TOXICOLOGICAL REVIEW

OF

CHLOROPRENE

(CAS No. 126-99-8)

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

October 2000

NOTICE

This document is an external review draft. It has not been formally released by the U.S. Environmental Protection Agency and should not at this stage be construed to represent Agency position on this chemical. It is being circulated for review of its technical accuracy and science policy implications.

U.S. Environmental Protection Agency
Washington, DC

DISCLAIMER

This document is a preliminary draft for review purposes only and does not constitute U.S. Environmental Protection Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use. Note: This document may undergo revisions in the future. The most up-to-date version will be made available electronically via the IRIS Home Page at http://www.epa.gov/iris.

CONTENTS—TOXICOLOGICAL REVIEW FOR CHLOROPRENE
(CAS No. 126-99-8)

CONTENTS (continued)

FOREWORD

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

In Section 6, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose-response. Matters considered in this characterization include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's Risk Information Hotline at 513-569-7254.

AUTHORS, CONTRIBUTORS, AND REVIEWERS

Chemical Manager

Mark Greenberg, U.S. Environmental Protection Agency, National Center for Environmental Assessment, Research Triangle Park, NC

Authors

Steven Donkin, Ph.D.
Sciences International, Alexandria, VA

Karen Hogan, Ph.D.
National Center for Environmental Assessment, U.S. Environmental Protection Agency, Washington, DC

Mark Greenberg
U.S. Environmental Protection Agency, National Center for Environmental Assessment, Research Triangle Park, NC

Internal U.S. EPA Reviewers

Jennifer Jinot, Ph.D.
National Center for Environmental Assessment, U.S. Environmental Protection Agency, Washington, DC

Aparna Koppikar, M.D.
National Center for Environmental Assessment, U.S. Environmental Protection Agency, Washington, DC

Lawrence Valcovic, Ph.D.
National Center for Environmental Assessment, Washington, DC

1. INTRODUCTION

This document presents background and justification for the hazard and dose-response assessment summaries in EPA's Integrated Risk Information System (IRIS). IRIS summaries may include an oral reference dose (RfD), inhalation reference concentration (RfC) and a carcinogenicity assessment.

The RfD and RfC provide quantitative information for noncancer dose-response assessments. The RfC is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis but may not exist for other toxic effects such as some carcinogenic responses. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer effects during a lifetime. The inhalation RfC is analogous to the oral RfD, but provides respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). It is generally expressed in units of mg/m^3.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral exposure and inhalation exposure. The information includes a weight-of-evidence judgement of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways. The *slope factor* is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg/day. The *unit risk* is the quantitative estimate in terms of either risk per ∶/L drinking water or risk per concentration providing cancer risks of 1 in 100,000; or 1 in 1,000,000.

Development of these hazard identification and dose-reponse assessments for chloroprene has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines that were used in the development of this assessment may include the following: the Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986a), Guidelines for the Health Risk Assessment of Chemical Mixtures (U.S. EPA, 1986b), Guidelines for Mutagenecity Risk Assessment (U.S. EPA, 1986C), Guidelines for Developmental Toxicity Risk Assessment (U.S. EPA, 1991), Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996a), Guidelines for Reproductive Toxicity Risk Assessment (U.S. EPA, 1996b), and Guidelines for Neurotoxicity Risk Assessment (U.S. EPA, 1998); (proposed) Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity (U.S. EPA, 1994a); Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (U.S. EPA, 1994b); Peer Review and Peer Involvement at the U.S. Environmental Protection Agency (U.S. EPA, 1994c); Use of the Benchmark Dose Approach in Health Risk Assessment (U.S. EPA, 1995b); Science Policy Council Handbook: Peer Review (U.S. EPA, 1998b); and memorandum from EPA Administrator, Carol Browner, dated March 21, 1995, Subject: Guidance on Risk Characterization.

Literature search strategy employed for this compound were based on the CASRN and at least one common name. At a minimum, the following databases were searched: RTECS, HSDB, TSCATS, CCRIS, GENETOX, EMIC, EMICBACK, DART, ETICBACK, TOXLINE, CANCER LINE, MEDLINE, and MEDLINE backfiles. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

$-Chloroprene ($C_4H_5Cl$) (hereafter referred to as chloroprene) is a volatile, flammable liquid monomer used exclusively in the manufacture of polychloroprene or neoprene rubber, the latter used to make diverse products such as tires, wire coatings, tubing, etc. While 90% of chloroprene is used to make the solid, polychloroprene, about 10% is converted to polychloroprene latex, a colloidal suspension of polychloroprene in water (IARC, 1999). Occupational exposure potential to chloroprene (2-chloro-1,3-butadiene) is confined to two facilities in the United States in which chloroprene is produced and converted to polychloroprene (both in extruded form and as a colloidal suspension (Lynch, 1999, personal communication). Any releases to the environment would be from these facilities. However, no measurements of chloroprene in ambient air have been made.

The starting material for the synthesis of chloroprene is 1,3-butadiene. Chloroprene is also a structural analogue of isoprene (2-methyl 1,3-butadiene) and resembles vinyl chloride as far as having a single carbon-bonded chlorine and a double-bonded carbon (alkene) backbone. However, whereas vinyl chloride contains only two carbons double-bonded to each other, chloroprene contains four carbons arranged with two double bonds (see Figure 1). Being volatile and highly reactive; chloroprene is not expected to bioaccumulate or persist in the environment (U.S. EPA, 1985).

Because of its high vapor pressure (174 mm Hg at 20NC), chloroprene is expected to readily evaporate from water and solid surfaces (U.S. EPA, 1985). Chloroprene vapor has an estimated ionization potential of 8.95±0.05 eV, and an estimated half-life in the atmosphere of less than 20 hours (Grosjean, 1990). Reactions with OH (to produce formaldehyde), O_3, and NO_3 are the expected pathways of removal, although no experimental data exist (Grosjean, 1991).

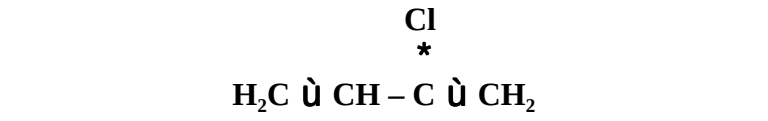

Figure 1. Structure of chloroprene.

Of particular relevance to any toxicological studies involving chloroprene is its propensity to oxidize and form dimers and other oxygenated species unless stabilizers are added. Uninhibited chloroprene must be stored under nitrogen at low temperatures (e.g., -20C). When bulk chloroprene with 5% n-octane added as an internal standard was stored at 55C for up to 6 hours, dimer content increased 62% and chloroprene monomer decreased 22% (NTP, 1996). Because these reaction products, if formed, may themselves account for the toxicity observed, toxicological studies that do not report storage or generation conditions may yield results that are suspect with relevance to chloroprene monomer. The toxicological studies in this Toxicological Review include a discussion of this aspect of chloroprene chemistry. A discussion of the polymerization process has been reported by Nystrom (1948), Stewart (1971), and in the Kirk-Othmer Encyclopedia of Chemical Technology (1993). Additional information on production and use has been reported by IARC (1999). Structures have been proposed for some of the chloroprene dimers (Stewart, 1971); some dimers result upon reaction at room temperature while others result after prolonged heating

In addition to volatilization, the potential fate of chloroprene that is released to soil is to leach into groundwater (HSDB, 1999). Breakdown via hydrolysis is not likely. It is only partially soluble in water (IARC, 1979). Chloroprene that is released to the water may only moderately adsorb to suspended sediments or particles, and there will be little bioaccumulation in aquatic organisms (K_{ow} = 2.06).

Table 1 presents some chemical and physical properties of chloroprene.

Table 1. Chemical and physical properties of chloroprene

Boiling point	59.4°C
Melting point	-130°C
Molecular weight	88.54
Density	0.9583
Log K_{OW}	2.06
Vapor pressure	174 mm Hg at 20°C
Henry's Law constant	3.2×10^{-2} atm-cu/mole at 25°C

Sources: HSDB, 1999; IARC, 1979.

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

3.1. ABSORPTION

Although no data exist on the absorption kinetics of chloroprene, it is assumed that absorption can occur through the lungs, gastrointestinal tract, or skin.

3.2. DISTRIBUTION

The many target sites exhibiting effects of chloroprene exposure (described elsewhere in this document) are evidence that distribution of absorbed chloroprene and/or its metabolic products is widespread within the body.

3.3. METABOLISM

A considerable number of older reports (1950-1973), principally of East European origin, appear to suggest a variety of effects of chloroprene on biochemical and metabolic parameters. References for these scientific articles were cited by Haley (1978). These reports have not been evaluated in this Toxicological Review, in part, because they are not in English and lack experimental details. Although there is very limited recent information about metabolism of chloroprene, it has been postulated to be mediated by the hepatic mixed-function oxidase system, with the production of epoxide intermediates (Haley, 1978). These intermediates may be then detoxified via the glutathione (GSH)-conjugation pathway, resulting in the excretion of conjugates in the urine. This hypothesis is supported, in part, by the results of a study in which when male Wistar rats were administered 100 or 200 mg/kg chloroprene by gavage, rapid depletion of hepatic GSH and a dose-dependent increase in excreted urinary thioethers (presumably GSH-conjugates) were observed (Summer and Greim, 1980). A dose-dependent depletion of GSH was seen as well as in isolated rat hepatocytes treated with chloroprene in this study. Pretreatment of rats or hepatocytes with phenobarbital or polychlorinated biphenyl congener mixture (Clophen A50), which induce the mixed-function oxidase enzymes, enhanced the GSH depletion effect. Additionally, metabolism may be similar to the biotransformation of butadiene, known to result in reactive epoxides (Himmelstein et al., 1996) and undergo bioactivation via CYP2E1 (Nieusma et al., 1998). Recently, in chamber studies with the B_6C_3 F_1 mouse and the Sprague-Dawley rat, Richardson et al. (1999) observed species differences in the nature of urinary metabolites excreted which led to investigators to conclude that the rat and mouse may metabolize butadiene by different metabolic pathways. Given the close structural similarity between chloroprene and butadiene, similar species differences may be involved in chloroprene metabolism.

Ongoing studies are examining if metabolic pathways of chloroprene by rodent species are similar to or different than those in humans. *In vitro* studies with rat, mouse and human microsomes recently initiated by Himmelstein et al.(2000a) indicate that chloroprene monoepoxide, a rather stable substance, is a principal chloroprene metabolite. Preliminary results indicate that the Fischer rat produces more monoepoxide than either the B6C3F_1 mouse or the Wistar rat. Human microsomes treated with chloroprene produced about 10-fold less monoepoxide than the Fischer rat, and about 4-fold less than the Wistar. However, the monoepoxide has not been detected in blood from the Fisher and Wistar exposed *in vivo.* Incubation of chloroprene monoepoxide with microsomal liver preparations indicates that hydrolysis of the epoxide is faster with hamster and human than with the mouse or rat (Himmelstein et al., 2000a). Hydrolysis was reduced upon inhibition of epoxide hydrolase. The

rate of intrinsic hepatic clearance of chloroprene epoxide by human liver microsomes is more similar to that of the Fischer or Wistar rat than it is to the mouse (most rapid) or hamster (least rapid). Subsequent reaction with GSH after epoxide hydrolysis is most rapid with hamster preparations compared to the rat or mouse; human microsomal preparations have not as yet been tested. Chloroprene metabolism does not involve direct conjugation with GSH.

Vitamin E was found to be protective against chloroprene-induced liver damage in rats (Liu et al., 1995). A single oral dose (150 mg/kg) of vitamin E, an antioxidant, administered 30 minutes prior to starting a 3-week, 80 mg/kg/day chloroprene oral exposure regime resulted in substantially decreased severity of necrosis seen in subsequently examined hepatocytes, compared with rats not pretreated with vitamin E. Indicators of oxidative stress and disrupted Ca^{2+} homeostasis were also increased by chloroprene treatment, but these effects were reduced by vitamin E pretreatment. Cytochrome P450 level and aminopyrine demethylase activity in the S9 fraction were also increased by vitamin E pretreatment, presumably ameliorating chloroprene's inhibitive effect on these enzymes and thus protecting the liver from damage. Hepatic lipid peroxidation and perturbed Ca^{2+} homeostasis were noted in rats administered chloroprene via intraperitoneal injection for 21 days, but pretreatment with vitamin E was again protective against these effects (Zhang et al., 1996). These observations, along with the observed protective effects of Aroclor 1254 (a mixed-function oxidase inducer) (Plugge and Jaeger, 1979), lend support to the proposed role of the mixed-function oxidase system as a detoxification pathway for liver effects.

3.4. EXCRETION

Although there is limited information, excretion of chloroprene appears to be rapid. Male Wistar rats that were administered 100 or 200 mg/kg chloroprene by gavage exhibited a dose-dependent, non-linear increase in excreted urinary thioethers (presumably glutathione-conjugates) which reached a threshold at 24 hours after dosing (Summer and Greim, 1980). In CD rats administered 40 mg chloroprene/kg in corn oil, no urinary metabolites were detected (Himmelstein, 2000).

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS—EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS

The reader should note that some studies that typically have associated chloroprene with potential health effects in humans have also involved exposure to polychloroprene, used in the manufacture of formed products.

4.1.1. Reproductive/Developmental

Sanotskii (1976) summarized an earlier Russian occupational study which reported reproductive effects among 143 male chloroprene workers. When compared with 118 unexposed controls, the cohort exhibited an increased incidence of "disturbed" sperm function and morphology, as well as an increased incidence of spontaneous abortion among workers' wives. This study has been questioned because of inadequate reporting of experimental details, and the fact that no subsequent studies replicating the results have been reported (Savitz et al., 1994; Schrag and Dixon, 1985). It also is unclear if these workers were involved in chloroprene production or in the use of polychloroprene. Because of the lack of details concerning storage conditions, analytical techniques, and chemical characterization, the information reported by Sanotskii (1976) are unreliable.

Roeleveld et al. (1990) reviewed studies that examined neurodevelopmental toxicity in children of parents occupationally exposed to various chemicals. However, the association between chloroprene exposure and teratogenic effects was deemed inconclusive.

4.1.2. Dermal

Alopecia (hair loss) from the scalp has been reported among men occupationally exposed to chloroprene during the manufacture of polychloroprene (Amblard et al., 1974; Ritter and Carter, 1948; Nystrom, 1948). This effect is temporary and was reported to be reversible upon cessation of exposure (Schwartz, 1945). Alopecia was a concentration-related effect of chloroprene exposure in the Wistar rat (Trochimowicz et al., 1998). This study is described in section 4.2.2.

4.1.3. Hematologic

It was reported that a Chinese study (cited by Dong et al.,1989) involving a micronucleus test of peripheral blood erythrocytes in chloroprene workers was positive. It is not known if these workers were exposed to chloroprene monomer because this report (Zhang et al., 1985) has not been translated.

4.1.4. Hepatic

A study of workers at a chloroprene polymerization plant evaluated the biochemical and hematological status of the following cohorts: 283 "never-exposed" workers, whose assigned work area was away from the chloroprene polymerization process; 227 "previously-exposed" workers, who were assigned to the polymerization area in the past, but were not currently assigned there; and 336 "currently-exposed" workers, who currently worked in the polymerization area (Gooch and Hawn, 1981). Cohorts were also subdivided by duration of exposure and level of potential exposure ("high," "moderate," "low," or "varied") for cross-comparisons. No statistically significant effects on a variety of biochemical and hematological

parameters were seen that could be associated with exposure to chloroprene. Paired analysis of workers before and after assignment to a potentially high-exposure area revealed significant changes only in serum glucose, cholesterol, and lactate dehydrogenase levels.

Ward et al. (1980) examined the potential hepatoxicity among chloroprene/polychloroprene production workers at a chemical plant in Texas. A sample of 81 individuals out of 225 workers were examined. Indices of hepatic function included liver enzyme activities (serum glutamate-oxaloacetate transaminase, serum glutamate-pyruvate transaminase, gamma glutamyl transpeptidase, alkaline phosphatase, cholinesterase), bilirubin levels (total and direct) and determination of prothrombin time. The study authors reported that four individuals in the sample exhibited clinically significant abnormalities. The increases in liver enzyme activities were found to be significantly related to alcohol consumption. However, the authors noted "some trend toward increased values among workers in the high exposure areas," and concluded that the results suggest that exposure to chloroprene may contribute to liver function abnormalities and that individuals who consume alcohol may be particularly at risk. This study was also limited by a lack of adequate exposure data, and the possible exposure of workers to chemicals other than chloroprene.

4.1.5. Cancer

Both case-control and cohort analyses, described as a preliminary trial, were performed using data on cancer deaths occurring between 1969 and 1983 among chloroprene/polychloroprene (neoprene) production workers in China (Shouqi et al., 1989). Most workers were reported to have been associated with chloroprene exposure since 1952. Wage roll workers were categorized according to likely exposure to chloroprene as determined by their occupations and opinions of workers and administrators as to levels of exposure. Those who could not be categorized were not included. The occupations included were the monomer workshop, the polymer workshop, and the laboratory. The only other known or possible carcinogens to which workers were reported to have been potentially exposed were benzene and anti-agar D, but exposure to these chemicals was reported to be extremely limited. Causes of death were reported to be ascertained from the medical records in city general and cancer hospitals, and it appears that histologic confirmation of the diagnoses was not made.

In the case-control study, 54/55 cancer deaths among plant workers (16 males had histories of chloroprene exposure with a median of 11 years) were matched with 54 noncancer deaths among plant workers according to sex, age at death, and date of death. A significantly increased risk of cancer death was reported to be associated with chloroprene exposure; 16/54 of the cancer deaths were attributed to chloroprene exposure, but only 4/54 of the noncancer deaths occurred among chloroprene workers. A statistically significant odds ratio of 13 ($P<0.005$) for all cancers was calculated from the paired data. The average age of death from cancer among workers exposed to chloroprene (41.95 ± 5.5 years) was significantly ($P<0.001$) less than that of unexposed workers (54.6 ± 9.5 years). There was no indication that alcohol use or smoking was considered in establishing the paired sets and thus represent limitations in interpreting results.

