FINAL

REVIEWER COMMENTS

External Peer Review Meeting on the *Toxicological Review of Chloroprene* (CAS No. 126-99-8)

Prepared for:
Allen Davis, M.P.H.
National Center for Environmental Assessment
U.S. Environmental Protection Agency
109 T.W. Alexander Drive
Research Triangle Park, NC 27711

Prepared by:
Versar, Inc.
Contract No. EP-C-07-025
Task Order 69

Peer Reviewers:
Herman J. Gibb, Ph.D., M.P.H.
Dale Hattis, Ph.D.
Ronald L. Melnick, Ph.D.
John B. Morris, Ph.D.
Avima M. Ruder, Ph.D.
Richard B. Schlesinger, Ph.D.

January 26, 2010

TABLE OF CONTENTS

I. INTRODUCTION

The Integrated Risk Information System (IRIS) is an EPA database containing Agency consensus scientific positions on potential adverse human health effects that may result from chronic (or lifetime) exposure, or in select cases less-than-lifetime exposures, to chemicals in the environment. IRIS currently provides health effects information on over 500 chemical substances. IRIS contains chemical-specific summaries of qualitative and quantitative health information in support of two steps of the risk assessment process, i.e., hazard identification and dose-response evaluation. IRIS information includes a reference dose (RfD) for noncancer health effects resulting from oral exposure, a reference concentration (RfC) for noncancer health effects resulting from inhalation exposure, and an assessment of carcinogenicity for both oral and inhalation exposures. Combined with specific situational exposure assessment information, the health hazard information in IRIS may be used as a source in evaluating potential public health risks from environmental contaminants.

The IRIS program developed a Toxicological Review of Chloroprene, an assessment which has not previously appeared in IRIS. Chloroprene was nominated for IRIS assessment in 1999. The draft document contains a chronic inhalation reference concentration (RfC) and a cancer inhalation unit risk.

Peer Reviewers:

Herman J. Gibb, Ph.D., M.P.H.
Tetra Tech Sciences
Arlington, VA 22201

Dale Hattis, Ph.D.
Clark University
Worcester, MA 01610

Ronald L. Melnick, Ph.D.
Ron Melnick Consulting, LLC
Chapel Hill, NC 27514

John B. Morris, Ph.D.
University of Connecticut
Storrs, CT 06269

Avima M. Ruder, Ph.D.
National Institute for Occupational Safety and Health (NIOSH)
Cincinnati, OH 45226

Richard B. Schlesinger, Ph.D.
Pace University
Pleasantville, NY 10570

II. CHARGE TO THE REVIEWERS

The U.S. Environmental Protection Agency (EPA) is seeking an external peer review of the scientific basis supporting the human health assessment of chloroprene that will appear on the Agency's online database, the Integrated Risk Information System (IRIS). IRIS is prepared and maintained by the EPA's National Center for Environmental Assessment (NCEA) within the Office of Research and Development (ORD). Currently an IRIS assessment of chloroprene does not exist on the database.

The draft health assessment includes a chronic reference concentration (RfC) and a carcinogenicity assessment. Below are a set of charge questions that address scientific issues in the assessment of chloroprene. Please provide detailed explanations for responses to the charge questions.

General Charge Questions:

1. Is the Toxicological Review logical, clear and concise? Has EPA clearly synthesized the scientific evidence for noncancer and cancer hazards?

2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of chloroprene.

Chemical-Specific Charge Questions:

(A) Oral Reference Dose (RfD) for Chloroprene

1. An RfD was not derived for chloroprene. Has the scientific justification for not deriving an RfD been clearly described in the document? Please identify and provide the rationale for any studies that should be selected as the principal study.

(B) Inhalation Reference Concentration (RfC) for Chloroprene

1. A chronic RfC for chloroprene has been derived from an inhalation toxicity study (NTP, 1998) investigating non-cancer effects in multiple organ systems. Please comment on whether the selection of this study as the principal study is scientifically justified. Please identify and provide the rationale for any other studies that should be selected as the principal study.

