and its metabolites across developmental stages and across organs have not been performed. In
5 addition, exposure prenatally may be altered based on whether formaldehyde or its metabolites
6 pass through the placenta, but placental transfer data are not available. Third, no quantitative
7 models have been developed to characterize these differences for formaldehyde. Formaldehyde-
8 specific PBPK models and their validation will aid in understanding the uncertainties associated
9 with formaldehyde exposure in children.

10 Given the large proportion of time that most individuals in the U.S. spend indoors,
11 exposure scenarios where indoor concentrations to formaldehyde are high (e.g., in homes or in
12 trailers; see Section 2.3.1) may play a significant role and may be of particular concern to the
13 elderly or health-impaired individuals who spend relatively more time at home. Further
14 evaluation of the effects of coexposures and pathways of exposure and aggregate risk is needed.
15 An estimate of the multiple exposure pathways is needed to know where along the dose-response
16 curve to place an incremental exposure to formaldehyde.

17 18 **4.6.7.2. *Uncertainties of Effect***

19 Studies specifically designed to evaluate effects after early and later life stage exposure
20 are needed in order to more fully characterize potential life-stage-related differences in
21 formaldehyde toxicity, including the defining of critical windows during development. For
22 example, life-stage-specific neurotoxic and pulmonary effects, particularly in the developing
23 fetus, need further evaluation. The preconceptional period may be a critical window for
24 formaldehyde exposure and reproductive and developmental effects, based on rodent studies of
25 reproductive, embryonic and gamete effects. Data specific to the carcinogenic effects of
26 formaldehyde exposure during early life stages do not exist. The reduction in fertility seen in
27 some studies (Gray and Wu, 2000; Taskinen et al., 1999; Hakim et al., 1995) is not adequately
28 described and a well-established MOA has not been identified, but some have been hypothesized
29 including altered sperm quality (Özen et al., 2002; Sarsilmaz et al., 1999; Woutersen et al.,
30 1987). Further, spontaneous abortion/fetal loss occurring early in gestation, prior to maternal
31 knowledge of the pregnancy, can lead to misclassification of the effect as infertility (see Sections
32 4.1.1.7 and 4.2.7).

33 More research is needed to clarify the role of genetic polymorphisms in formaldehyde
34 metabolism. Similarly, data gaps pertaining to gender differences remain. A potential impact of
35 nutritional status and iron deficiency on formaldehyde toxicity needs further investigation.

1 A fair body of evidence suggests that asthmatics are more susceptible to formaldehyde exposure
2 than the general population, however, the mechanism of action for this increased susceptibility is
3 unknown.

4 In the studies discussed above, there are a number of examples of studies that assessed
5 multiple susceptibility factors that are worth noting. For example, the Krzyzanowski et al.
6 (1990) study reported asthma and chronic bronchitis cases for two interacting potential
7 susceptible groups, in children and those with high exposure (due to living in homes with
8 formaldehyde levels that were higher than 60 ppb). Similarly, the Garrett et al. (1999) study
9 assessed the same two interacting potential susceptible groups.

10 The study of Senichenkova and Chebotar (1996) assessed developmental effects in
11 mouse fetuses after *in utero* iron-deficiency and formaldehyde exposure. Thus, the study
12 findings must be considered in light of possible interactions between life stage exposure
13 differences and nutritional status differences.

14 Studies to understand the nature of the interactions between the various susceptibility
15 factors for formaldehyde have not been performed.

17 4.6.8. Summary of Potential Susceptibility

18 There is some evidence to demonstrate susceptibility for various populations exposed to
19 formaldehyde. Available data are summarized in Table 4-95 where formaldehyde susceptibility
20 factors are presented by those with data for increased formaldehyde susceptibility and those with
21 data for differences but with an unknown impact on formaldehyde susceptibility.

22 Exposure to formaldehyde during early developmental and later life stages may be of
23 concern. However, human exposure to the developing fetus is unknown since it is not known
24 whether formaldehyde or one of its metabolites crosses the placenta. However, there is very
25 limited life-stage-specific information regarding the TK of formaldehyde. Life-stage-specific
26 TK has not been characterized, and, thus, no PBPK models exist to effectively evaluate the risk
27 to early life stages. Children may be more susceptible to noncancer health effects as a result of
28 inhalation exposure to formaldehyde due to increased respiratory rates. There are no studies to
29 evaluate whether formaldehyde exposure in early life (e.g., pregnancy) is associated with an
30 increased risk of childhood cancer.

31 The weight of evidence supports a plausible association between formaldehyde exposure
32 and aggravated asthmatic responses in humans and this association is corroborated by limited
33 evidence from animal studies. Formaldehyde does not appear to directly induce airway
34 hyperreactivity but may sensitize airways to subsequent exposures. One issue in interpreting the
35 available studies that assessed the relationship between asthma and formaldehyde could not
36

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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
<p>Life stage</p> <ul style="list-style-type: none"> ▪ Preconception ▪ Prenatal ▪ Postnatal 	<p>Developmental effects reported suggesting that critical windows of exposure may be relevant:</p> <ul style="list-style-type: none"> ▪ Reproductive outcomes (Taskinen et al., 1999; Maronpot et al., 1986) ▪ Embryo effects (Kitaev et al., 1984) ▪ Structural- and functional developmental outcomes (Martin, 1990; Saillenfait et al., 1989; Sheveleva, 1971; Seninchenkova, 1991) ▪ Lung function outcome (Krzyzanowski et al., 1990; Rumchev et al., 2002; Garrett et al., 1999) ▪ Developmental neurotoxicity (Weiler and Apfelbach, 1992) 	<ul style="list-style-type: none"> ▪ 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) ▪ 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)
<p>Disease status</p>	<ul style="list-style-type: none"> ▪ 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) 	<ul style="list-style-type: none"> ▪ 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)
<p>Nutritional status/diet</p>	<ul style="list-style-type: none"> ▪ Iron-deficiency <i>in utero</i> (Seninchenkova and Chebotar, 1996). 	
<p>Genetics</p> <ul style="list-style-type: none"> ▪ Polymorphisms 	<ul style="list-style-type: none"> ▪ 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) 	<ul style="list-style-type: none"> ▪ Differences among ADH3 alleles and asthma outcome (Wu et al., 2007) ▪ Differences among ethnic groups in ADH3 alleles (Hedberg et al., 2001)
<p>Gender</p>		<ul style="list-style-type: none"> ▪ Gender differences in incidence of nasopharyngeal carcinoma following formaldehyde exposure (Luce et al., 2002)

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6

1 distinguish between the cases of asthma that were due to earlier formaldehyde exposure vs. those
2 without a direct link to formaldehyde exposure.

3 No direct link exists between formaldehyde exposure and differential susceptibility in
4 different ethnic groups, although genetic polymorphisms in the enzymes involved with
5 formaldehyde metabolism, ADH3 and ALDH2, provide some support for differential
6 susceptibility to alcoholism in a number of ethnic groups. The evidence for differential gender
7 responses to formaldehyde exposure is equivocal. Coexposures may result in altered metabolism
8 and clearance, but there is no evidence that coexposures are a critical part of formaldehyde-
9 mediated differential susceptibility.

10 Thus, given the available data, increased susceptibility to adverse effects of
11 formaldehyde is most strongly supported for three populations: (1) Preconception and perinatal
12 exposure based on reproductive and developmental effects; (2) children, whose exposure may be
13 higher by virtue of their increased activity level and respiratory rate; and (3) asthmatics who may
14 exhibit exacerbation of response to formaldehyde.

- End of Volume II -