In the cohort portion of the study, 1,213 individuals (955 males, 258 females) exposed to chloroprene at the plant were assigned to the following exposure groups: exposure to chloroprene for over 15 years (n=852), exposure for over 20 years (n=381), and exposure for over 25 years (n=149). This group of 1,213 included all retirees (except for 23) and transferred employees (except for 22 lost to follow-up). In the opinion of workers and administrators, exposure levels were much higher in the years prior to 1964, especially the years before 1958 when the production lines were first established. The standard mortality ratio (SMR) for all cancers in the whole cohort was 2.38 ($P<0.01$). SMRs were calculated for the period July 1, 1969 through June 30, 1983, based on sex- and age-specific cancer mortality in the local area in 1973-1975. Confidence intervals were not presented. Smoking and alcohol use represent confounding factors inasmuch as it is not known if the cohort differed from the reference population in this regard. Occupations associated with high-level chloroprene exposure (e.g., monomer workshop, monomer maintenance mechanic, polymer operator, researcher) had SMRs significantly higher than expected. Those occupations associated with neoprene production had SMRs significantly higher than expected only for polymer operators. Only the maintenance mechanics, the group in the monomer production area with the highest risk of cancer, had significantly increased SMRs for liver (16.67), lung (50.0), and malignant lymphoma (100.0). There was only one death each attributed to lung cancer and lymphoma in the mechanics. In the monomer workshop as a whole, the SMR for liver cancer (4 observed cases) was 4.82 ($P<0.05$), for lung (one observed case) 7.14 (not significant), for malignant lymphoma 12.5 (not significant; only one observed), and for pancreas, eyes, and tonsils 13.33 ($P<0.05$). SMRs for liver and lung in the neoprene workers was also elevated above those expected, but not significantly so. In contrast to the maintenance mechanics in the monomer area, there were no cancer deaths in the neoprene mechanics; however, it was stated that the numbers of these latter mechanics may have been too small. There were no female cancer deaths which the investigators suggested may have been due to shorter duration of exposure and exposure to lower levels than males. Other factors such as lowered smoking rates and alcohol use were not discussed. The authors concluded that, based on both the case-control and cohort studies, chloroprene was probably the causal agent behind the excessive cancer deaths at the plant. While this study raises concern that there may be a link between exposure to chloroprene monomer and multi-site cancer mortality, other causes or contributory factors cannot be ruled out. Alcohol consumption and smoking are two potential confounding factors that could partially explain liver cancer (6 cases/cohort) and lung cancer (2 cases/cohort), respectively. Another is the extent to which co-exposure to chloroprene oligomers may have contributed to mortality and tumor incidence. Thus, the associations reported in this study should be regarded as inconclusive that chloroprene monomer causes cancer in humans.

In a retrospective cohort study of 2,314 workers (1,897 men, 417 women) employed in an Armenian chloroprene monomer production plant between 1940 and 1988, Bulbulyan et al. (1999) found a duration of exposure-related increase in the standard incidence ratio (3.27, 95% C.I. 1.47-7.27) for liver cancer compared to the overall Armenian population. Four of six cases of liver cancer occurred in workers with 20+ years of employment. The total cohort was followed for cancer incidence for the years 1979-1990 and for mortality for 1979-1988. Incidence for all cancer and for mortality was below those expected. Causes of death were

abstracted from death certificates and coded according to the 9th revision of the International Classification of Diseases. Measured air levels before 1980 ranged from about 1 mg/m^3 to over 700 mg/m^3. After 1980, maximum air level was reported as 23 mg/m^3. Study limitations which preclude a positive association between exposure to chloroprene and liver cancer include (1) lack of follow-up prior to 1979 which could seriously bias the incidence ratios, (2) lack of accounting for alcohol use, (3) lack of histologic confirmation, and (4) possible co-exposure to other chemicals.

Another study by Bulbulyan et al. (1998) examined cancer mortality in Moscow shoe workers who were reported to be exposed to chloroprene from glue and from polychloroprene latex (colloidal suspension of polychloroprene in water). The extent to which workers were exposed to chloroprene monomer and analytical methods were not stated. The study comprised a total of 5,185 workers (4,569 women) who were employed for at least two years during 1960-1976 and followed-up during 1979-1993. Causes of death were ascertained from death certificates and classified according to the 9th revision of the International Classification of Diseases. There was no histologic confirmation of cause of death. A total of 131 were lost to follow-up. Workers were assigned to three exposure groups based on industrial hygiene data from the 1970s: no exposure, medium exposure (0.4–1 mg/m^3 chloroprene), and high exposure (20 mg/m^3 chloroprene). Both the medium and high exposure groups were exposed to other solvents. The authors found the mortality due to all causes (SMR = 1.03; 95% C.I. 0.97–1.10) to be comparable to that of the general Moscow population during the 1979-1993 period, but the mortality due to all cancers was higher than expected (SMR = 1.22; 95% C.I. 1.07–1.37). Significantly increased mortalities were also seen for liver cancer (SMR = 2.4; 95% C.I. 1.1–4.3) and leukemia (SMR = 1.9; 95% C.I. 1.0–3.3). [For liver, the reference mortality rate for the year 1992 was used and may have resulted in an overestimation of the SMR] Mortality from leukemia was associated with the high chloroprene exposure group, a group also believed to have been exposed to benzene. Lung cancer was increased in men (SMR=1.7; 95% C.I. 1.0-2.7), but not women [the SMR for both men and women was 1.1]. Elevated relative risks were seen among the medium exposure group for stomach, liver and kidney cancer, and among the high-exposure group for stomach, liver, kidney, pancreas and colon cancer, and leukemia. None of these effects was statistically significant. When analyzed according to employment duration (1–9 years, 10–19 years, 20+ years), a significant linear trend was seen in mortality rates from liver cancer and leukemia (only in the high exposure group) with increased duration of employment. Limitations of this study include (1) possible confounding effect from co-exposure of workers to benzene and other chemicals during part of their employment, (2) the lack of reliable data on chloroprene and polychloroprene exposure levels throughout the entire study period, and (3) a lack of control for smoking and alcohol use. Overall, the reported possible associations between exposure to chloroprene monomer and cancer should be regarded as inconclusive.

Du Pont de Nemours examined cohorts from two of its neoprene (polychloroprene) manufacturing plants to evaluate lung cancer mortality (Pell, 1978). Causes of death were obtained from death certificates and coded according to the 7th and 8th revised editions of the International Classification of Diseases Adapted for Use in the United States. The first cohort

("LouisvilleWorks Cohort") consisted of 1,576 males on the wage rolls in June 1957. This cohort was followed until December 31, 1974. All employees who were exposed to chloroprene, but who terminated before June 30, 1957 were excluded. Only 17 individuals were lost to follow-up. Mortalities that occurred were compared to rates seen among du Pont male wage roll employees and retirees as well as to U.S. males. Worker exposures to chloroprene were classified only as "high," "moderate," "low," and "varied," based on job description (there were no quantitative estimates of exposure to chloroprene monomer). There were 51 cancer deaths in the cohort compared to 44.7 expected for du Pont employees and 52.8 based on U.S. mortality. Sixteen of the 193 total deaths were reported as due to lung cancer (13 of whom had a smoking history). Four of the 16 deaths were maintenance mechanics, three of whom were known smokers. Seven deaths were associated with cancer of the lymphatic and hematopoietic systems (no details given) and were non-significantly higher than those expected, after excluding those that occurred before an assumed latent period of 15 and 20 years. This is suggestive evidence of an exposure-related effect. There was no indication of an increasing mortality trend with exposure after the latent period.

The second cohort originally consisted of 270 males ("Chamber Works Cohort") believed to be exposed between 1931 and 1948 and followed through December 31, 1974. Follow-up was complete for 240. Efforts were made to identify employees at work during this period from recollections of current employees since work history records were not maintained for all in this cohort. The observation period, during which latency in tumor induction could be analyzed, was 30-40 years from date of first exposure. The total number of deaths was 55. Of these, 13 occurred before 1957 (the starting point of observation assuming a 15-year latency period) and were excluded from analyses. There were 39 deaths (3 from lung cancer) that occurred from 1957 to 1974, which were slightly higher than the du Pont comparison population and less than the U.S. population. This cohort, although smaller in number than the first cohort, had a long latency period. It was concluded that chloroprene exposure in both cohorts did not increase the risk of lung cancer. There were 5 observed cases of cancer of the bladder (3) and kidney (2) which were significantly elevated ($P<0.01$) compared to either the du Pont or U.S. Comparison populations; bladder cancer was attributed to beta-naphthylamine exposure. The leading cause of death in the cohort was ischemic heart disease (14/39).

A reanalysis of the Pell (1978) data for the first cohort (1,575 males) was performed by NIOSH (Leet and Selevan, 1982). The reanalysis supported the overall findings of Pell (1978). Cancer mortality data was analyzed with respect to latency and duration of exposure, each stratified into 10-year intervals. There were no statistically significant trends in numbers of deaths from malignant neoplasms either for latency or duration of exposure. The investigators stated that the statistical power of the study was limited because of cohort selection factors and stratification. The cohort did not include employees who terminated employment before 1957, the start of the study.

A case-control study of respiratory cancer deaths involving workers at the same plant was subsequently reported (Chen, 1990). Two controls were matched with each lung cancer death

from plant rosters matched by payclass, year of birth and year of death. It was found there were 54 respiratory cancer deaths occurred among male employees (excluding pensioners) during 1957 through 1986 and this represented a significant increase over that expected (40.4). However, only 8% of cases were nonsmokers compared to 46% of controls, suggesting that smoking may have been a significant confounding factor as was prior illness and age at time of first hire. Because of these confounding factors as well as the finding that those most highly exposed had an odds ratio less than those with a lesser degree of exposure, this study is of limited value in assessing exposure to chloroprene in relation to lung cancer.

4.1.6. Mortality

In a retrospective cohort and a nested case-control study (Romazini et al., 1992) of French workers (599 males and 61 females) who worked for at least two years between 1966 and 1989 in a plant that produced polychloroprene, there were only 32 mortalities in the cohort of 642 (18 were lost to follow-up). Because of the low number of mortalities, unsubstantiated exposure groupings, and incomplete accounting for confounding factors, no conclusions can be drawn.

4.2. PRECHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

4.2.1. Oral

BD IV rats were given lifetime oral exposure to chloroprene to investigate possible carcinogenicity through this route (Ponomarkov and Tomatis, 1980). Seventeen pregnant rats were administered a single gavage dose of 100 mg/kg chloroprene in olive oil on day 17 of gestation, and their offspring (81 males, 64 females) were given weekly doses of 50 mg/kg chloroprene for 120 weeks. The purity of the chloroprene was 99%, but storage conditions and whether or not oxidation inhibitors were used during this 120-week study was not reported. The oral LD_{50} for chloroprene in this strain of rat had previously been determined to be 900 mg/kg. Fourteen pregnant control rats were given olive oil on gestation day 17, and their offspring (53 males, 53 females) were given weekly doses of olive oil for 120 weeks. No significant effects of treatment on survival rates, body weights, or total tumor incidence were seen. Of those given chloroprene, 9/16 dams were tumor-bearing vs. 5/14 controls. The treated dams also had a two-fold higher incidence of multiple tumors/rat vs. Controls. Severe congestion of the lungs and kidneys was observed in progeny treated weekly that died after 23–25 weeks, and multiple liver necroses were seen in treated animals (unclear if progeny only) that died after 80–90 weeks.

The types of tumors observed were cited in a footnote to a table that was not keyed to the footnote. Those tumors included: uterine squamous cell carcinoma, transitory-cell carcinoma of the urinary bladder, forestomach papilloma, lymphomas and others (e.g., tumours of the mammary gland). However, the data in the paper do not make it clear which tumors occurred in treated or control animals, with the exception of mammary tumors.

Because of the uncertainties involving data presentation and the lack of reporting on storage conditions, this study is insufficient for drawing conclusions for health hazard characterization.

4.2.2. Inhalation

The National Toxicology Program conducted 16-day, 13-week, and 2-year inhalation exposure studies with chloroprene in F344/N rats and $B6C3F_1$ mice, described in the following paragraphs (NTP, 1998). Results of the 13-week study were reported by Melnick et al. (1996) while the carcinogenic results of the 2-year study were discussed separately by Melnick et al. (1999) in relation to observations noted with 1,3-butadiene in mice. All exposure regimes consisted of 6-hour whole-body exposures each day, 5 days per week, and group sizes were 10 animals per sex per group in the 16-day and 13-week studies, and 50 animals per sex per group in the 2-year studies. The actual concentrations were within 99% of target concentrations. There was no degradation of bulk chemical (stored under nitrogen at -20°C) and total impurities in the distribution line during exposure were less than 0.1 %. The stability of the bulk chloroprene was monitored throughout the study. No dimer peaks were observed by gas chromatography in the samples drawn from the distribution lines. Vapor was generated in the 2-year studies from chloroprene held an evaporation flask kept at 66 °C followed by a metered flow of nitrogen into the base of a temperature-controlled condenser column (attached to top of flask). The temperature of the chloroprene vapor in the condenser column was monitored by a sensor. Histopathology was performed by study pathologist and reviewed by a quality assurance pathologist and the Pathology Working Group.

In the 13-week studies, complete histopathology was performed on controls, 200 ppm (724 mg/m^3) rats, and 80 ppm (290 mg/m^3) mice. The nasal cavity of rats exposed to the three lowest concentrations were also examined microscopically as was the liver of 80 ppm (290 mg/m^3) rats. Separate groups of rats and mice were evaluated for effects of exposure on sperm parameters and vaginal cytology parameters using an NTP (1984) protocol. In addition, separate groups of rats and mice were evaluated for nonprotein sulfhydryl determinations and for hematology, clinical chemistry and urinalysis evaluations. A battery of seven neurobehavorial tests were performed on surviving rats only during week 11.

In the 16-day study, rats were exposed to 0, 32, 80, 200, or 500 ppm (0, 116, 290, 724 or 1811 mg/m^3) chloroprene. Histopathological examination was performed on controls, 80 ppm female rats, both sexes of rats at 200 and 500 ppm (724 and 1811 mg/m^3); tissues examined included brain, liver, kidney, lung, bone marrow, thymus, spleen and testes. Sperm morphology and vaginal cytology were not evaluated. Among males in the high-dose group, 3/10 died by day 3. Females in this dose group had significantly decreased body weight gain, which was also observed in males at 200 ppm (724 mg/m^3) Irritation of the respiratory tract was seen in all dose groups, and hepatocellular necrosis was observed in males at 500 ppm (1811 mg/m^3) and females at 200 ppm (724 mg/m^3). Hematological and clinical chemistry parameters indicated increased serum enzyme (alanine aminotransferase, glutamate dehydrogenase, sorbitol

dehydrogenase) activities, as well as anemia and thrombocytopenia (decreased platelet count) in the 200 and 500 ppm (724 and 1811 mg/m^3) groups, on day 4 only. In females, significant increases in kidney weights (right kidney only) were seen at 80 and 500 ppm (290 and 1811 mg/m^3), and significantly increased liver weights were seen at 200 and 500 ppm (724 and 1811 mg/m^3). Incidence of olfactory degeneration in the nasal cavity of animals in all exposure groups was increased significantly relative to controls. Other than nasal lesions, no other effects were observed in the 32 ppm (116 mg/m^3) group.

In the mouse portion of the 16-day study, exposure levels were 0, 12, 32, 80 and 200 ppm (0, 43, 116, 290, and 724 mg/m^3). Histopathology was performed on all 0, 80, and 200 ppm animals and on selected target organs in other groups. Tissues examined were those performed on the rat. All animals died in the high-dose group, exhibiting signs of narcosis, hepatocellular and thymic necrosis, and hypertrophy of the myocardium. Significantly decreased body weight gains (compared to controls) were seen in males at 32 and 80 ppm (116 and 290 mg/m^3). Significant decreases in thymus weights were seen in 80 ppm (290 mg/m^3) males and females, and significantly increased liver weights were seen in 80 ppm (290 mg/m^3) females.

In the 13-week range-finding study in the rat, exposure groups were 0, 5, 12, 32, 80 and 200 ppm (0, 18, 43, 116, 290, and 724 mg/m^3). No effects on final mean body weights were seen. Activities of serum alanine amino transaminase (ALT), glutamine dehydrogenase (GDH), and succinic dehydrogenase (SDH) were elevated on day 22 in both sexes of the 200 ppm (724 mg/m^3) group, but enzyme levels returned to control levels by the end of the exposure period. However, at week 13, alkaline phosphatase enzymeuria occurred in males of the 32, 80, and 200 ppm (116, 290, and 724 mg/m^3) groups, and only in females of the 200 ppm (724 mg/m^3) group. Significant increases in kidney weights were seen in both sexes at 200 ppm (724 mg/m^3), and in females at 80 ppm (290 mg/m^3). In addition, at week 13, both males and females in the 200 ppm (724 mg/m^3) presented evidence of a normocytic, nonresponsive anemia. As in the 16-day study, hepatocellular centrilobular necrosis was seen particularly in females at 200 ppm (724 mg/m^3). Hemosiderin pigmentation was significantly increased compared to controls in both sexes at this exposure level. There was no exposure-related effects on motor activity, forelimb/hindlimb grip strength, or startle response.

Other exposure-related effects observed included (1) significantly decreased sperm motility in males of the 200 ppm (724 mg/m^3), (2) increased incidence of olfactory degeneration in 32 and 80 ppm (116 and 290 mg/m^3) females (4/10 and 9/10, resp.), (3) significantly lower liver nonprotein sulfhydryl concentrations in both sexes of the high exposure group, and (4) increased horizontal activity in those animals exposed to 32 ppm (116 mg/m^3) and higher .

In the 13-week mouse range-finding study, exposure groups were 0, 5, 12, 32 and 80 ppm (0, 18, 43, 116, and 290 mg/m^3). There was no increased mortality. Final mean body weights in males at 80 ppm (290 mg/m^3) were significantly decreased compared to controls. Among the few effects observed were (1) an increase in squamous epithelial hyperplasia of the forestomach in both sexes at 80 ppm (290 mg/m^3) and (2) significantly lower hematocrits and elevated platelet

counts in females of the 32 and 80 ppm (116 and 290 mg/m^3) groups compared to controls. Sperm morphology of exposed males were similar to controls and there was no effect on the estrus cycle. Decreases in hepatic nonprotein sulfhydryl levels were not associated with histopathological changes in the liver.

In the 2-year rat study, exposure groups were 0, 12.8, 32, and 80 ppm (0, 46, 116, and 290 mg/m^3). In the histopathological analyses, the following evaluations were not performed on either the rat or mouse: (1) sperm morphology and vaginal cytology, (2) neurobehavioral (3) nonprotein sulfhydryl determinations, (4) hematology, (5) clinical chemistry, and (6) urinalysis. Survival in the 32 and 80 ppm (116 and 290 mg/m^3) groups of males was significantly lower than control by life table pairwise comparison. Survival of female rats was not significantly affected. The occurrence of neoplasms was considered incidental to the cause of death or not rapidly lethal. The cause of the low survival in the exposed male groups is not readily apparent. Although mortality in the low exposure group was not statistically significant (it was in the higher exposure groups), the trend is significant (P=0.013); thus, the low exposure concentration likely represents a frank-effect-level (FEL). Percent probability of survival at end of study was similar to historical control data for males. Body weight gain was not significantly reduced over the span of the study.