2. An increase in the incidence of degenerative nasal lesions in male rats, characterized by olfactory epithelial atrophy and/or necrosis with increasing severity, was selected as the critical effect. Please comment on the scientific justification for combining the incidence of atrophy and necrosis and for selecting this endpoint as the critical effect. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

3. Benchmark dose (BMD) modeling was used to define the point of departure (POD) for the derivation of the RfC. The POD was based on increased incidence of degenerative nasal lesions in male rats at a benchmark response (BMR) of 10% extra risk. Has the BMD approach been appropriately conducted? Is the BMR selected for use in deriving the POD (i.e., 10% extra risk of degenerative nasal lesions of less than moderate severity) scientifically justified? Please identify and provide the rationale for any alternative approaches (including the selection of the BMR, model, etc.) for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.

4. Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD for the derivation of the RfC. If changes to the selected UFs are proposed, please identify and provide a rationale(s).

(C) Carcinogenicity of Chloroprene

1. Under the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (www.epa.gov/iris/backgr-d.htm), the Agency concluded that chloroprene is *likely to be carcinogenic to humans* by all routes of exposure. Please comment on the cancer weight of evidence characterization. Is the cancer weight of evidence characterization scientifically justified?

2. A two-year inhalation cancer bioassay in B6C3F1 mice (NTP, 1998) was selected as the basis for derivation of an inhalation unit risk (IUR). Please comment on whether the selection of this study for quantification is scientifically justified. Please identify and provide the rationale for any other studies that should be selected as the basis for quantification.

3. A mutagenic mode of carcinogenic action is proposed for chloroprene. Please comment on whether the weight of evidence supports this conclusion. Please comment on whether this determination is scientifically justified. Please comment on data available for chloroprene that may support an alternative mode(s) of action.

4. Data on hemangiomas/hemangiosarcomas (in all organs) and tumors of the lung (bronchiolar/alveolar adenomas and carcinomas), forestomach, Harderian gland (adenomas and carcinomas), kidney (adenomas), skin and mesentery, mammary gland and liver in B6C3F1 mice were used to estimate the inhalation unit risk. Please comment on the scientific justification and transparency of this analysis. Has the modeling approach been appropriately conducted? Please identify and provide the rationale for any alternative approaches for the determination of the inhalation unit risk and discuss whether such approaches are preferred to EPA's approach.

5. Lung tumors have been alternatively treated as systemic or portal-of-entry effects in the modeling of cancer endpoints. Please comment on the scientific justification for this modeling approach. Please comment on whether the rationale for this decision has been transparently and objectively described. Please comment on data available

for chloroprene that may support an alternative method for modeling the observed lung tumors in mice.

6. An oral slope factor (OSF) for cancer was not derived for chloroprene. Is the determination that the available data for chloroprene do not support derivation of an OSF scientifically justified?

III. GENERAL IMPRESSIONS

Herman J. Gibb

In general, the document lays out its arguments well. The discussion of the epidemiology, however, should be more transparent and perhaps could be better organized (studies of a facility where cohorts overlap or could overlap discussed together). Elaboration on the transparency is provided in my response to Question C1. The epidemiologic studies should be evaluated more rigorously.

Dale Hattis

Overall, the judgments made in the draft IRIS document for chloroprene are sound. However the modeling of the cancer risk can be improved by taking into account the existing evidence for partial saturation of metabolic activation of chloroprene in the dose range studied in the NTP cancer bioassay. Using a simple Michaelis Menten dose response equation to model this approach to saturation indicates that low dose cancer risks in both the male and female mouse bioassays are likely to be 2-3 fold greater than the risks indicated by application of a straight linear dose response model, as was done using the Weibull equation in the current cancer slope factor analysis. For the final assessment it would be desirable either to incorporate the Michaelis-Menten saturating form into the Weibull model or (less desirably) to multiply the Weibull model result by a factor derived from the Michaelis-Menten analysis of the lifetime tumor incidence information. The former approach is preferable because it will simultaneously take into account the time-to-tumor information and the apparent saturation of activating metabolism indicated by the incidence data.