Concentration-dependent increases in the incidence of squamous cell carcinoma and papilloma of the oral cavity were observed in both males and females with combined effects achieving statistical significance at 80 ppm (290 mg/m^3). It is not known if this primarily was a result of preening activity. Follicular cell adenoma or carcinoma of the thyroid for females showed less of a concentration dependence than males; statistical significance was achieved in males for the combined effects at both 32 (116 mg/m^3) and 80 ppm (290 mg/m^3) and the trend was positive. The incidence of alveolar/bronchiolar carcinoma in males reached statistical significance at 80 ppm (290 mg/m^3) with little indication of a concentration-related trend while the incidence of hyperplasia of the alveolar epithelium (in both sexes) was statistically significant at all exposure concentrations compared to controls. An increase (not statistically significant) in the incidence of alveolar/bronchiolar adenomas at was seen in females exposed to 80 ppm (290 mg/m^3) that exceeded the incidence in historical controls, but no adenomas were seen in the two lower concentration groups. There were no carcinomas. In females, the incidences of multiple fibroadenomas in the mammary gland of all exposed groups were greater than controls.

The incidence of renal adenomas/carcinomas was significantly greater than controls in males from all exposure groups and there was a concentration-related positive trend when evaluated histopathologically by step sections. In the urinary bladder, there was a slight increase in transitional epithelium carcinoma in 80 ppm (290 mg/m^3) females and males at 32 ppm (116 mg/m^3). Additionally 1/50 males at 80 ppm (290 mg/m^3) had a transitional cell papilloma. All these incidences exceeded the historical control ranges; no such neoplasms have been observed in historical controls. The findings in the bladder were considered by the NTP to be of uncertain significance although were noteworthy because no such neoplasms have been seen in either male

or female control F344/N rats. Incidence for neoplastic lesions are shown in Table 4-1. The NTP concluded that, overall, there was clear evidence of carcinogenicity.

Prominent among nonneoplastic lesions were atrophy, basal cell hyperplasia, metaplasia, and necrosis of the olfactory epithelium in 32 and 80 ppm (116 and 290 mg/m^3) males and females. Atrophy and necrosis were elevated significantly above controls in males in all exposure groups. In females, the incidence of atrophy and necrosis was statistically significant in the two highest concentration groups only. Because of low survival of males (compared to controls) in the low exposure group, it can be concluded that there is no NOAEL. The low exposure group is represented as an FEL.

Table 4-1. Incidence of neoplasms in male and female rats exposed to chloroprene for 2 years (NTP, 1998)

Site:neoplasm	Incidence	Exposure concentration (ppm) 0	12.8	32	80	Historical range
oral cavity:	male	0	4	10*	24**	0-6%
squamous cell	adjusted %[a]	0	5.7	15.8*	35.5**	
papilloma or	female	2	6	10	22**	0-6%
carcinoma	adjusted %	2.4	7.0	12.3	27.0**	
Lung:alveolar/	male	4	4	8	12	0-10%
bronchiolar	adjusted %	5.5	5.8	12.8	18.7	
adenoma or	female	2	0	0	6	0-4%
carcinoma	adjusted %	2.4	0	0	7.3	
Kidney:	male	2	16**	12**	16**	0-16%
adenoma or	adjusted %	2.8	22.2*	19.5*	25.1**	
carcinoma	female	0	0	0	8	0-4%
	adjusted %	0	0	0	9.7**	
Thyroid:follicular	male	0	4	8*	10*	0-4%
cell adenoma or	adjusted %	0	5.7	12.9*	15.4*	
carcinoma	female	2	2	2	10	0-6%
	adjusted %	2.4	2.3	2.5	12.2	
Mammary gland:	female[b]	49	64	72*	72*	16-42%
fibroadenoma	adjusted %	54.0	70.3	78.1**	79.4**	

[a] Survival-adjusted incidence values were based on the Poly-3 quantal response method listed on page 119 of NTP (1998). This method is now used by NTP in lieu of the Kaplan-Meier method to adjust for intercurrent mortality.
[b] Includes animals with single or multiple fibroadenomas.
Asterisks in the exposed group columns indicate significant differences from the control groups:
$^{*}p \sim 0.05$ and $^{**}p \sim 0.01$.

In the 2-year mouse study, exposure groups were 0, 12.8, 32 and 80 ppm (0, 46, 116 and 290 mg/m^3). Increased incidences of neoplasms of the lung, circulatory system (hemangiomas and hemangiosarcomas), Harderian gland, forestomach, mammary gland (females only), and kidney (males only) were seen. Incidences are tabulated in Table B-1 of Appendix B. Survival of females was significantly lower than controls in all exposure groups and in the two highest groups of males. Many early deaths and moribund sacrifices were stated to be associated with treatment-related neoplasms. Although there was an increased incidence of adenomas/carcinomas and hemangiosarcomas of the liver in male mice, these lesions were judged to have been influenced by Heliobacter hepaticus infection which may have resulted in hepatitis. Increased incidences of hepatocellular carcinoma were seen in all exposed females, but was not considered

to be a result of Heliobacter infection. An increased incidence of Zymbal's gland carcinoma, which metastasized to the lung, was seen in the 80-ppm (290 mg/m^3) females. Non-neoplastic effects reported by NTP (1998) in mice included (1) increased incidences of bronchiolar hyperplasia (all concentrations) and histiocytic cell infiltration in the lung (mainly in high concentration group), (2) epithelial hyperplasia in the forestomach (high concentration only), (3) renal tubule hyperplasia (males only; no concentration-related response), and (4) atrophy of the olfactory epithelium (high concentration only). Because of statistically significant treatment-related high mortality in females at all concentration levels, it is concluded that there is no NOAEL. The low exposure group is represented as an FEL. Significant non-neoplastic effects and their associated incidences are shown in Table 4-2.

In a 7-month inhalation study, groups of 77 to 132 Kunming albino mice were exposed to 0, 2.9, 19.0 or 189.0 mg/m^3 (0, 0.8, 5.3 or 52.5 ppm) chloroprene (99.8% pure) 4 hours per day, then sacrificed at the end of month 8 and examined for induced lung tumors (Dong et al., 1989). There was no discussion of the vapor generating system and the level of chloroprene dimers to which the animals were exposed. This represents a study shortcoming. This mouse strain was reported to have a low spontaneous lung tumor rate. No lung tumors were seen before month 6. A concentration-related, statistically significant increase in lung tumor incidence was seen, with 1.3 percent of the mice exhibiting tumors at 0 mg/m^3, 8.1 percent at 2.9 mg/m^3 (0.8 ppm), 9.4 percent at 19.0 mg/m^3 (5.3 ppm), and 19.7 percent at 189.0 mg/m^3 (52.5 ppm). The number of mice with multiple tumors also increased with dose; the 0 and 2.9 mg/m^3 groups had no such mice, the 19.0 mg/m^3 group had one mouse with two tumors, and the 189.0 mg/m^3 group had six mice with two tumors and two mice with three tumors. Most tumors were papilloadenomas, and a few were adenomas. There was no mention of carcinomas.

In a 4-week range-finding assay for a future lifetime exposure assay (see below, Trochimowicz et al., 1998), groups of 10 male and 10 female Wistar rats were exposed to mean concentrations of 0, 39, 161 or 625 ppm (0, 140, 580, 2250 mg/m^3) freshly-distilled chloroprene for 5 days per week, 6 hours per day (Clary et al., 1978). While no mortality was seen in the control and 39 ppm groups, 3 males died by week 4 in the 161 ppm group, and 5 males and 3 females died in the 625 ppm group. Gross pathology of those animals that died included dark, swollen livers (also in survivors of high exposure and grayish lungs with hemorrhagic areas.

Table 4-2. Incidences of significant nonneoplastic lesions in b6c3f1 mice exposed to chloroprene for 2 years (NTP, 1998)

Lesion		Controls	12.8 ppm	32 ppm	80 ppm
Lung:					
bronchiolar	male	0/50	10/50*	18/50**	23/50**
hyperplasia	female	0/50	15/49**	12/50**	30/50**
Nose:					
olfactory epithelium					
atrophy	male	7/50	8/48	7/50	49/50**
	female	6/50	5/49	4/49	47/50**
Kidney:					
renal tubular					
hyperplasia	male	2/50	12/49**	16/50**	17/50**
(extended evaluation)					
Forestomach:					
epithelial	male	4/50	6/48	7/49	29/50**
hyperplasia	female	4/50	3/49	8/49	27/50**

* Significantly different from controls at $p \sim 0.05$.
** Significantly different from controls at $p \sim 0.01$.

Mean body weights were significantly less than controls in all exposure groups beginning at the first week of exposure; retarded growth showed an exposure-related trend. Significant, concentration-related decreases in liver and spleen-to-body weight ratios were seen; brain-to-body ratios increased across exposure groups. Microscopic examination revealed centrilobular liver degeneration and necrosis, slightly enlarged tubular epithelial cells in kidneys, and hemorrhaging and edema in the lungs of animals in the high exposure group. There were no adverse liver, kidney, or lung effects in the 39 ppm group. There were no adverse hematological findings in any exposure group.

This exposure protocol was used for a similar 4-week range-finding study in Syrian golden hamsters, with mean concentration levels of 0, 39, 162 and 630 ppm (0, 140, 583, and 2268 mg/m^3). While there was no exposure-related mortality in the 0 and 39 ppm groups, one male and 3 females died in the first week of exposure at 162 ppm, and all animals died in the first week of exposure at 630 ppm. These early mortalities exhibited reddish or grayish fluid-filled areas in their lungs. Most survivors in the mid-exposure group exhibited localized necrosis and degeneration and focal pallor of the liver. Some animals in the two lower exposure groups

exhibited mucous membrane irritation around the nasal cavity which presented as flattening and thinning of the olfactory epithelium. These signs were less apparent in the high exposure group. Mean body weights were significantly less than controls in the 162 ppm group beginning at the first week of exposure.

A whole-body, chronic inhalation exposure study (Trochimowicz et al., 1998) exposed Wistar rats (100/sex/exposure group) to actual concentrations of 0, 10, or 50 ppm (0, 36, or 181 mg/m^3) chloroprene for 6 hours per day, 5 days per week, for 24 months. There is also an unpublished final report of the rat study (EPA/OTS, 1985c). Syrian golden hamsters (100/sex/group) were exposed similarly to 0, 10, and 49 ppm (0, 36, or 177 mg/m^3 for 18 months. There is also an unpublished final report of the hamster study (EPA/OTS, 1985a). Stock solutions of freshly-distilled chloroprene were stored under nitrogen at -20C and vapors were generated from vessels kept at 0C. Purity was 99.6% $-chloroprene. Chamber atmospheres were monitored every half-hour. Microscopic examination was conducted on all organs and tissues from controls and animals in the high exposure groups. In addition, histological examination of liver, spleen, pituitary, thyroid, adrenals and tumors were conducted on animals in the lower exposure groups. Clinical chemistry, however, was not part of the protocol and hematological and immunological assessments were not made.

At week 72, a chamber failure caused the accidental deaths of 87 male and 73 female rats in the 10 ppm group. Mortality rates in the 50 ppm group were similar to controls. Rats in the 50 ppm group exhibited (1) a concentration-related increase in the severity of and an increased incidence of alopecia (greater in females than in males), (2) increased relative liver weight (females only), (3) lower relative spleen and thyroid weights (females only), (4) decreased lung weight (both sexes), 10% growth retardation (i.e., body weight gain), and increased incidence of clear hepatocellular foci (males) and a combination of basophilic, clear and mixed-cell type foci in females. The livers of the 10 ppm group that accidentally died were slightly to moderately autolytic precluding histological findings. There were no statistically significant compound-related effects on the kidney, spleen, and thyroid of either sex.

The only statistically significant neoplastic finding was an statistically significant increase in mammary fibroadenomas in female rats in the 50 ppm treatment group, particularly in those animals that were sacrificed or died prior to terminal sacrifice. The incidence of thyroid follicular adenomas in females in the 50 ppm group was 3/100 while the incidence of papillary carcinoma was 2/100; no neoplasms were found in the thyroid for female controls. Papillary carcinoma was not observed in any male rats. The incidence of Zymbal's gland adenoma was 1/100 in 50 ppm females. The incidence of nasal squamous-cell carcinoma in males of the 50 ppm group was 3/100. One such carcinoma was found in a control group female. The incidence in males was reported to be within the historical range (0-3.4%) for the Wister rat. Thus, the investigators that the occurrence of this neoplasm as not treatment-related. The incidence of transitional-cell carcinoma of the urinary bladder was 1/100 males in the 50 ppm group.

Microscopic evaluation was carried out on all organs and tissues of controls and 50 ppm hamsters. While alopecia was noted, there appeared to be no statistical correlation attributed to exposure. There were no remarkable differences in gross or microscopic pathology of hamsters nor were there any significant concentration-related increases in neoplasm incidence. Nearly all neoplasms were equally distributed between test and control animals. Clinical observations revealed no adverse findings.

The investigators concluded that chloroprene is not carcinogenic in either rats or hamsters under these exposure conditions. This conclusion for the rat differs considerably from the findings in the NTP study in which multi-site tumors were observed in the high concentration group. Trochimowicz et al. (1998) suggest that this may relate to the difference in the high concentrations in the two studies or may relate to species and/or strain differences.

Studies performed for E. I. duPont de Nemours and Co. with Wistar rats (40 per sex per group) exposed to 0, 10, 33 or 100 ppm (0, 36, 119, or 360 mg/m^3 chloroprene for 26 weeks (EPA/OTS, 1985b) found no evidence of exposure-related tumor induction. There was no mortality. Only a few animals (2/20 and 4/20, respectively) in the 10 and 33 ppm group exhibited focal areas of alopecia. Testicular bilateral atrophy was observed in 2/20 animal in the 100 ppm group, but this was not considered exposure-related. The two-year study confirmed that there was no exposure-related testicular atrophy (Trochimowicz et al., 1998). There were no adverse histopathological or hematological findings attributed to exposure.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

A study of reproductive and developmental endpoints in Charles River rats exposed to chloroprene via inhalation was performed by Culik et al. (1978). The test material was 99.9% pure, was kept under nitrogen at -20C before use, and contained less than 50 ppm dimers. No decomposition was observed during the experiment. In a reproduction study, male rats (5 per group) were exposed to 0 or 25 ppm (91 mg/m^3) for 22 consecutive days, 4 hours per day, prior to mating with untreated virgin females. No adverse effects on reproductive capability, as measured by number of successful matings, pup survival, and pups per litter were seen. Groups of 25 pregnant rats were exposed to 0, 1, 10 or 25 ppm (0, 4, 36, or 91 mg/m^3) chloroprene on gestation days 3 through 20 (4 hours per day) to assess teratogenic effects. Developing fetuses were examined on gestation day 21. No adverse effects were observed. Finally, groups of 50 pregnant rats were exposed to 0, 1, 10 or 25 ppm (0, 4, 36, or 91 mg/m^3) chloroprene on gestation days 1 through 12 (4 hours per day). No embryotoxic effects were seen in embryos examined on gestation day 17. The lack of effects in this study contradicts the positive effects seen in previous Soviet studies (Salnikova, 1968; Salnikova and Fomenko, 1973) at an exposure level of 1 ppm and lower. Culik et al. (1978) speculate that this is due to insufficient control for impurities in the chloroprene samples used by the Soviet investigators.

A study by E. I. duPont de Nemours and Co. in which two generations of Wistar rats (40 per sex per group) were exposed to actual mean concentrations of 0, 10, 33 or 100 ppm (0, 36,

119, or 360 mg/m^3) chloroprene found no evidence of exposure-related reproductive effects, measured as female fertility, litter size, male/female ratio, and offspring mortality (EPA/OTS, 1985d). Chloroprene was freshly purified and test atmospheres were generated from stock material kept at 0C. The F_0 generation male and female rats were exposed to chloroprene for 6 hours/day, 5 days/week for 13 weeks, then paired with unexposed mates; the F_1 rats were subsequently exposed in a similar exposure regimen for 10 weeks. Although alopecia was observed in some animals, including controls, there was no apparent exposure-related response. Discussion of the results of this study were also reported in unpublished form by Appleman and Dreef-van der Muelen (1979). A study by E. I. duPont de Nemours and Co. with pregnant Charles River rats exposed to 0, 1, 20 or 25 ppm (0, 4, 72, or 90 mg/m^3) chloroprene for 4 hours per day during gestation days 6–15 found no evidence of embryotoxic effects related to exposure (EPA/OTS, 1985e).

Exposure of pregnant New Zealand white rabbits to chloroprene was found not to have adverse effects on reproductive and developmental parameters (Mast et al., 1994). Artificially inseminated rabbits (15/ exposure group) were exposed to chloroprene at 0, 10, 40 or 175 ppm, 6 hours/day, 7 days/week on day 6 through 28 of gestation. Purity of chloroprene in generation flask was 98.5% with a total dimer content of 5-6%. Purity at end of exposure day was substantially lower than at beginning of day. However, dimers were not detected in distribution lines or in chamber samples. There were no overt signs of maternal toxicity. There was no effect on: (1) number of implantations, (2) mean % of live pups per litter, (3) fetal sex ratio, (4) fetal body, kidney or liver weights, or (5) incidence of resorptions per litter. There was no exposure-related effect on fetal malformations or variations.

In an unpublished report, Koeter (1979) reported that pregnant rats (strain unspecified) were exposed to 0, 10, 25, 75 or 175 ppm chloroprene (purity unstated) for 6 hours/day from days 4-16 of gestation. No signs of embryotoxicity or teratogenicity were observed. However, the number of live fetuses were significantly reduced in the 75 and 175 ppm groups compared to controls. Significant differences were noted in other endpoints as well, but the authors did not consider any to be related to treatment.

4.4. OTHER STUDIES

4.4.1. Lethality

An approximate lethal concentration (ALC) for chloroprene inhalation was determined in Charles River rats by exposing groups of 6 males to 530, 1,690, 2,280, 3,535 or 3,610 ppm for 4 hours, then observing the animals for 14 days (Clary et al., 1978). A dose-related increase in mortality was seen, with no deaths in the two lowest exposure groups, 1 death occurring at 2,280 ppm (the ALC), and 2 deaths each in the two highest exposure groups. In separate tests involving exposure to 530 ppm chloroprene via inhalation, or to 50 mg/kg via ingestion, no mortality was observed (Clary et al., 1978).

4.4.2. Dermal

Male Charles River rats exposed dermally to a 4-hour exposure to 200 mg/kg chloroprene exhibited mild to moderate skin erythema (redness) accompanied by edema (fluid accumulation) (Clary et al., 1978). Similar results were seen after a 2-day exposure of male albino rabbits to 200 mg/kg chloroprene in a study by E. I. duPont de Nemours and Co., leading the authors to conclude that chloroprene is a moderate primary irritant (EPA/OTS, 1985f).

4.4.3. Hepatic

Glutathione conjugation of epoxide byproducts of chloroprene oxidative metabolism in the liver is thought to play an important role in minimizing toxic effects of chloroprene exposure. Several studies have examined the effect of nutritional state, which may affect GSH content in tested rats, on the resulting toxicology of chloroprene. Male Holtzman rats that were fasted (in which case their liver GSH content was substantially diminished) prior to inhalation exposure to 500–10,000 ppm chloroprene exhibited significantly increased serum alanine "-ketoglutarate activity and associated mortality than rats fed a normal diet (which maintained GSH content at a high level) (Jaeger et al., 1975).

Male Sprague-Dawley rats were fasted (38-40 hours prior to sacrifice) and exposed via inhalation for 4 hours to time-weighted average air concentrations of 0, 110, 151, 212 or 307 ppm (0, 396, 544, 763, or 1105 mg/m^3) chloroprene (Plugge and Jaeger, 1979). A significant, exposure-related increase in liver-to-body weight ratio was seen at the highest three exposure levels. Activity of serum sorbitol dehydrogenase (SDH), an enzyme indicative of liver damage, was significantly increased at the two highest exposure levels. Significantly increased levels of non-protein sulfhydryl (NPSH) were measured in liver 24 hours after exposure at all chloroprene levels, while significantly decreased levels of lung NPSH were seen at all chloroprene exposure levels. Pretreatment of rats with polychlorinated biphenyl congener mixture (Aroclor 1254), an inducer of mixed-function oxidase enzymes, resulted in a protective effect with no significant liver enlargement or SDH or NPSH elevation.

Zhang et al. (1996) administered 99.9% pure chloroprene dissolved in sesame oil to male Wistar rats (6 animals/group) in daily i.p. injections for 21 consecutive days at doses of 0, 8, 40, and 200 mg/kg. The left liver lobe was dissected for histopathological examination. Hydropic degeneration, centrilobular necrosis and macrophage infiltration was seen in the 40 and 200 mg/kg groups (incidence not reported). Serum SDH, as well as ALT, AST, and alkaline phosphatase were unaffected by treatment. Erythrocyte GSH peroxidase and superoxide dismutase were significantly reduced in the 40 and 200 mg/kg groups. In a separate experiment, pretreatment with Vitamin E before i.p. injections of 60 mg chloroprene/kg reduced chloroprene-induced increases in cholylglycine, reported to be an indicator of liver function, as well as malonadialdehyde, an indicator of lipid peroxidation.

4.4.4. Genotoxic

As part of the 2-year bioassay of chloroprene, NTP (1998) also evaluated possible oncogene-activating mechanisms for lung and Harderian gland neoplasms in the B6C3F1 mouse. The results were published by Sills et al. (1999). After isolation and amplification of DNA from the neoplasms, H-*ras* and K-*ras* mutations were identified. A higher frequency (80%) of K-*ras* codon 61 mutations were detected in chloroprene-induced lung neoplasms than in spontaneous neoplasms of control mice (30%). The predominant mutation was an A ÿ T tranversion (CAA ÿ CTA). They appeared in an inverse dose-response relationship. This pattern of *ras* mutations was observed with isoprene-induced lung neoplasms, but not in those induced by butadiene. Rare point mutations, not seen in spontaneous lung neoplasms, were detected at codon 12. No consistent morphological pattern or type of neoplasm was associated with specific K-*ras* mutations. A higher incidence (100%) of both K- and *H-ras* codon 61 mutations was detected in chloroprene-induced Harderian gland neoplasms than those in control mice (56%) or in neoplasms (69%) from butadiene-exposed mice. The predominant mutation was also a CAA ÿ CTA transversion. The concentration-response was similar across exposure groups. It was suggested that the large number of *ras* mutations at A:T base pairs after exposure to chloroprene, isoprene, and butadiene may indicate an interaction of metabolic intermediates with DNA to form adenine adducts, that may be important for tumor induction.

Notwithstanding the increased incidence of mutations at codon 61, it should be noted that all exons of the *ras* gene were not amplified in this study nor were possible mutations in other oncogenes or tumor suppressor genes examined. In addition, the finding of K-*ras* mutations is not evidence of interaction of chloroprene (or its metabolites) on DNA. Thus, it is far from conclusive that the chloroprene-induced mutations observed in this study are contributory to the lung and Harderian gland neoplasms.

Cytogenetic tests using chloroprene have been negative. In studies performed by Brookhaven National Laboratories for the NTP, sister chromatid exchanges and chromosomal aberrations (mouse bone marrow cells) and the frequency of micronuclei in peripheral blood erythrocytes were evaluated in mice exposed by inhalation to chloroprene in the NTP bioassay (NTP, 1998). Results have been separately published by Shelby (1990) and by Tice et al. (1988). [Duration of exposure was 12 days in a 16-day period]. There was no exposure-related effect in male mice, compared to controls, in either sister chromatid exchange numbers, chromosomal aberrations, or micronuclei frequency in either polychromatic or normochromatic erthyrocytes (NTP, 1998; Shelby, 1990). Detailed protocol for these experiments were provided in Tice et al. (1988). Tice et al. (1988) did report that the mitotic index (frequency of cells in metaphase) in mouse bone marrow cells was elevated in chloroprene-exposed animals with the increase being significant in the 80-ppm group. Tice (1988) suggested that the lack of chloroprene-induced genotoxicity in bone marrow may imply that any carcinogenic activity attributable to chloroprene would be very site-specific.

Neither was the frequency of micronucleated cells in peripheral blood erythrocytes affected when mice were exposed to chloroprene for 13 weeks (NTP, 1998). The detailed protocol for this experiment was provided by MacGregor et al. (1990). Although Sanotskii (1976) reported an increase in chromosomal aberrations in bone marrow cells of mice exposed for two months to chloroprene concentrations of 3.5 mg/m^3 (1 ppm) and less, protocol details and information about the purity and storage of chloroprene was not provided. Thus, these results cannot be adequately evaluated. Chloroprene did not induce micronuclei in bone marrow from Wistar rats exposed by inhalation to 100 ppm for 5 consecutive days, 6 hours per day (EPA/OTS, 1985g).

In a separate screening study (Shelby and Witt, 1995), chloroprene was negative in both the *in vivo* mouse bone marrow micronucleus test and the chromosomal aberration test in which male B6C3F1 mice were injected i.p. with chloroprene in corn oil three times at 24-hour intervals.

Vogel (1979) evaluated chloroprene (99% pure with negligible dimer content) dissolved in DMSO (final DMSO concentration=1%) in a feeding experiment to see if it induced recessive-lethal mutations on the X-chromosome of male *Drosophila melanogaster* (Berlin-K). Storage conditions and the elapsed time between receipt and use was not reported; these represent study limitations since aged chloroprene may have been used. After mating, the F_3 brood was evaluated for recessive lethals. Pooled data from experiments at five different concentrations were statistically compared against data from seven control experiments. Chloroprene was found to induce a significantly higher percentage at $P<0.01$. As shown in the experiments with *S. typhimurium* by Westphal (1994) (see below), the interaction of chloroprene with DMSO may result in genotoxic degradation products. In studies by Foureman et al. (1994), chloroprene (purity not reported) dissolved in ethanol was negative in its ability to produce sex-linked recessive lethal mutations in postmeiotic and meiotic germ cells of adult male *D. melanogaster* (Canton S) when exposed through either the injection or feeding route. The F_2 broods were examined. However, the investigators suggested that the discrepancy between their negative findings and those of Vogel (1979) may be due to (1) differences in purity of the chloroprene sample, (2) differences between the Berlin-K and Canton-S strains, (3) differences in sample sizes, and (4) possible genetic drift within the female populations used by the two groups of investigators. Based on the experiments by Gahlmann et al. (1993), one could include a fifth possibility that chloroprene in ethanol is less genotoxic than if dissolved in DMSO.

In an extension of earlier studies (Bartsch et al.,1975), Bartsch et al. (1979) exposed *S. typhimurium* strain TA 100 to 0.5 to 8% (v/v) of chloroprene in air, for 0 to 4 hours at 37°C, in the absence of S9, causing a concentration-related increase in the mutagenic response. Chloroprene purity was 99% and contained a negligible amount of dimers. Batch solutions were kept at -20°C and were freshly prepared before use. S9 liver fractions were obtained from rats and mice pretreated with phenobarbital. When either S9 fractions from either phenobarbital-pretreated or untreated mice was used, a several-fold increase in the number of revertants was observed. At a 20% vapor concentration, toxicity was severe. The investigators also tested a

mixture of chloroprene dimers (dissolved in DMSO) in a plate incorporation assay with TA 100 and TA 1535. The dimers caused only a small increase in the number of revertants and this was paralleled by an increase in toxicity. When a more purified chloroprene solution (99.7%) was used, the S9-mediated mutagenicity with TA 100 was similar to that using the 99% pure material. It was thus concluded that the dimers were ruled out as a cause of the mutagenicity. The isomeric dimer mixture included: 9% dichlorodivinyl- and dichlorovinylcyclobutanes, 68% chlorovinylchloro- and vinyldichlorocyclohexenes, 19% dichlorocyclooctadienes, and 4% dimers of unspecified structure. It was suggested that mutagenicity was caused by the formation of an epoxide intermediate upon oxidative metabolism, possibly explaining the enhanced effect when S9 was present. This was based upon detection of a chloroprene adduct with the trapping agent, 4(4-benzyl)pyridine, after chloroprene vapor was passed through a microsomal suspension in the absence of an NADPH-generating system; absorbance was increased 4-fold suggesting the formation of a reactive intermediate. It is not as well established that the dimers tested are without mutagenic potential because of uncertainties involving potential interactions of the compounds with the DMSO vehicle, with the result that degradation products may have, in part, caused toxicity.

Willems (1980) also found that chloroprene (purity not stated) was mutagenic with TA100 as well as TA1535, in the presence or absence of S9, under vapor exposure conditions; it was negative with TA98. Petri plates were incubated at 37°C in dessicators for 24 hours, removed and then incubated for another 24 hours. Positive controls were used. Four dimers (chemical characterization not stated) were also tested under the same conditions. Three of the four were mutagenic against both *Salmonella* strains; the fourth was not found to be mutagenic.

On the other hand, Westphal et al. (1994) reported that freshly distilled chloroprene (from a 50% in xylene solution) was negative in the Ames assay with TA 100 with and without S9. The distillates, stored at -20°C, were checked for purity immediately before testing. The assays were performed in gas-tight chambers to prevent chloroprene volatilization. S9, TA 100, and liquid chloroprene were preincubated at 37C for 2 hours in gas-tight vials. Vials were left open to allow chloroprene to evaporate before plating. Propylene oxide and benzo(a)pyrene were used as positive controls. When freshly distilled chloroprene was compared to aged chloroprene, it was found that the latter was mutagenic in this assay and mutagenicity increased linearly with age of the distillate. GSH, both with and without S9, reduced mutagenicity of the aged chloroprene, but was less effective as the amount of decomposition products increased. Chloroprene diluted in DMSO was markedly more toxic and more mutagenic than chloroprene dissolved in ethanol. Analysis of aged chloroprene by gas chromatography revealed the presence of decomposition products such as cyclic dimers, which may have been responsible for the mutagenic effects that were absent when fresh chloroprene was tested.

Ames assays performed by E. I. duPont de Nemours and Co. found that chloroprene (dissolved in DMSO) in a plate incorporation assay was mutagenic in *Salmonella* strains TA1535 and TA100 upon S9 activation, but not in strains TA98, TA1537 and TA1538 (EPA/OTS, 1985h). Positive controls were used, but the chloroprene purity was not stated. Preliminary

evidence reported by du Pont de Nemours indicates that chloroprene monoepoxide is mutagenic in strains 100 and 1535 and less so in strains 97A and 98 without arochlor-induced S9 activation (Himmelstein et al., 2000); inclusion of S9 lowered the mutagenic response in all tester strains. In their summarization of results from Ames plate incorporation assays performed by SRI, Zeiger et al. (1987) noted that chloroprene (dissolved in DMSO; purity not stated) was nonmutagenic when tested with *S. typhimurium* strains TA100, TA1535, TA1537, and TA98. These tests were performed using positive controls and with or without Arochlor-induced rat or hamster liver S9.

Chloroprene (99% pure) was evaluated for mutagenic potential in V79 Chinese hamster cells in the presence of a liver supernatant (S15 fraction) from phenobarbitone-pretreated rats and mice (Drevon and Kuroki, 1979). Vapor exposures were conducted up to 10% (v/v) chloroprene for 5 hours. No positive controls were reported to have been used. While it was found to be toxic, it was not mutagenic under the assay conditions. Recent preliminary evidence obtained in experiments by du Pont de Nemours indicates that chloroprene has no clastogenic response in Chinese hamster V79 cells when evaluated up to toxic concentrations (Himmelstein et al., 2000b).

Chloroprene was also tested in a dominant lethal assay with male Swiss mice (Immel and Willems, 1978). Groups of 12 males each were exposed to 0, 10, or 100 ppm chloroprene for 6 hours/day, 5 days/week for two weeks. Immediately after exposure each male was mated with two virgin females for 7 days. Females were replaced each week for 8 weeks. There was no sign of dominant lethal mutations or adverse effects on mating performance or fertility

4.4.5. Toxicity Prediction With Chloroprene Dimers

The carcinogenic potential of dimers formed from aged chloroprene or upon heating is not known. To explore this potential three dimers associated with chloroprene were evaluated using the TOPKAT® system (personal communication, R.M. Bruce). This method of toxicity prediction uses validated literature-derived data for known oral carcinogens to evaluate carcinogenicity (as well as other endpoints) based on structure and electrotopological parameters of a given substance. The strengths and limitations of TOPKAT® have been discussed by Dearden et al. (1997). The three dimers evaluated were: (1) 1-chloro-5-(1-chloroethenyl)-cyclohexene, (2) 1-chloro-4-(1-chloroethenyl)-cyclohexene, and (3) 1,6-dichloro-1,5-cyclooctadiene. When structural and electronic parameters of these compounds were evaluated against the database of compounds known to exhibit a carcinogenic response in both sexes of rats and mice, TOPKAT® predicted compounds (1) and (3) to be carcinogenic to females of both species; in (1) and (3) the data was insufficient to predict for the male rat whereas with (1) it was negative for male mouse. Dimer (2) was predicted negative for female mice and positive for the male rat (female rat negative). Data were insufficient to make a prediction for the male mouse.

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION (IF KNOWN)—ORAL AND INHALATION

Little is known about the effects of chloroprene monomer in humans through epidemiological studies. Alopecia has been a commonly reported finding in chloroprene workers, but there is no quantitative information on the exposure conditions under which this occurs. Alopecia has also been observed in both the rat and mouse, but appears to be reversible. In the few epidemiological studies available that have examined non-cancer endpoints, neither hematological, reproductive, or neurological adverse effects have been reported or substantiated.

Nor have any significant neurological or hematological effects been noted in long-term exposures of rats and mice. Mild anemia, considered to be a result of acute blood loss, was observed in $B6C3F_1$ mice exposed at 32 and 80 ppm for 13 weeks; however, neither hematological or immunological parameters were part of the chronic study protocol (NTP, 1998). There was high mortality in all but one of the mouse exposure groups and mortality appeared to be causally associated with the high incidence of neoplastic lesions. Bronchiolar hyperplasia was the principal lesion noted in the mouse chronic study; although it was statistically elevated above controls at all exposure concentrations, this endpoint is best regarded as part of the continuum leading to adenomas/carcinoma of the lung. In chronic exposure of the F344/N rat (NTP, 1998), the principal non-neoplastic histopathological lesion was atrophy and degeneration of the olfactory epithelium. In contrast, neither nasal toxicity, nonneoplastic lesions in other organs, nor mortality was observed in the Wistar strain under chronic exposure conditions (Trochimowicz et al., 1998). The mode of action whereby chloroprene results in nasal lesions is largely unknown, but may relate to metabolism by P450 isozymes in which chloroprene is converted to the monoepoxide, a principal metabolite of chloroprene which has been detected in rat, mouse, and human liver microsomal preparations (Himmelstein, 2000). Microsomes from the 344/N rat appears to produce considerably more epoxide than the Wistar or human microsomes. Neither hematological, immunological, or neurological evaluations were performed in any of these chronic studies. Because of low survival in the two highest dose groups of F344/N rats and in all female $B6C3F_1$ exposure groups, only an FEL can be identified, precluding derivation of an inhalation RfC for either species.

Several studies (EPA/OTS, 1985d; EPA/OTS, 1985e, Mast et al., 1994; Culik et al., 1978) found chloroprene, at levels of 100 ppm and below, not to have effects on reproductive or developmental endpoints in either two strains (Wistar and Charles River)of rats, New Zealand rabbits or male Wistar rats exposed to chloroprene before mating. In one unpublished study (Koeter, 1979), however, exposure of an unspecified strain of pregnant rats to 75 and 175 ppm (purity of chloroprene not stated) was reported to have caused a significant decrease in the number of live fetuses and other endpoints. The author did not consider these effects exposure-related, but did not offer a basis for this conclusion. Reduced sperm motility was noted in male rats exposed to 200 ppm for 13 weeks (NTP, 1996), but this endpoint was not evaluated in the chronic portion of the study. Further research on possible reproductive effects in male laboratory animals is recommended.

4.6. WEIGHT-OF-EVIDENCE VALUATION AND CANCER CHARACTERIZATION

4.6.1. Human

Although the case-control and cohort study (Shouqi et al., 1989) of workers exposed to chloroprene/polychloroprene have reported increased cancer mortality associated with liver and lung neoplasms and lymphoma (only one observed), the evidence should be regarded as inconclusive. Lack of information on smoking and alcohol use confounds cause-effect interpretation. The studies by Bulbuyan et al. (1998,1999) purported to show an association between chloroprene/polychloroprene exposure and liver cancer; however, possible confounding by alcohol use was not addressed. The study by Pell (1978) of chloroprene/polychloroprene workers did not show increased mortality due to cancer although cohort selection factors may have reduced the statistical power of the study. Further studies of workers exposed to chloroprene monomer will be needed to better assess the role of exposure with cancer mortality and incidence.