Ronald L. Melnick

The draft document is a well-written, comprehensive review and assessment of published studies on the health effects of chloroprene in humans and in experimental animals. The information is clearly presented and the conclusions are generally scientifically justified and consistent with EPA policy. One exception is the rationale for the selection of 10% extra risk for the benchmark response. Specific areas for improvement of this review are described below in my response to the “chemical-specific charge questions.”

John B. Morris

From my perspective as an inhalation toxicologist with expertise in rodent studies, the Toxicological Review of Chloroprene provides an in depth review of the toxicological literature on this compound. In many ways it is quite clear and thorough. The available database appears to be presented accurately and objectively. The overall conclusion, that chloroprene is an animal carcinogen whose mechanism(s) may include genotoxicity and mutagenesis, appears well founded. In some aspects, the document is confusing and perhaps lacks transparency. For example, information is provided in the summary and synthesis sections that have not been discussed previously. There are some apparent

contradictions in interpretive approaches, for example the potential for systemic blood delivery for the pulmonary but not nasal effects. The importance of some findings has gone unrecognized. For example, the extraordinarily high pulmonary metabolism rates in the mouse calls into question the relevance of this species with respect to pulmonary injury. Overall, the fundamental conclusions appear sound; however, the document could be significantly improved with respect to clarity and interpretive issues.

It is interesting that there are no charge questions relating to the toxicokinetics of chloroprene. Since the mode of action includes activation to an epoxide as the first step, the toxicokinetics becomes an issue of great importance. The toxicokinetic section describes the available information, but could provide much more information. Moreover, the toxicokinetic data is not adequately synthesized in the overall mode of action relative to potential species differences and extrapolation to man. PBPK modeling would be a highly appropriate way to incorporate kinetic data into the risk assessment. The published model of Himmelstein may provide a useful structure. Because it includes both nasal and tracheobronchial airway compartments the styrene model of Sarangapani may be a superior approach.

Avima M. Ruder

I can only validate accuracy for the section I compared to the original papers, that on human epidemiology. There are some key relevant references that were not cited and some points that should have been discussed (latency, age at diagnosis, etc.) that were not touched on (see 2.1).

The conclusions about the human hazard potential do not evaluate the role of genetic polymorphism in genes coding for glutathione *S*-transferases, epoxide hydrolase, and other metabolic enzymes in clearing epoxide metabolites from the body. Approximately half the human population is clears those metabolites at a much slower rate [Musak, et al. 2008], presumably making them more vulnerable to exposure. The conclusion also should point out that the noncancer effects (page 6-1, lines 24-33) were observed at levels lower than the current Permissible Exposure Limit.

The statements of conclusions in section 6 are less clear than those in section 4.7. It is appropriate to include all relevant caveats about the conclusions, and all the details of the studies that support those conclusions, but the conclusions themselves should be succinctly stated.

Richard B. Schlesinger

The background information that is provided to support the selection of the key studies is clearly and accurately presented. However, the derivation of some of the quantitative factors, as noted in subsequent comments in this document, could be made more transparent. In general, the overall conclusions appear to be sound.

IV. RESPONSE TO CHARGE QUESTIONS

General Charge Questions:

1. Is the Toxicological Review logical, clear and concise? Has EPA clearly synthesized the scientific evidence for noncancer and cancer hazards?

Herman J. Gibb

In general, the Toxicological Review is logical, clear and concise. A more rigorous and transparent evaluation of the epidemiologic studies and an objective evaluation of how the epidemiologic studies integrate with the rest of the data should be performed, however. The descriptor of "likely to be carcinogenic to humans" is justified based on the animal and genotoxicity information, but the document overstates the human evidence.

Dale Hattis

Generally, yes. I have some reservations and suggestions for incremental improvement, as will be apparent below. But the overall evaluation in the proposed IRIS document is sound.