4.6.2. Animal

The lifetime inhalation cancer study conducted for the NTP (1998) in rodents exposed to chloroprene demonstrated clear evidence of carcinogenicity in the F344/N rat and B6C3F$_1$ mouse with the mouse regarded as the most sensitive species since tumor incidence and multi-site distribution was greater than with the rat. There was decreased survival in both the rat and mouse treated groups with decreased survival in the mouse significantly associated with the neoplastic response; mortality in the rat had no obvious basis. In rats, increased incidences of neoplastic lesions primarily occurred in (1) oral cavity, (2) lung (males only), kidney, and mammary gland (female only). In mice, increased incidences in neoplasms occurred in (1) lung, (2) circulatory system, (3) Harderian gland, (4) forestomach, (5) liver, skin and mesentery (females only), and (6) kidney (males only). In contrast to the neoplastic findings in the F334/N rat, no neoplastic responses were observed. in the Wistar rat or in Syrian Golden hamsters (Trochimowicz et al., 1998).

There is no unequivocal explanation for why the results for the rat differ between these two studies. The stability of the bulk material in the NTP study was monitored throughout using gas chromatography coupled with flame ionization detection and the material was analyzed for peroxide content. In addition, stabilizer concentrations were in an acceptable range and no dimer peaks were found in the distribution lines leading to the exposure chamber. In the study in the Wistar rat by Trochimowicz et al. (1998), there was no evidence of degradation of the freshly-distilled chloroprene and dimer concentrations were stated to be less than the limit of detection. Thus, it appears unlikely that the bulk materials or generated atmospheres differed to an extent that would have caused the differences in results.

Plausible reasons that may account for the divergent results may relate to the difference in the high exposure concentrations (80 *vs.* 50 ppm) between the studies, in susceptibility via metabolic differences (e.g., species difference in the production of the monoepoxide), or as a result of breeding pedigree (F344/N *vs*. Wistar). For example, the highest incidence of neoplasms in the NTP study were observed at 80 ppm, a level 30 ppm higher than in the Trochimowicz et al. (1998) study.

The inhalation study by Dong et al. (1989) found that a 7-month exposure of the Kunming strain of albino mice, a strain reported to have a low spontaneous rate of lung tumor formation, resulted in a chloroprene-associated increase in lung tumors. Although quality assurance procedures regarding histopathology were not reported, these study results are considered to support the findings in the $B6C3F_1$.

In the only long-term oral cancer study (an F_1 generation of inbred BD IV rats given weekly doses of 50 mg chloroprene/kg by gavage), no significant neoplastic effects were reported to have been observed (Ponomarkov and Tomatis, 1980). The number of tumor-bearing animals was similar to controls. Because of the uncertainties involving data presentation and the lack of reporting on chloroprene storage conditions, this study is insufficient for drawing conclusions for risk characterization.

4.6.3. Mode of Action

There are insufficient data to provide a clear indication as to the mode of action either for neoplasm induction or non-neoplastic toxicity. Preliminary results from ongoing in vitro and in vitro research suggests that the species differences in the amount of chloroprene monoepoxide produced (Himmelstein, 2000) may play a key role.

Tumors induced by chloroprene in the lung and Harderian gland of $B6C3F_1$ mice were found to have higher frequencies of K-*ras* (in lung and Harderian gland) and H-*ras* mutations (Harderian gland) in codon 61 than controls (NTP, 1998; Sills et al., 1999). However, the role that these mutations may have in relation to chloroprene-induce increased incidence of these neoplasms is unknown since mutations in other exons of the *ras* gene were not examined nor were mutations in tumor suppressor genes. Chloroprene was without effect on sister chromatid exchange, chromosomal aberrations bone marrow, or assays in micronuclei formation in peripheral blood erythrocytes (NTP, 1996) or micronuclei formation from bone marrow cells (EPA/OTS, 1985g). Similarly, i.p. injection also was without effect on micronuclei formation (bone marrow) and chromosomal aberrations in male $B6C3F_1$ mice (Shelby and Witt, 1995).

In vitro studies with *S. typhimurium* exposed to purified chloroprene in air gave conflicting results with strain TA100 (with and without S9): positive in the study by Bartsch et al. (1979) and Willems (1980) and negative in the study by Westphal et al. (1994). Chloroprene (dissolved in DMSO) also was reported negative in plate incorporation assays with TA100, TA1535, TA1537, and TA98 (Zeiger et al., 1987), while the plate incorporation study

(chloroprene dissolved in DMSO) referenced in EPA/OTS (1985h) reported positive results with TA100 and TA1535, and negative results with TA1538 and TA98. The potential for interaction between DMSO and chloroprene leading to mutagenic products cannot be discounted (Westphal et al., 1994). Both positive (Vogel, 1979) and negative (Foureman et al., 1994) effects of chloroprene on induction of recessive lethal mutations in *D. melanogaster* were reported.

Because of conflicting evidence in *in vitro* assays in bacteria and in *Drosophila* coupled with the inconclusive role of *ras* mutations in mouse neoplasms, solid evidence for the genotoxic potential of chloroprene remains to be established.

4.7. SUSCEPTIBLE POPULATIONS

4.7.1. Possible Childhood Susceptibility

No evidence has been found that suggests children are more susceptible to chloroprene effects than adults. Exposures of children have not been reported and the metabolic fate of chloroprene in humans has not been sufficiently characterized as of yet.

4.7.2. Possible Gender Differences

In lifetime studies conducted in the rat, mouse and hamster, chloroprene was not shown to exhibit any remarkable gender-related differences in effects with the exception of a more pronounced neoplastic response in $B6C3F_1$ female mice compared to males.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

The available data are inadequate to derive an oral RfD for chloroprene. There are no human data involving oral exposure and the only lifetime oral study exposed rats to only one dose (Ponomarkov and Tomatis, 1980).

5.2. INHALATION REFERENCE CONCENTRATION (RFC)

The inhalation reference concentration (RfC) is based on the assumption that thresholds exist for certain toxic effects, such as cellular necrosis, but may not exist for other toxic effects, such as carcinogenicity. In general, the RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

5.2.1. Choice of Principal Study and Critical Effect—With Rationale and Justification

No human epidemiological or occupational studies of chloroprene are available that (1) adequately describe inhalation exposure concentrations, (2) conclusively demonstrate that exposure causes nonneoplastic effects, or (3) account for possible confounding factors such as smoking, alcohol use and exposures to other chemicals.

Inhalation data on subchronic and chronic toxicity of chloroprene are available from several rodent studies (Trochimowicz et al. 1998; Dong et al., 1989; NTP, 1998), but only the NTP (1998) study with F344/N rats and $B6C3F_1$ mice, utilizing both 13-week and 2-year exposures, resulted in a variety of nonneoplastic effects.

Because of low survival in the male F344/N and female $B6C3F_1$ mouse (NTP, 1998) showing a positive trend with increasing exposure, the derivation of an RfC is deemed inadvisable since only an FEL was identified. Survival in study controls was consistent with historical controls. Survival in both the $B6C3F_1$ mouse and F344/N rat is shown in Table 5-1.

The mild behavioral effects and reduced sperm motility seen in high-exposure male rats in the subchronic NTP study were not evaluated in the chronic study nor have they been confirmed by other studies.

5.2.2. Methods of Analysis

Not applicable (see Section 5.2.1).

5.2.3. RfC Derivation—Including Application of Uncertainty Factors (UF) and Modifying Factors (MF)

Not applicable (see Section 5.2.1).

Table 5-1. Animal survival at the end of the NTP (1998) 2-year inhalation exposure study with chloroprene

Chloroprene exposure concentration	0 ppm	12.8 ppm	32 ppm	80 ppm
Male mouse	27/50*	27/50	14/50	13/50
Female mouse	35/50	16/50	1/50	3/50
Male rat	13/50	9/50	5/50†	4/50†
Female rat	29/50	28/50	26/50	21/50

* Number surviving/number exposed.

† p=0.025.

5.3. CANCER ASSESSMENT

The are no adequate human studies that are appropriate for deriving cancer risk because of (1) a lack of reliable exposure data for chloroprene monomer and (2) confounding factors such as smoking, alcohol use, and exposure to other chemicals that limit interpretation of the findings.

The animal data include 2-year inhalation studies in F344/N rats and B6C3F1 mice (NTP, 1998), in which multiple neoplasms (adenomas and carcinomas) were induced at all exposure levels (12.8, 32, and 80 ppm, equal to 46.1, 115, and 288 mg/m^3, respectively), and a 7-month inhalation study in Kunming mice (Dong et al., 1989), in which primarily papilloadenomas (no carcinomas) were induced at all exposure levels (2.9, 19.0, and 189.0 mg/m^3). Because this latter study provided no details of the vapor generating system, storage conditions, or the level of dimers in the test atmospheres, it was not considered adequate for the estimation of risk, despite an apparent effect at exposure levels lower than those used in the NTP study. A 2-year inhalation study in Wistar rats and an 18-month inhalation study in Syrian golden hamsters produced negative results at all exposure levels (10 and 50 ppm) (Trochimowicz et al., 1998). Similarly, a lifetime oral exposure study in BD IV rats produced negative results at the only dose tested (50 mg/kg/week) (Ponomarkov and Tomatis, 1980).

5.3.1. Choice of Study/Data With Rationale and Justification

The NTP (1998) study is the only one of two inhalation lifetime exposure of laboratory animals to chloroprene in which results indicated a treatment-related increase in the incidence of a multiplicity of neoplastic (including carcinogenic) endpoints. The mouse was selected for calculation of the unit risk because (1) early mortality was related to neoplasms, (2) the number of neoplastic sites was greater than in the rat, (3) the dose-response was significantly higher than in the rat and (4) EC10 calculations performed by Melnick et al. (1999) on rat data indicated that values were considerably higher than for the mouse. Groups of male and female B6C3F$_1$ mice were exposed to chloroprene concentrations of 0, 12.8, 32, or 80 ppm chloroprene for 6 hours/day, 5 days/week, for up to 105 weeks. Statistically significant increases in tumor incidence were observed at multiple sites: the circulatory system (hemangiomas, hemangiosarcomas), lung (bronchiolar/alveolar adenomas and carcinomas), forestomach, Harderian gland, kidney (males), skin (females), liver (females), and mammary gland (females). These incidences are provided in Table B-1. Note that statistically significant increases in hemangioma or hemangiosarcoma (male and female), lung cancer incidence (male and female), liver (female), and skin (female) were observed at chloroprene exposure levels down to 12.8 ppm, the lowest level tested (NTP, 1998). Furthermore, survival for all chloroprene-exposed female mice and for male mice in the two higher exposed groups was statistically significantly lower than the corresponding control mice.

5.3.2. Dose-Response Data

Dose-response analysis for carcinogenicity can be an iterative process, especially as in the case of multiple tumor sites associated with the NTP (1998) 2-year mouse study. Quantal dose-response analysis of the more significant tumor sites was carried out as a baseline. Since decreased survival was significantly associated with chloroprene exposure, time-to-tumor analysis is an essential component of the dose-response assessment of the carcinogenic potential of chloroprene. The calculations for the cancer assessment are presented in Appendix B.

For both approaches to dose-response analysis, the exposure concentrations, presented in ppm units in the report, were weighted by time (5 days exposure × 1 week/7 days, 6 hours exposure × 1 day/24 hours) to obtain equivalent continuous exposure, or duration-adjusted, concentrations (see Table B-2). There were no relevant data characterizing internal doses of reactive chloroprene metabolites, or for chloroprene absorption. Under EPA's proposed new cancer risk assessment guidelines (U.S. EPA, 1996), the default adjustment to convert animal exposure concentrations to human equivalent concentrations (HECs) depends upon the critical target (U.S. EPA, 1994b). Under the default methodology (U.S. EPA, 1994b), chloroprene is a Category 3 gas, having thoracic effects, that is, bronchiolar/ alveolar adenomas and carcinomas, and extra-respiratory or systemic effects.

5.3.3. Extrapolation Methods

The GLOBAL86 linearized multistage (LMS) computer algorithm was used to calculate the upper limit on Q(1). The parameters used were the extra risk option and degree of polynomial determined by the algorithm of GLOBAL86. The general model used for the time-to-tumor (or time-to-response) analyses was the multistage Weibull model. The latter analyses were conducted using the computer software TOX_RISK version 3.5 (Crump et al., ICF Kaiser International, Ruston, LA), which is based on Weibull models taken from Krewski et al. (1983). Parameters are estimated using the method of maximum likelihood. Details and results for both models are presented in Appendix B. No oral cancer risk was calculated, due to a lack of adequate data.

5.3.4. Inhalation and Oral Slope Factors and Inhalation Unit Risk

The strongest site-specific dose-response patterns were judged to be the lung tumor incidence for female mice and the hemangiosarcoma and hemangioma incidence for male mice. Under EPA's proposed new cancer risk assessment guidelines (U.S. EPA, 1996), unit cancer risk estimates (quantal approach) for genotoxic chemicals would be derived by straight linear extrapolation to 0 from the LEC_{10} (estimated 95% UCL on the dose corresponding to a 10% extra cancer risk). Using the LEC_{10} generated for the LMS model by GLOBAL86 for the male mouse circulatory system tumors yields a unit cancer risk of 3.2×10^{-5} per $\mu g/m^3$, very similar to the q_1^* (3.4×10^{-5} per $\mu g/m^3$). Using the LEC_{10} for the combined, less common extra-respiratory female mouse tumors yields a unit cancer risk of $8.0 \times 10^{-5}/(\mu g/m^3)$ compared to the corresponding estimate from the female lung tumors of $2.4 \times 10^{-5}/(\mu g/m^3)$. Similar unit estimates (8.6 and $2.6 \times 10^{-5}/\mu g/m^3$, respectively) result when the q_1^* is used. Because single site

evaluations may underestimate the carcinogenic potential, all tumor sites were combined (see table B-4). The q_1* for humans calculated from the combined, less common extra-respiratory female mouse tumors is 6.4 x 10^{-5}/(∶g/m^3), a value slightly lower than the unit risk when tumors were not combined. Similarly, for combined less common male mouse tumors, the q_1* was 4.6 x 10^{-5}/∶g/m^3 compared to the q_1* of 3.4 x 10^{-5}/∶g/m^3 for the cicrculatory system tumors alone.

However, quantal incidence data for total tumor-bearing mice in each exposure group does not fully characterize the cancer potency. Single site evaluations may underestimate the carcinogenic potential of chloroprene, especially in the female mouse. The mouse inhalation bioassay results demonstrate different dose-response relationships for different tumor sites. To assess the characteristics of the dose-response relationships for different tumor sites, time-to-tumor analyses were performed to adjust for competing mortality from cancer at other sites. Complete details are presented in Appendix B. Hemangiomas/hemangiosarcoma in male mice and lung tumors in both sexes were found to convey the greatest amount of extrapolated risk to humans. Hemangioma/hemangiosarcoma in male mice resulted in a q_1* = 6.5 x 10^{-5}/(∶g/m^3) whereas for lung tumors (direct mode of action) the q_1* = 5.9 x 10^{-5}/(∶g/m^3) (females) and 4.1 x 10^{-5}/ (∶g/m^3) (males). Assuming a systemic mode of action, the q_1* = 1.4 x 10^{-4}/(∶g/m^3)(males) and 1.9 x 10^{-4}/ (∶g/m^3). These unit risks are about two-fold higher than those cited above for the quantal analyses.

To get some indication of the total unit risk from multiple tumor sites, assuming the multiple sites are mechanistically independent, the MLEs (and variances) of the unit potency from the Weibull time-to-tumor models were summed (for female and male mouse data) across tumor sites and estimates of the 95% upper bound on the summed unit potency were calculated. The results of these summation analyses are summarized in Table B-8. The resulting 95% UCLs on the unit potency (extra risk) for the total unit risk for female data were 0.48/ppm or 1.3 x 10^{-4}/(∶g/m^3) (direct-mode) and 0.92/ppm or 2.6 x 10^{-4}/(∶g/m^3) (systemic-mode). These latter unit risk estimates represent the best estimates for an upper bound on human extra cancer risk from continuous lifetime exposure to chloroprene, derived from animal data. They reflect the time-to-tumor response as well as the exposure-response relationships for the multiple tumor sites in the most sensitive species.

No potency slope for oral exposure was derived due to lack of adequate data.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

Chloroprene is a volatile, flammable liquid used as a monomer in the manufacture of neoprene rubber, food packaging, automobile parts, and wire coatings. Human toxicity data on chloroprene are limited and are confined mostly to occupational exposures in which levels of exposure (to both monomer and polychloroprene) were generally not known, and in which a variety of confounding factors were present, including possible co-exposure to other chemicals. Collectively, these studies have not provided evidence that exposure to chloroprene monomer leads to neoplastic or non-neoplastic responses. However, a clear association between exposure to chloroprene monomer and evidence of carcinogenicity is apparent in both the F344/N rat and $B6C3F_1$ mouse, but not in the Wistar rat nor in hamsters. Preliminary unpublished data indicates that there are species differences in the amount of chloroprene monoepoxide, a principal metabolite, produced in *in vitro* systems. Significantly less appears to be produced in human liver microsomes than either the rat or mouse.

The mode(s) of action associated with chloroprene carcinogenicity are unknown, but chloroprene has limited genotoxic potential. Genotoxicity studies involving Ames assays and micronucleus tests have been negative with the exception of conflicting results with regard to *S. typhimurium* strain TA100, which may relate to interaction of monomer with the carrier solvent, dimethyl sulfoxide. The observation that chloroprene monomer caused mutations in mouse K-*ras* codons suggests that there may be interaction between metabolic metabolites and DNA that may be important for tumor induction. Because chloroprene is similar in structure to vinyl chloride, butadiene, and isoprene, which have all been demonstrated to cause cancer in animals (vinyl chloride is a known human carcinogen as well), this structure-activity relationship represents additional cause for concern that chloroprene may be carcinogenic in humans.

Based on the weight of evidence (inconclusive evidence in humans, positive results in at least two animal species, carcinogenic evidence in structural analogues, and evidence of oncogene activation in the mouse), chloroprene can be classified as a probable human carcinogen (B2) according to the 1986 guidelines (U.S. EPA, 1986a). According to the 1996 proposed guidelines (U.S. EPA, 1996), inhaled chloroprene is characterized as a an agent that is "likely to produce cancer in humans due to the production or anticipated production of tumors by modes of action that are relevant or assumed to be relevant to human carcinogenicity." The human carcinogenic potential of ingested chloroprene cannot be determined because of inadequate data.

6.2. DOSE RESPONSE

A quantitative estimate of human risk as a result of chronic chloroprene inhalation exposure is based on animal studies because no adequate human inhalation exposure data are available. The lungs and respiratory tract appear to be primary targets for chloroprene toxicity and carcinogenicity in rodents. A quantitative estimate of human risk from chronic oral exposure to chloroprene was not derived due to inadequate information in both animals and humans.

An RfC could not be derived because of treatment-related mortality in all concentration groups of the F344/N male rat and in both sexes of the $B6C3F_1$ mouse. No RfD was determined because of a lack of oral chronic studies.

To estimate the human cancer risk to chloroprene, the Agency has assumed that, in general, the risk is proportional to the dose, and the quantitative risk of cancer which might appear in humans is the same as the risk of lung cancer in the mouse. The best estimate for an upper bound on human extra cancer risk ranges from 0.48/ppm (1.3×10^{-4} per $\mu g/m^3$) to 0.92/ppm (2.6×10^{-4} per $\mu g/m^3$) depending upon whether the mode of action for generating lung tumors involves direct or systemic exposure to chloroprene. These estimates reflect the time-to-tumor response as well as the exposure-response relationships for the multiple tumor sites in the most sensitive species. No cancer assessment for chloroprene ingestion was performed due to lack of adequate studies.

7. REFERENCES

Amblard, P; Faure, J; Martel, J; et al. (1974) Alopecia in a factory making polychloroprene elastomers. Bulletin de la Societe Francaise de Dermatologie et de Syphiligraphie 81:114-115.

Appleman, LM; Dreef-van der Meulen, HC. (1979) Reproduction Study with $-chloroprene vapour in Rats. Report No. 6225. Central Institute for Nutrition and Food Research (CIVO), Netherlands. Unpublished.

Bartsch, H; Malaveille, C; Montesano, R; et al. (1975) Tissue-mediated mutagenicity of vinylidene chloride and 2-chlorobutadiene in *Salmonella typhimurium*. Nature 255:641-643.

Bartsch, H; Malaveille, C; Barbin, A; et al. (1979) Mutagenic and alkylating metabolites of haloethylenes, chlorobutadienes and dichlorobutenes produced by rodent or human liver tissues: Evidence for oxirane formation by P450-linked microsomal mono-oxygenases. Arch Toxicol 41:249-277.

Bascom, R; Fisher, JF; Thomas, RJ; et al. (1988) Eosinophilia, respiratory symptoms and pulmonary infiltrates in rubber workers. Chest 93:154-158.

Bruce, RM (1999) Personal communication to Mark Greenberg, U.S.EPA, July 13.
Bulbulyan, MA; Changuina, OV; Zaridze, DG; et al. (1998) Cancer mortality among Moscow shoe workers exposed to chloroprene (Russia). Cancer Causes Control 9:381-387.

Bulbulyan, MA; Margaryan, AG; Ilychova, SA; et al. (1999) Cancer incidence and mortality in a cohort of chloroprene workers from Armenia. Int J Cancer 81:31-33.

Chen, JL (1990) Case-control study of respiratory cancer deaths among male employees at Louisville plant. du Pont de Nemours and Company, February 1990.

Clary, JJ; Feron, VJ; Reuzel, PGJ. (1978) Toxicity of $-chloroprene (2-chlorobutadiene-1,3): Acute and subacute toxicity. Toxicol Appl Pharmacol 46:375-384.

Culik R; Kelly, DP; Clary, JJ. (1978) Inhalation studies to evaluate the teratogenic and embryotoxic potential of $-chloroprene (2-chlorobutadiene-1,3). Toxicol Appl Pharmacol 44:81-88.

Dearden, JC; Barratt, MD; Benigni, R; et al. (1997) The Development and validation of expert systems for predicting toxicity: The report and recommendations of an ECVAM/ECB workshop (ECVAM workshop 24). ATLA 25: 223-252.

Dong, QA; Xiao, BL; Hu, YH; et al. (1989) Short-term test for the induction of lung tumor in mouse by chloroprene. Biomed Environ Sci 2:150-153.

Drevon, C; Kuroki,T. (1979) Mutagenicity of vinyl chloride, vinylidene chloride and chloroprene in V79 Chinese hamster cells. Mut Res 67:173-182.

EPA/OTS. (1985a) 18-Month inhalation carcinogenicity study of beta-chloroprene in Syrian golden hamsters. Doc #878215012. Contract Number: 878215012. NTIS/OTS0206752.

EPA/OTS. (1985b) Subchronic (26-week) inhalation toxicity study with beta-chloroprene in rats. Doc #878215014. Contract Number: 878215014. NTIS/OTS0206752.

EPA/OTS. (1985c) 2-Year inhalation carcinogenicity study of chloroprene in rats. Doc #878215006. Contract Number: 878215006. NTIS/OTS0206752.

EPA/OTS. (1985d) Reproduction study with beta-chloroprene vapour in rats (preliminary report). Doc #878215011. Contract Number: 878215011. NTIS/OTS0206752.

EPA/OTS. (1985e). Embryotoxicity and teratogenicity studies on inhaled beta-chloroprene. Doc #878214999. Contract Number: 878214999. NTIS/OTS0206752.

EPA/OTS. (1985f) Class B poison test by skin absorption in rabbits. Doc #878214992. Contract Number: 878214992. NTIS/OTS0206752.

EPA/OTS. (1985g) Examination of the potential mutagenicity of beta-chloroprene using the micronucleus test. Doc #878215009. Contract Number: 878215009. NTIS/OTS0206752.

EPA/OTS. (1985h) Mutagenic activity of 1,3-butadiene, 2-chloro in the *Salmonella*/microsome assay. Doc #878215003. Contract Number: 878215003. NTIS/OTS0206752

Foureman, P; Mason, JM; Valencia, R; et al. (1994) Chemical mutagenesis testing in *Drosophila*: X. Results of 70 coded chemicals tested for the National Toxicology Program. Environ Mol Mutagen 23:208-227.

Gahlman, R; Herbold, B; Ruckes,A; et al. (1993) Untersuchungen zur Stabilitat aromatischer Diisocyanate in dimethylsulfoxid (DMSO): Toluylendiisocynat (TDI) und Diphenylmethandiisocyanat (MDI)_ im Ames-test. Zbl Arbeitsmed 43:34-38

Gooch, JJ; Hawn, WF. (1981) Biochemical and hematological evaluation of chloroprene workers. J Occup Med 23:268-272.

Grosjean, D. (1990) Atmospheric chemistry of toxic contaminants 1. Reaction rates and atmospheric persistence. J Air Waste Manag Assoc 40:1397-1402.

Grosjean, D. (1991) Atmospheric chemistry of toxic contaminants. 5. Unsaturated halogenated aliphatics: Allyl chloride, chloroprene, hexachlorocyclopentadiene, vinylidene chloride. J Air Waste Manage Assoc 41:182-189.

Haley, TJ. (1978) Chloroprene (2-chloro-1,3-butadiene) –what is the evidence for its carcinogenicity? Clin Toxicol 13:153-170.

Himmelstein, MW; Turner, MJ; Asgharian,B; et al. (1994) Comparison of blood concentrations of 1,3-butadiene and butadiene epoxides in mice and rats exposed to 1,3-butadiene by inhalation. Carcinogenesis 15: 1479-1486.

Himmelstein, MW; Carpenter,SC; Hinderliter, PM; et al. (2000a) Chloroprene Metabolism and Toxicokinetic Modeling. Presentation at the International symposium for Evaluation of Butadiene, Isoprene, and Chloroprene health Risks. London, September 12-14.

Himmelstein, MW; Gladnick, NL; Donner EM; et al. (2000b) Abstract. In vitro genotoxicity of (1-chloroethenyl)oxirane, a metabolite of chloroprene. Haskell Laboratory for Toxicology and Industrial Medicine, du Pont de Nemours Inc. Paper to be submitted to Chem Biol Interactions.

IARC. International Agency for Research on Cancer. (1999) IARC Monograph Volume 71: Re-evaluation of some Organic Chemicals, Hydrazine and Hydrogen Peroxide. World Health Organization.

Immels, HE; Willems, MI. (1979) Dominant lethal Assay with $-chloroprene in Male Mice. Report No. 5756. Central Institute for Nutrition and Food Research (CIVO), Netherlands. Unpublished.

Jaeger, RJ; Conolly, RB; Reynolds, ES; et al. (1975) Biochemical toxicology of unsaturated halogenated monomers. Environ Health Perspect 11:121-128.

Kirk-Othmer. (1993) Chloroprene. In Kirk-Othmer Encyclopedia of Chemical Technology, Vol 6. John Wiley. pp 70-78.

Koeter, HBWM. (1979) Inhalation Embryotoxicity/teratogenicity Studies with $-chloroprene in Rats (Summary and Tables). CIVO Institute, Netherlands. Unpublished.

Krewski, D; Crump KS, Farmer J; et al. (1983) A comparison of statistical methods for low dose extrapolation utilizing time-to-tumor data. Fundam Appl Toxicol 3: 140-160.

Leet, TL; Selevan, SG. (1982) Mortality analysis of workers exposed to chloroprene. Final report for E.I. Du Pont de Nemours and Company, Inc.

Liu, X-F; Zhong, L-F; Xia, Y-X. (1995) Protective effect of vitamin E on liver damage induced by 2-chloro-1,3-butadiene in rats. Chinese J Pharmacol Toxicol 9:283-286.

Liu, Y-F; Xiao, B-L; Liu, Y-Q; Li, S-Q; Zhan, C-L. (1988) The use of isolated rat hepatocyte in prescreening of hepatotoxicants: Observation on toxic effects of 14 compounds. Biomed Environ Sci 1:308-315.

Lynch, M. du Pont-Dow elastomers. (1999) Personal communication to M. Greenberg, U.S. EPA.

MacGregor, JT; Wehr, CM; Henika, PR; et al. (1990) The in vivo erythrocyte micronuclease test: Measurement at steady state increases assay efficiency and permits integration with toxicity studies. Fundam Appl Toxicol 14:513-522.

Mast, TJ; Evanoff, JJ; Westerberg, RB; et al. (1994) Inhalation developmental toxicology studies: developmental toxicity of chloroprene vapors in New Zealand white rabbits. Prepared for the National Institute of Environmental Health Sciences by Battelle Pacific Northwest Laboratories. PNL-7458.

Melnick, RL; Elwell, MR; Roycroft, JH; et al. (1996) Toxicity of inhaled chloroprene (2-chloro-1,3-butadiene) in F344 rats and B6C3F1 mice. Toxicology 108:79-91.

Melnick, RL; Sills, RC; Portier, CJ; et al. (1999). Multiple organ carcinogenicity of inhaled chloroprene (2-chloro-1,3-butadiene) in F344/N rats and $B6C3F_1$ mice and comparison of dose-response with 1,3-butadiene in mice. Carcinogenesis 20(5):867-878.

National Research Council (1983) Risk Assessment in the Federal Government: Managing the Process. Washington, DC: National Academy Press.

Nieusma, JL; Claffey, DJ; Koop, DR; et al. (1998) Oxidation of 1,3-butadiene to (R-) and (S)-butadiene monoxide by purified recombinant cytochrome P450 2E1 from rabbit, rat and human. Toxicol Lett 95: 123-129.

NTP (1998) Toxicology and Carcinogenesis Studies of Chloroprene (CAS No. 126-99-8) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). National Toxicology Program, NTP TR 467.

NTP (1984) Technical protocol for Sperm Morphology and Vaginal Cytology Evaluations in Toxicity Testing for Rats and Mice. National Toxicology program. 10/31/82 version (updated January, 1984).

Nystrom, AE (1948) Health hazards in the chloroprene rubber industry and their prevention. Acta Med Scand 132 (suppl 219):1-125.

Pell, S. (1978) Mortality of workers exposed to chloroprene. J Occup Med 20(1):21-29.

Plugge, H; Jaeger, RJ. (1979) Acute inhalation toxicity of 2-chloro-1,3-butadiene (chloroprene): Effects on liver and lung. Toxicol Appl Pharmacol 50:565-572.

Ponomarkov, V; Tomatis, L. (1980) Long-term testing of vinylidene chloride and chloroprene for carcinogenicity in rats. Oncology 37:136-141.

Portier,CJ; Hedges, JC; Hoel, DG. (1986) Age-specific models of mortality and tumor onset for historical control animals in the National Toxicology program's carcinogenicity experiments. Cancer Res 46:4372-4378.

Richardson, KA; Peters, MMCG; Wong, BA; et al. (1999) Quantitative and qualitative differences in the metabolism of ^{14}C-1,3-butadiene in rats and mice: Relevance to cancer susceptibility. Toxicol Sci 49: 186-201

Ritter, WL; Carter, AS. (1948) Hair loss in neoprene manufacture. J Ind Hyg Toxicol 30:192-195.

Roeleveld, N; Zielhuis, GA; Gabreëls, F. (1990) Occupational exposure and defects of the central nervous system in offspring: Review. Br J Ind Med 47:580-588.

Romazini, S.; Lavdevant, G; Lutz, JM; et al. (1992) Mortality study in occupational exposure to chloroprene. Arch Mal Prof 8:721-725.

Salnikova, LS (1968) Embryotrophic effect of volatile substances from Mairit latexes. Toksikol. Nov prom Khim Veshchetsv 11: 106-111.

Salnikova, LS; Fomenko, VN (1973) Effect of chloroprene on embryogenesis. Gig Tr Prof Zabol 8: 23-26.

Sanotskii, IV. (1976) Aspects of the toxicology of chloroprene: Immediate and long-term effects. Environ Health Perspect 17:85-93.

Savitz, DA; Sonnenfeld, NL; Olshan, AF. (1994) Review of epidemiologic studies of paternal occupational exposure and spontaneous abortion. Am J Ind Med 25:361-383.

Schrag, SD; Dixon, RL. (1985) Occupational exposures associated with male reproductive dysfunction. George, R. And R.Okun (Ed.). Ann Rev Pharmacol Toxicol 25:567-592.

Schwartz, L. (1945) Skin hazards in the manufacture and processing of synthetic rubber. JAMA 127:389-391.

Shelby, MD. (1990) Results of NTP-sponsored mouse cytogenetic studies on 1,3-butadiene, isoprene, and chloroprene. Environ Health Perspect 86:71-73.

Shelby, MD; Witt, KL. (1995) Comparison of results from mouse bone marrow chromosome aberration and micronucleus tests. Environ Mol Mutagen 25:302-313.

Shouqi, L; Quinan, D; Yingfei, L. (1989) Epidemiologic study of cancer mortality among chloroprene workers. Biomed Environ Sci 2:141-149.

Sills, RC; Hong, HL; Melnick, RL; et al. (1999) High frequency of codon 61 K-*ras* A-T transversions in lung and Harderian gland neoplasms of B6C3F1 mice exposed to chloroprene (2-chloro-1,3-butadiene) for 2 years, and comparisons with the structurally related compounds isoprene and 1,3-butadiene. Carcinogenesis 20:657-662.

Stewart, CA, Jr. (1971) Dimerization of chloroprene and related dimers. J Am Chem Soc 93: 4815-4821.

Summer, KH; Greim, H. (1980) Detoxification of chloroprene (2-chloro-1,3-butadiene) with glutathione in the rat. Biochem Biophys Res Commun 96:566-573.

Tice, RR. (1988) The cytogenetic evaluation of *in vivo* genotoxic and cytotoxic activity using rodent somatic cells. Cell Biol Toxicol 4:475-486.

Tice, RR; Boucher, R; Luke, CA; et al. (1988) Chloroprene and isoprene cytogenetic studies in mice. Mutagenesis 3:141-146.

Trochimowicz, HJ; Löser, E; Feron, VJ; et al. (1998) Chronic inhalation toxicity and carcinogenicity studies on $-chloroprene in rats and hamsters. Inhalation Toxicol 10:443-472.

U.S. EPA. (1986a) U. S. Environmental Protection Agency. Guidelines for carcinogen risk assessment. Federal Register 51 (185):33992-34003.

U.S. EPA. (1986b) U. S. Environmental Protection Agency. Guidelines for the health risk assessment of chemical mixtures. Federal Register 51(185):34014-34025.

U.S. EPA. (1986) U. S. Environmental Protection Agency. Guidelines for mutagenicity risk assessment. Federal Register 51(185):34006-34012.

U.S. EPA. (1988) U. S. Environmental Protection Agency. Recommendations for and documentation of biological values for use in risk assessment. Document no. EPA 600/6-87/800, NTIS PB88-179874/AS.

U.S. EPA. (1991) U. S. Environmental Protection Agency. Guidelines for Developmental Toxicity Risk Assessment. Federal Register 56:63798-63826, Dec. 5.

U.S. EPA. (1994a) U. S. Environmental Protection Agency. Interim policy for particle size and limit concentration issues in inhalation toxicity studies. Washington, DC: Office of Prevention, Pesticides and Toxic Substances. February 1.

U.S. EPA. (1994b) U. S. Environmental Protection Agency. Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry. EPA/600/8-90/066F.

U.S. EPA. (1994c) U. S. Environmental Protection Agency. Peer Review and Peer involvement at the U.S. Environmental Protection Agency. Signed by the U.S. EPA Administrator, Carol A. Browner, June 7.

U.S. EPA. (1995) U. S. Environmental Protection Agency. Use of the Benchmark Dose Approach in Health risk Assessment. EPA/630/R-94/007

U.S. EPA. (1996a) U. S. Environmental Protection Agency. Proposed Guidelines for Carcinogen Risk Assessment. EPA/600/P-92/003C. Washington, DC: National Center for Environmental Assessment.

U.S. EPA. (1996b) U. S. Environmental Protection Agency. Reproductive Toxicity Risk Assessment Guidelines. Federal Register 61(212):56274-56322.

U.S. EPA. (1998a) U. S. Environmental Protection Agency. Science Policy Council Handbook:

Peer Review. EPA/100/B-98/001. Washington, DC: U.S. EPA Office of Science Policy, Office of Research and Development.

U.S. EPA. (1998c). Health risk assessment of 1,3-butadiene. National Center for Environmental Assessment - Washington, DC. NCEA-W-0267.

Vogel, E. (1979) Mutagenicity of chloroprene, 1-chloro-1,3-trans butadiene, 1,4-dichlorobutene-2 and 1,4-dichloro-2,3-epoxybutane in *Drosophila melanogaster*. Mut. Res. 67: 377-381

Ward, JB, Jr; Whorton, EB; Gilmer, PR, Jr; et al. (1982) Liver function among neoprene workers. NTIS/PB82-231044.

Westphal, GA; Blaszkewicz, M; Leutbecher, M; et al. (1994) Bacterial mutagenicity of 2-chloro-1,3-butadiene (chloroprene) caused by decomposition products. Arch Toxicol 68:79-84.

Willems, MI. (1980) Evaluation of $-chloroprene and four chloroprene dimers in the Ames test by atmospheric exposure of the tester strains. Report Number R6392. Central Institute for Nutrition and Food Research (CIVO).

Zeiger, E; Anderson, B; Haworth, S; et al. (1987) *Salmonella* mutagenicity tests: III. Results from the testing of 255 chemicals. Environ Mutagen 9(Suppl 9):1-110.

Zhang,,HE; Qiao, CB; Chen, YL; et al. (1985) Cytogenetic effects on human by chloroprene. Chem Ind Safety Health 1: 10. (In Chinese).

Zhang, R; Zhong, L-F; Xia, Y-X. (1996) Protective effect of vitamin E on liver damage induced by 2-chloro-1,3-butadiene. Biomed Environ Sci 9:71-80.

APPENDIX A. SUMMARY OF EXTERNAL PEER COMMENTS AND DISPOSITION

This appendix will be added after the external peer review.

APPENDIX B. INHALATION CANCER ASSESSMENT CALCULATIONS

In the 1998 NTP inhalation study, groups of male and female B6C3F$_1$ mice were exposed to chloroprene concentrations of 0, 12.8, 32, or 80 ppm chloroprene for 6 hours/day, 5 days/week, for up to 105 weeks. Statistically significant increases in tumor incidence were observed at multiple sites: the circulatory system (hemangiomas, hemangiosarcomas), lung (bronchiolar/alveolar adenomas and carcinomas), forestomach, harderian gland, kidney (males), skin (females), liver (females), and mammary gland (females). These incidences are provided in Table B-1. Note that statistically significant increases in hemangioma or hemangiosarcoma (male), lung cancer incidence (male and female), liver (female), and skin (female) were observed at chloroprene exposure levels down to 12.8 ppm, the lowest level tested (NTP, 1998). Furthermore, survival for all chloroprene-exposed female mice and for male mice in the two higher exposed groups was statistically significantly lower than for the corresponding control mice.

Dose-response analysis for carcinogenicity can be an iterative process, especially in the case of multiple tumor sites, as here. Quantal dose-response analysis of the more significant tumor sites was carried out as a baseline, and for comparison with other chemicals assessed for carcinogenicity, mainly through quantal analysis. Since decreased survival was significantly associated with chloroprene exposure, however, time-to-tumor analysis is an essential component of the dose-response assessment of its carcinogenic potential. Both phases are detailed below.

Exposure Conversions to Human Equivalent Concentrations

For both approaches to dose-response analysis, the exposure concentrations, presented in ppm units in the report, were weighted by time (5 days exposure × 1 week/7 days, 6 hours exposure × 1 day/24 hours) to obtain equivalent continuous exposure, or duration-adjusted, concentrations (see Table B-2). There were no relevant data characterizing internal doses of reactive chloroprene metabolites, or for chloroprene absorption. Under EPA's proposed new cancer risk assessment guidelines (U.S. EPA, 1996), the default adjustment to convert animal exposure concentrations to human equivalent concentrations (HECs) depends upon the critical target (U.S. EPA, 1994).

The HEC for thoracic effects is derived by multiplying the duration-adjusted concentrations by an interspecies dosimetric adjustment for gas:respiratory effects in the thoracic region of the lung, according to the following calculation (U.S. EPA, 1994b):

$$RGDR(TH) = (MV_a/S_a)/(MV_h/S_h)$$

where

RGDR(TH) = regional gas dose ratio for the thoracic (tracheobronchial and pulmonary) area of the lung

MV_a = animal minute volume (mouse = 0.061 m^3/day)

MV_h = human minute volume (20 m^3/day)
S_a = surface area of the thoracic region of the animal lung (mouse = 503.5 cm^2)
S_h = surface area of the thoracic region of the human lung (543,200 cm^2).

Using these default values, the RGDR(TH) = (0.061/503.5)/(20/543200) = 3.3.

For extra-respiratory effects, no adjustment of the duration-adjusted concentrations was made, since the air:blood partition coefficients for mice and humans for chloroprene are unknown, and dosimetry defaults to equivalence of inhalation concentrations across species (U.S. EPA, 1994). By analogy with butadiene (U.S. EPA, 1998), it is possible that the respiratory effects could have resulted from systemic exposure to chloroprene and its derivatives, in which case the HECs used for the extra-respiratory effects would also apply for the lung tumor analysis. The HECs for both direct respiratory effects and systemic effects are listed in Table B-2.

Quantal Dose-Response Analysis

When EPA estimates cancer risks for humans from rodent bioassay data, the risk estimates are most often calculated from the incidence of rodents of the most sensitive species, strain, and sex bearing tumors at any of the sites displaying treatment-attributable increases. For chloroprene, mice were the more sensitive species, with both sexes showing similar dose-response patterns for several tumor types (hemangiomas or hemangiosarcomas, alveolar/ bronchiolar adenomas and carcinomas), with female mice having additional tumor types.

When survival is not significantly affected by exposure, EPA uses the linearized multistage (LMS) model to estimate a 95% upper confidence limit (UCL) incremental lifetime unit cancer risk (extra risk) for humans. The multistage model has the form:

$$P(d) = 1 - \exp [-(q_0 + q_1 d + q_2 d^2 + ... + q_k d^k)],$$

where P(d) represents the lifetime risk (probability) of cancer at dose (i.e., human equivalent exposure concentration, in this case) d, and parameters $q_i \geq 0$, for i=0, 1, ..., k. Note that modest impacts on survival can be addressed by omitting the animals in each treatment group who died before the first occurrence of the tumors being analyzed. Extra risk over the background tumor rate is defined as

$$[P(d) - P(0)] / [1 - P(0)].$$

Point estimates of the dose coefficients (q_is), and consequently the extra risk function, at any dose d are calculated by maximizing the likelihood function with respect to the tumor incidence data. The incremental lifetime unit cancer risk for humans (q_1^*) is defined as the 95% UCL on the parameter q_1, which is the linear dose coefficient, for extra risk. This 95% UCL represents a plausible upper bound for the true risk. The 95% UCL was calculated using the computer program GLOBAL86 (Van Landingham and Howe, 1990). Both the model and the curve-fitting methodology used are described in detail by Anderson et al. (1983).

The strongest site-specific dose-response patterns were judged by inspection to be the lung tumor incidence for female mice (Table B-2), and the hemangiosarcoma and hemangioma incidence for male mice (Table B-3). GLOBAL86 inputs, using the HECs described above and survival-adjusted incidence rates, are also listed in these tables. The q_1* for humans, for continuous lifetime inhalation exposure to chloroprene, calculated from the female lung tumors is 0.31/ppm (8.6×10^{-5} per ：g/m^3) if the mode of action involves systemic exposure, or 0.093/ppm (2.6×10^{-5} per ：g/m^3) if chloroprene acts before entering the circulatory system (Table B-2). Table B-3 shows the q_1* for humans calculated from the male mouse circulatory system tumors is 0.12 per ppm (3.4×10^{-5} per ：g/m^3). These risk estimates are fairly similar, with the q_1* based on male mouse circulatory tumors differing from each of the female mouse lung tumor unit risks by about a factor of two, but closer to the direct-mode lung tumor unit risk. Note that if chloroprene's mode of action for the female lung tumors were a combination of direct and systemic exposure, the unit risk would more likely be intermediate between these two estimates, and still quite similar to the male mouse circulatory system tumor unit risk. Based on this single-site per sex analysis, neither species is clearly more sensitive than the other.

Under EPA's proposed new cancer risk assessment guidelines (U.S. EPA, 1996), unit cancer risk estimates for genotoxic chemicals would be derived by straight linear extrapolation to 0 (no exposure) from the LED_{10} (estimated 95% lower confidence limit the dose corresponding to a 10% extra cancer risk). Using the LEC_{10} generated for the LMS model by GLOBAL86 for these tumors yields unit cancer risks very similar to the q_1^*s already calculated.

So many sites demonstrated significant tumor increases attributable to chloroprene that single site evaluations may underestimate the carcinogenic potential of chloroprene, especially in the case of the female mice. When all of these tumor sites are combined, however, overall background incidence levels for these sites obscure the effects of chloroprene. This 'flattening' of the dose-response relationship results from the inability of the LMS model to allow for (primarily) single tumors in control animals and multiple tumors in treated animals. One approach to assessing the risk of multiple tumor types is to derive risk estimates from responsive sites with low background tumor incidence in female mice: hemangiomas or hemangiosarcomas, mammary gland adenocanthomas or carcinomas, liver carcinomas, and skin and mesentery sarcomas. Under the direct mode of action hypothesis, lung tumors were omitted, since the higher exposure level could not be accommodated by the LMS procedure. Consequently, this combined estimate could still underestimate the overall carcinogenic potential of chloroprene. As in the previous GLOBAL86 analyses, deaths occurring before the earliest occurrence of any of these tumors were omitted from the calculations. In addition, the lung tumors were included in a second analysis, assuming a systemic mode of action is appropriate for all of the tumor types considered. The GLOBAL86 inputs for fitting both sets of incidences are given in Table B-4.

The results of analyzing these combined incidences are provided in Table B-4. The q_1* for humans calculated from the combined, less common extra-respiratory female mouse tumors is 0.23 per ppm (6.4×10^{-5} per ：g/m^3), for continuous lifetime inhalation exposure to chloroprene. This is slightly lower, but similar to the earlier systemic-mode lung tumor-based unit risk (0.31/ppm, or 8.6×10^{-5} per ：g/m^3), and about twofold higher than the unit risk for lung

tumors alone. The unit risk resulting from modeling the combined incidences, and also assuming a systemic mode of action for lung tumors, is 0.40/ppm, a 30% increase over the corresponding unit risk for lung tumors alone.

A similar analysis was carried out for less common tumors in male mice: circulatory system hemangiomas and hemangiosarcomas, forestomach adenomas and carcinomas, harderian gland adenomas and carcinomas, and renal tubule adenomas. Lung tumors were specifically omitted because of their higher background rate in the control animals (13/50=26%, Table B-1). Table B-5 summarizes the inputs and results. The combined unit risk for these tumors was 0.17/ppm (4.6×10^{-5} per $\mu g/m^3$), a 40% increase over the unit risk based on circulatory system tumors alone. The female mouse unit risks accounting for multiple tumors are clearly higher.

The unit cancer risk estimates (95% UCL) derived above are intended to be plausible upper limits on the risk of developing any chloroprene-attributable tumor over a full (70-year) lifetime. They also provide points of comparison with assessments of other chemicals with similar dose-response patterns. However, as noted above, using the quantal incidence data for total tumor-bearing mice in each exposure group does not fully characterize the cancer potency reflected by the mouse bioassay results. First, the methodology does not take into account the fact that many of the mice in the higher exposure groups had tumors at multiple significant sites, only that at least one tumor was observed. Second, the methodology ignores the fact that survival was significantly decreased in female mice exposed to 12.8 ppm or more chloroprene as a result of chloroprene-attributable tumors. The omission of deaths occurring before the first relevant tumor is only a crude adjustment, and does not allow for the possible accelerated occurrence of tumors with increasing exposure. Time-to-tumor analyses conducted for specific tumor sites are presented below and can be used to evaluate the time component of the cancer risk.

Time-to-Tumor Dose-Response Analysis

The mouse inhalation bioassay results demonstrate different dose-response relationships for different tumor sites. To assess the characteristics of the dose-response relationships for different tumor sites, time-to-tumor analyses were performed to adjust for competing mortality from cancer at other sites. These time-to-tumor analyses were conducted from the individual mice data, for sites demonstrating an increased cancer incidence, as noted in the NTP report. Benign and malignant tumors were combined for sites where appropriate. Thus time-to-tumor analyses were performed for lung alveolar/ bronchiolar adenomas or carcinomas; hemangiomas and hemangiosarcomas; harderian gland adenomas; forestomach squamous cell papillomas or carcinomas; and hepatocellular carcinomas, skin sarcomas and mammary gland carcinomas (females). Kidney renal tubule adenomas (males) were not analyzed because the additional mice with tumors detected in the extended evaluation were not individually identified in the NTP report. Tumor types were not combined across sites prior to modeling, because this would interfere with elucidating the different time courses of each tumor type.

The general model used for the time-to-tumor (or time-to-response) analyses was the multistage Weibull model, which has the form

$$P(d,t) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + ... + q_k d^k)*(t - t_0)^z]$$

where P(d,t) represents the probability of a tumor (or other response) by age t (in bioassay weeks) for dose d (i.e., human equivalent exposure), and parameters $z \geq 1$, $t_0 \geq 0$, and $q_i \geq 0$ for i=0, 1, ..., k, where k = the number of dose groups - 1. The parameter t_0 represents the time between when a potentially fatal tumor becomes observable and when it causes death (see below). The analyses were conducted using the computer software TOX_RISK version 3.5 (Crump et al., ICF Kaiser International, Ruston, LA), which is based on Weibull models taken from Krewski et al. (1983). Parameters are estimated using the method of maximum likelihood.

Tumor types were categorized by tumor context as either fatal or incidental tumors, in order to adjust appropriately for competing risks. Incidental tumors are those tumors thought not to have caused the death of an animal, while fatal tumors are thought to have resulted in animal death. Hemangiomas and hemangiosarcomas were treated as fatal tumors, unless observed at the terminal sacrifice, in which case they were considered incidental. Furthermore, these tumors were considered rapidly fatal, and t_0 was set equal to 0, as there were insufficient data to reliably estimate t_0 in any event. Tumors at all other sites were treated as incidental. This is consistent with the determination made by EPA for 1,3-butadiene (U.S. EPA 1998). The work of Portier et al. (1986) analyzing tumor types in NTP historical controls lends support to these tumor context assumptions.

Specific n-stage Weibull models were selected for the individual tumor types for each sex based on the values of the log-likelihoods according to the strategy used by EPA (U.S., 1998). If twice the difference in log-likelihoods was less than a chi-square with degrees of freedom equal to the difference in the number of stages included in the models being compared, then the models were considered comparable and the most parsimonious model (i.e., the lowest-stage model) was selected. Parameter estimates for the time-to-tumor analyses for each tumor type are presented in Table B-6. For all tumor types except the hemangiosarcomas and hemangiomas in female mice, the one-stage Weibull was the preferred model. The hemangiosarcomas and hemangiomas in female mice were best described by the two-stage Weibull model.

Human unit cancer risk (or potency) estimate results (extra risk) are presented in Table B-7. Lung tumors in female mice convey the greatest amount of extrapolated risk to humans, whether or not the mode of action is assumed to be direct, at 0.21/ppm (5.9×10^{-5} per $\mu g/m^3$)) or systemic, at 0.69/ppm (1.9×10^{-4} per $\mu g/m^3$). Hemangiomas/hemangiosarcomas and lung tumors in male mice also convey a similar amount of extrapolated risk to humans: hemangioma/hemangiosarcoma $q_1^* = 0.23$/ppm (6.5×10^{-5} per $\mu g/m^{3)}$ chloroprene exposure; lung tumor $q_1^* = 0.15$/ppm (4.1×10^{-5} per $\mu g/m^3$) by the direct-mode, or 0.49/ppm (1.4×10^{-4} per $\mu g/m^3$) by the systemic mode. Note that the time-to-tumor unit risks for male hemangiomas and hemangiosarcomas, and for female lung tumors, are about twofold higher that their quantal analysis counterparts.

Although the time-to-tumor modeling does help account for decreased survival times in the mice, considering the tumor sites individually still does not convey the total amount of risk potentially arising from the sensitivity of multiple sites. To get some indication of the total unit risk from multiple tumor sites, assuming the multiple sites are mechanistically independent, the MLEs of the unit potency from the Weibull time-to-tumor models were summed across tumor sites and estimates of the 95% upper bound on the summed unit potency were calculated. The TOX_RISK software provides MLEs and 95% UCLs for human risk at various exposure levels, allowing for the calculation of unit potency estimates at those exposure levels.

When the MLEs of unit potency from the female mouse data (Table B-7) were summed across the mouse tumor sites, the MLE of the total unit risk was 0.35/ppm (direct mode of action assumed for the lung tumors) or 0.71/ppm (systemic mode of action for the lung tumors), assuming continuous lifetime chloroprene exposure. Summing the q_1*s across the female mouse tumor sites yielded 0.63/ppm and 1.1/ppm, respectively; this approach is statistically incorrect, however, resulting in overestimates of the upper bounds. A statistically correct 95% upper bound for the total potency was calculated by assuming a normal distribution for the risk estimates, deriving the variance of the risk estimate for each tumor site from its 95% UCL according to the formula

$$95\%\ \text{UCL} = \text{MLE} + 1.645\sigma,$$

where the standard deviation σ is the square root of the variance. The variances were summed across tumor sites to obtain the variance of the sum of the MLEs. The 95% UCL on the sum of the MLEs was calculated from the variance of the sum using the same formula. The resulting 95% UCLs on the unit potency for the total unit risk were 0.48/ppm (direct-mode) and 0.92/ppm (systemic-mode). The results of these summation analyses are summarized in Table B-8.

These unit potencies for the female mouse data were also summed using a Monte Carlo analysis and the software Crystal Ball version 4.0 (Decisioneering, Denver, CO). Normal distributions were assumed for the unit potency for each tumor site, with the mean equal to the MLE and σ as calculated from the above formula. A distribution around the sum of the MLEs was then generated by simulating the sum of unit potencies picked from the distributions for each tumor site (according to probabilities determined by those distributions) 10,000 times. The mean for the sum and the 95th percentile on the distribution were the same as the sum of MLEs and 95% UCL calculated above, as they should be. However, a sensitivity analysis (based on contribution to variance) for the sum incorporating the direct-mode lung tumor unit risk, revealed that variability associated with the circulatory system tumors unit potency estimate was contributing about 50% of the variance in the sum, while the unit risk contributed essentially nothing to the overall sum. Excluding the circulatory system tumors yielded the same MLE of total risk, 0.35/ppm, while 95% UCL decreased slightly to 0.44/ppm. The lung tumors, which contributed the most to that sum, contributed about 42% of the variance, followed by the liver with 35%. For the overall sum incorporating the systemic-mode lung tumor unit risk, the lung unit risk contributed the most to the sum and the variance, at 72%. The other sites had little

impact on the MLE of risk and less on the upper bound. The results of these summation analyses are summarized in Table B-8.

The same analyses were performed summing the estimates of unit potency derived from the male mouse data for the different tumor sites (from Table B-7). The resulting MLE for the total unit risk was 0.36/ppm lifetime chloroprene exposure, with a 95% UCL of 0.51/ppm., incorporating a direct-mode of action for lung tumors. Circulatory system tumors contributed about 41% to the variance of this sum, and about half of the sum. Consequently, the unit risk for circulatory system tumors was retained in the sum. Alternatively, for a systemic mode of action for lung tumors, the MLE for the total unit risk was 0.61/ppm lifetime chloroprene exposure, with a 95% UCL of 0.76/ppm. As with the parallel analysis for female mice, this site was the single most significant contributor to the total unit risk, assuming a systemic mode of action. The results of these summation analyses are also summarized in Table B-8.

Discussion

Based on the analyses discussed above, the best estimate for an upper bound on human extra cancer risk from continuous lifetime exposure to chloroprene, derived from animal data, is about 0.48/ppm (1.3×10^{-4} per $\mu g/m^3$), or 0.92/ppm (2.6×10^{-4} per $\mu g/m^3$) depending upon whether the mode of action for generating lung tumors involves direct or systemic exposure to chloroprene. These estimates reflect the time-to-tumor response as well as the exposure-response relationships for the multiple tumor sites in the most sensitive species.

Note that Melnick et al. (1999) have reported the EC10 for chloroprene to be 0.3 ppm, based on a analysis of female mouse lung tumors, adjusted for survival. The corresponding EC10 in this analysis is reported in Table B-2 at 0.4 ppm, LEC10 at 0.3 ppm. When time-to-tumor was incorporated in the analysis, the EC10 for lung tumors (systemic-mode) decreased to 0.2 ppm (0.7 mg/m^3, Table B-7). On a site-specific basis, this analysis is in general agreement with that of Melnick et al. (1999).

The greatest source of uncertainty in these estimates is from the interspecies extrapolation of risk from the mouse to humans. The two rodent species for which bioassay data were available—the mouse and the rat—varied significantly in their carcinogenic responses to chloroprene, in terms of both site specificity and magnitude of response. The mouse was the more sensitive species to the carcinogenic effects of chloroprene exposure and, hence, the more conservative (i.e., public health protective) for the extrapolation of risk to humans. Note that EPA's risk assessment for 1,3-butadiene included some human data which resulted in unit risk of 0.03/ppm, while the tumor-specific unit risks based on animal data were very similar to those calculated for chloroprene in this analysis (U.S. EPA, 1998).

In addition to uncertainties pertaining to the relevance of the rodent models to human risk, there is uncertainty in quantitatively scaling the animal risks to humans. Ideally, a PBPK model for the internal dose(s) of the reactive metabolite(s) would decrease some of the quantitative uncertainty in interspecies extrapolation; however, none is available.

Another major source of uncertainty in the unit potency estimates is the extrapolation of high-dose risks observed in the mouse bioassay to lower doses that would be of concern from human environmental exposures. A multistage Weibull time-to-tumor model was the preferred model because it can take into account the differences in mortality between the exposure groups in the mouse bioassay; however, it is unknown how well this model is predicting the low-dose extrapolated risks for chloroprene.

References

Krewski, D; Crump KS; Farmer J; et al. (1984) A comparison of statistical methods for low dose extrapolation utilizing time-to-tumor data. Fundam. Appl. Toxicol. 3: 140-160.

Melnick, RL; Sills, RC; Portier, CJ; et al. (1999). Multiple organ carcinogenicity of inhaled chloroprene (2-chloro-1,3-butadiene) in F344/N rats and B6C3F_1 mice and comparison of dose-response with 1,3-butadiene in mice. Carcinogenesis. 20(5):867-878.

NTP (1998) Toxicology and Carcinogenesis Studies of Chloroprene (CAS No. 126-99-8) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). National Toxicology Program, NTP TR 467.

Portier, CJ; Hedges, JC; Hoel, DG. (1986) Age-specific models of mortality and tumor onset for historical control animals in the National Toxicology Program's carcinogenicity experiments. Cancer Res 46:4372-4378.

U.S. EPA. (1994) U. S. Environmental Protection Agency. Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry. EPA/600/8-90/066F.

U.S. EPA. (1996) U. S. Environmental Protection Agency. Proposed Guidelines for Carcinogen Risk Assessment. EPA/600/P-92/003C. Washington, DC: National Center for Environmental Assessment

U.S. EPA. (1998). Health risk assessment of 1,3-butadiene. National Center for Environmental Assessment - Washington, DC. NCEA-W-0267.

Van Landingham, C.; Howe, RB. (1990) GLOBAL86. ICF Kaiser International, Ruston, LA.

Table B-1. Tumor incidence in female and male mice exposed to chloroprene via inhalation (NTP, 1998)

Sex	Tissue	Administered Chloroprene Concentration			
		Control	12.8 ppm	32 ppm	80 ppm
Females	Circulatory system: hemangioma or hemangiosarcoma	4/50	6/49	18/50	8/50
	Lung: alveolar/bronchiolar adenoma, carcinoma	4/50	28/49	34/50	42/50
	Liver: hepatocellular carcinoma	4/50	11/49	14/50	19/50
	Forestomach: squamous cell papilloma or carcinoma	1/50	0/49	0/50	3/50
	Harderian gland: adenoma	1/50	3/49	3/50	8/50
	Mammary gland: carcinoma and adenocanthoma	3/50	5/49	10/50	14/50
	Skin: sarcoma	0/50	11/49	11/50	18/50
	Mesentery: sarcoma	0/50	4/49	8/50	3/50
Males	Circulatory system: hemangioma or hemangiosarcoma (excluding liver)	1/50	12/50	18/50	17/50
	Lung: alveolar/bronchiolar adenoma, carcinoma	13/50	28/50	36/50	43/50
	Forestomach: squamous cell papilloma	1/50	0/50	2/50	4/50
	Harderian gland: adenoma, carcinoma	2/50	5/49	10/50	12/50
	Kidney: renal tube adenoma, standard and extended evaluations combined	0/50	2/49	3/50	9/50

Table B-2. Summary of quantal analysis of female mice lung tumor incidence, adjusted for survival

Dose-response data	Administered Concentration (ppm)	Adjusted incidence[a]:	Human Equivalent Concentration (ppm)	
			for Systemic Effects[b]	for Direct Respiratory Effects[c]
	0 12.8 32 80	4/49 28/47 34/49 42/48	0 2.3 5.7 14.3	0 7.5 18.9 47.1
Results[d]	MLEs of dose coefficients[e]:		q_0 =0.1008 q_1 =0.2380/ppm	q_0 =0.1008 q_1 =0.0722/ppm
	p-value for chi-square goodness of fit		0.015	0.015
	q_1* (95% UCL on extra risk, at 1 ∶g/m^3)		3.1 x 10^{-1}/ppm, or 8.6 x 10^{-5}/(∶g/m^3)[d]	9.3 x 10^{-2}/ppm, or 2.6 x 10^{-5}/(∶g/m^3)
	MLE of extra risk at 1 ∶g/m^3		6.6 x 10^{-5}/(∶g/m^3)	2.0 x 10^{-5}/(∶g/m^3)
	EC_{10}		0.4 ppm, or 1.6 mg/m^3	1.5 ppm, or 5.3 mg/m^3
	LEC_{10} (lower 95% bound on exposure at 10% extra risk)		0.3 ppm, or 1.2 mg/m^3	1.1 ppm, or 4.1 mg/m^3
	$0.1/LEC_{10}$ (slope from POD to background)		2.8 x 10^{-1}/ppm, or 8.0 x 10^{-5}/(∶g/m^3)	8.8 x 10^{-2}/ppm , or 2.4 x 10^{-5}/(∶g/m^3)

[a] Deaths occurring before the first observed tumor, Week 47 for lung tumors, were omitted.
[b] Adjusted to continuous exposure by multiplying by 6/24 (hours) x 5/7 (days) = 0.178.
[c] Multiplied continuous exposure by RGDR(TH) = 3.3.
[d] Results of fitting the 3 lower dose groups, using GLOBAL86; the model fit was poor when the high dose was included (p=0.008).
[e] $P(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + ... + q_k d^k)]$, where d is ppm chloroprene.
[f] 1 ppm chloroprene = 3.6 mg/m^3.

Table B-3. Summary of quantal analysis of male mice hemangioma and hemangiosarcoma incidence, adjusted for survival

Dose-response data	Administered concentration (ppm)	Adjusted incidence[a]	Human Equivalent Concentration (ppm)
	0	1/50	0
	12.8	12/49	2.3
	32	18/48	5.7
	80	17/48	14.3
Results[b]	MLEs of dose coefficients[c]:		q_0 =0.0230 q_1 =0.0885/ppm
	p-value for chi-square goodness of fit		0.20
	q_1* (95% UCL on extra risk, at 1 ∶g/m^3)		1.2 x 10^{-1}/ppm, or 3.4 x 10^{-5}/(∶g/m^3)[d]
	MLE of extra risk at 1 ∶g/m^3		2.5 x 10^{-5}/(∶g/m^3)
	EC_{10}		4.3 mg/m^3
	LEC_{10} (lower 95% bound on exposure at 10% extra risk)		3.1 mg/m^3
	$0.1/LEC_{10}$ (slope from POD to background)		1.2 x 10^{-1}/ppm , or 3.2 x 10^{-5}/(∶g/m^3)

[a] Deaths occurring before the first observed tumor, Week 65, were omitted.
[b] Results of fitting the 3 lower dose groups; the model fit was poor when the high dose was included (p=0.004).
[c] $P(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + ... + q_k d^k)]$, where d is ppm chloroprene.
[d] 1 ppm chloroprene = 3.6 mg/m^3.

Table B-4. Summary of quantal analysis of female mice, multiple tumor incidence[a] adjusted for survival

Dose-response data	Administered concentration (ppm)	HEC (ppm)	Survival adjusted incidence[a]:	
			Extra-respiratory tumors	and lung tumors
	0 12.8 32 80	0 2.3 5.7 14.3	9/49 29/50 37/50 44/49	14/49 37/50 43/50 48/49
Results	MLEs of dose coefficients :		q_0 =0.2553[b] q_1 =0.1779/ppm	q_0 =0.3780 q_1 =0.3035/ppm
	p-value for chi-square goodness of fit		0.09	0.17
	q_1* (95% UCL on extra risk, at 1 μg/m^3)		2.3 x 10^{-1}/ppm, or 6.4 x 10^{-5}/(μg/m^3)[c]	4.0 x 10^{-1}/ppm, or 1.1 x 10^{-4}/(μg/m^3)
	MLE of extra risk at 1 ppb		4.9 x 10^{-5}/(μg/m^3)	8.4 x 10^{-5}/(μg/m^3)
	EC_{10}		2.1 mg/m^3	1.2 mg/m^3
	LEC_{10} (lower 95% bound on exposure at 10% extra risk)		1.7 mg/m^3	1.0 mg/m^3
	$0.1/LEC_{10}$ (slope from POD to background)		2.2 x 10^{-1}/ppm, or 6.0 x 10^{-5}/(μg/m^3)	3.8 x 10^{-1}/ppm, or 1.0 x 10^{-1}/(μg/m^3)

[a] Extra-respiratory tumors: circulatory system hemangiomas and hemangiosarcomas, mammary adenocanthomas and carcinomas, liver carcinomas, and skin and mesentery sarcomas. Deaths occurring before Week 31, when the first hemangiosarcoma was observed, were omitted.

[b] $P(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + ... + q_k d^k)]$, where d is ppm chloroprene.

[c] 1 ppm chloroprene = 3.6 mg/m^3.

Table B-5. Summary of quantal analysis of male mice, extra-respiratory tumor incidence, adjusted for survival

Dose-response data	Administered concentration (ppm)	HEC (ppm)	Adjusted incidence[a]:
	0 12.8 32 80	0 2.3 5.7 14.3	3/50 16/49 25/48 25/48
Results[b]	MLEs of dose coefficients[c]:		q_0 =0.0656 q_1 =0.1255/ppm
	p-value for chi-square goodness of fit		0.30
	q_1* (95% UCL on extra risk, at 1 $\mu g/m^3$)		1.7×10^{-1}/ppm , or $4.6 \times 10^{-5}/(\mu g/m^3)$[d]
	MLE of extra risk at 1 $\mu g/m^3$		$3.5 \times 10^{-5}/(\mu g/m^3)$
	EC_{10}		3.0 mg/m^3
	LEC_{10} (lower 95% bound on exposure at 10% extra risk)		2.2 mg/m^3
	$0.1/LEC_{10}$ (slope from POD to background)		1.6×10^{-2}/ppm , or $4.5 \times 10^{-5}/(\mu g/m^3)$

[a] Circulatory system hemangiomas and hemangiosarcomas, forestomach adenomas and carcinomas, harderian gland adenomas and carcinomas, and renal tubule adenomas. Deaths occurring before Week 65, when the first hemangiosarcoma was observed, were omitted.

[b] Results of fitting the 3 lower dose groups; the model fit was poor when the high dose was included ($p<0.01$).

[c] $P(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + ... + q_k d^k)]$, where d is ppm chloroprene.

[d] 1 ppm chloroprene = 3.6 mg/m^3.

Table B-6. Parameter estimates for multistage Weibull time-to-tumor model based on female mouse tumor incidence

Tumor type		Model Parameter Estimates[a]			
		q_0	q_1	q_2	z
Female mice					
Circulatory system: hemangioma or hemangiosarcoma[b]		4.15×10^{-14}	0.0	1.66×10^{-14}	6.10
Lung: alveolar/bronchiolar adenoma, carcinoma	Direct-mode	2.46×10^{-9}	3.79×10^{-9}	-	3.77
	Systemic mode	2.63×10^{-9}	1.33×10^{-8}	-	3.75
Liver: hepatocellular carcinoma		1.09×10^{-8}	7.91×10^{-9}	-	3.48
Forestomach: squamous cell papilloma or carcinoma		2.15×10^{-9}	1.18×10^{-9}	-	3.33
Harderian gland: adenoma		8.94×10^{-9}	8.29×10^{-9}	-	3.18
Mammary gland: carcinoma		6.61×10^{-4}	2.67×10^{-4}	-	1.00
Skin: sarcoma		0.0	4.85×10^{-5}	-	1.54
Male mice					
Circulatory system: hemangioma or hemangiosarcoma		3.49×10^{-22}	5.64×10^{-22}	-	10.0
Lung: alveolar/bronchiolar adenoma, carcinoma	Direct-mode	4.01×10^{-8}	7.56×10^{-9}	-	3.46
	Systemic mode	4.01×10^{-8}	2.50×10^{-8}	-	3.46
Forestomach: squamous cell papilloma		3.06×10^{-6}	1.32×10^{-6}	-	1.79
Harderian gland: adenoma		3.28×10^{-13}	2.03×10^{-13}	-	5.57

[a] $P(d,t) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + ... + q_k d^k)*(t - t_0)^z]$, where d is ppm chloroprene, t is weeks until death with tumor.
[b] High dose dropped due to poor fit

Table B-7. Human unit cancer risk estimates (extra risk, computed for risks of 10^{-6}) based on mouse tumor incidences, using multistage Weibull time-to-tumor model

Tumor Type		MLE		q_1*		EC_{10}, mg/m^3	LEC_{10}, mg/m^3	0.1/LEC_{10}	
		/ppm	/(:g/m^3)	/ppm	/(:g/m^3)			/ppm	/(:g/m^3)
Female mice									
Circulatory system		1.9×10^{-4}	5.2×10^{-8}	9.1×10^{-2}	2.5×10^{-5}	6.3	3.5	1.0×10^{-1}	2.8×10^{-5}
Lung: alveolar/bronchiolar adenoma, carcinoma	Direct-mode[a]	1.5×10^{-1}	4.3×10^{-5}	2.1×10^{-1}	5.9×10^{-5}	2.5	1.8	2.0×10^{-1}	5.6×10^{-5}
	Systemic-mode[a]	5.1×10^{-1}	1.4×10^{-4}	6.9×10^{-1}	1.9×10^{-4}	0.7	0.6	6.6×10^{-1}	1.8×10^{-4}
Liver: hepatocellular carcinoma		8.4×10^{-2}	2.3×10^{-5}	1.4×10^{-1}	3.8×10^{-5}	4.5	2.8	1.3×10^{-1}	3.6×10^{-5}
Forestomach: squamous cell papilloma or carcinoma		6.4×10^{-3}	1.8×10^{-6}	2.0×10^{-2}	5.6×10^{-5}	59.7	18.8	1.9×10^{-2}	5.3×10^{-6}
Harderian gland: adenoma		2.3×10^{-2}	6.3×10^{-6}	4.8×10^{-2}	1.3×10^{-5}	16.8	7.9	4.5×10^{-2}	1.3×10^{-5}
Mammary gland: adenocanthoma or carcinoma		2.8×10^{-2}	7.8×10^{-6}	4.3×10^{-2}	1.2×10^{-5}	13.5	8.9	4.0×10^{-2}	1.1×10^{-5}
Skin: sarcoma		6.3×10^{-2}	1.7×10^{-5}	9.2×10^{-2}	2.6×10^{-5}	6.0	4.1	8.8×10^{-2}	2.4×10^{-5}
Male mice									
Circulatory system		1.8×10^{-1}	5.1×10^{-5}	2.3×10^{-1}	6.5×10^{-5}	2.1	1.6	2.2×10^{-1}	6.2×10^{-6}
Lung: alveolar/bronchiolar adenoma, carcinoma	Direct-mode[a]	1.1×10^{-1}	3.1×10^{-5}	1.5×10^{-1}	4.1×10^{-5}	3.5	2.5	1.4×10^{-1}	3.9×10^{-5}
	Systemic-mode[a]	3.6×10^{-1}	1.0×10^{-4}	4.9×10^{-1}	1.4×10^{-4}	1.0	0.8	4.7×10^{-1}	1.3×10^{-4}
Forestomach: squamous cell papilloma		5.9×10^{-3}	1.6×10^{-6}	2.3×10^{-2}	6.3×10^{-6}	64.1	16.7	2.2×10^{-2}	6.0×10^{-6}
Harderian gland: adenoma		6.8×10^{-2}	1.9×10^{-5}	1.1×10^{-1}	3.0×10^{-5}	5.6	3.5	1.0×10^{-1}	2.9×10^{-5}

[a] See Table B-2 for human equivalent doses.

Table B-8. Unit potency estimates (extra risk) summed across tumor sites

Tumor set		Estimates of Extra Risk (calculated at 1 ppb)		
		Sum of MLEs of extra risk, /ppm	**Sum of q_1*s, /ppm**	**95% UCL on sum, /ppm**
Female mice	All (lung, direct-mode) [a]	0.35	0.63	0.48
	All (lung, direct-mode) except circulatory system tumors	0.35	0.54	0.44
	All (lung, systemic-mode)	0.71	1.12	0.92
Male mouse	All (lung, direct-mode[b]	0.36	0.51	0.44
	All (lung, systemic-mode)	0.61	0.85	0.76

[a] Circulatory system, lung, liver, forestomach, harderian gland, mammary gland, skin.
[b] Circulatory system, lung, forestomach, harderian gland