Ronald L. Melnick

While the Toxicological Review is clear and comprehensive, it is not obvious why a particular dose response model was selected for the determination of the benchmark dose for noncancer hazards, if more than one model provided an adequate fit to the data. The rationale for the selection of 10% extra risk for the benchmark response for non-cancer effects is not adequately justified.

Based on the animal data, mechanistic findings, and "the reasonably consistent" evidence of increased risk of liver cancer mortality "among workers exposed to chloroprene in different cohorts in different continents," it is not clear why consideration was not given to the conclusion that chloroprene is "carcinogenic to humans."

John B. Morris

In many ways, the toxicological review is logical and clear; however, the document could be significantly improved in this regard. See my specific comments (below) for more detail on this concern.

Avima M. Ruder

The review is logical but less clear and concise than it could be. In the section on human carcinogenicity, the discussion should have been consolidated by population and recommendations for additional analyses (by age at onset/death, with lags) and substudies (nested case-control) should have been included. Such analyses should be done as very

early age at cancer onset/death has been associated with occupational exposure [Kreuzer, et al. 1999; Ward, et al. 1988] and lagged analyses focus on exposure in time periods that are most relevant for the development of solid tumors [Villeneuve and Steenland 2010]. All the studies on the Louisville plant should have been discussed together. The original study includes ages at death from lung cancer for 16 workers, including four who died in their forties [Pell 1978], but no analysis of whether the ages at onset were earlier than expected (in another chloroprene cohort, earlier ages at onset among exposed workers were reported [Li, et al. 1989]). The NIOSH walk-through survey of the plant, which was not referenced in the Toxicological Review, provides useful details on plant history, processes, and personnel, noting that "there is a complete pre-employment physical" plus periodic re-examinations (presumably those who did not meet some standard of health were excluded from employment; no details were presented on how the periodic re-examinations impacted continued employment [Jones, et al. 1975]. The NIOSH re-analysis of DuPont demographic data included recommendations for improving the epidemiologic studies by including all plant employees from 1942 on [Leet and Selevan 1982]. Blood draws from 846 of the workers employed in 1977 were compared for biochemical and hematological markers, with no significant differences in age-adjusted analyses [Gooch and Hawn 1981] and workers and plant sites were monitored for exposure, and workers interviewed [McGlothlin, et al. 1984](neither referenced in the Toxicological Review).

One of the more recent University of Pittsburgh papers (not referenced in the Toxicological Review), presents SMRs for the Louisville cohort using the DuPont worker mortality database; these are significantly elevated for all causes of death, all cancers, respiratory cancers, and liver cancer [Leonard, et al. 2007]. Kentucky cancer mortality is significantly higher than U.S. national cancer mortality [U.S. Cancer Statistics Working Group 2009], and the incidence of lung cancer in both Jefferson county and all of Kentucky is almost 50% higher than the U.S. rate [Kentucky Institute of Medicine 2007], so comparisons of a working population to the population at large will show a pronounced healthy worker effect. Presumably an employment-based database would control for the healthy worker effect to some extent. The most recent studies are more comprehensive but could have included additional analyses by age at diagnosis/death, lagged analyses, comparisons with the DuPont employee mortality database, and inclusion of the pre-1949 PYAR [Marsh, et al. 2007a; Marsh, et al. 2007b]. Some discrepancies should be explored; for example, Jones stated that approximately 8000 hourly and 1000 salaried (one-third foremen) employees had been employed to the time of the 1975 visit and over 1000 workers were employed in 1975; the Marsh analysis includes 5507 employees 1949-2000 [Jones, et al. 1975; Marsh, et al. 2007a].

Some discrepancies between the report of a 1985 NIOSH walk-through of the Pontchartrain, Louisiana, plant (neoprene production from 1968, 1264 workers to 1985) and the recent epidemiologic studies (chloroprene from 1969, 1258 workers to 2000) also need to be resolved [Fajen and Ungers 1985; Marsh, et al. 2007a; Marsh, et al. 2007b].

The studies of the plant in Grenoble, Isère, France, should also have been assessed together [Colonna and Laydevant 2001; Marsh, et al. 2007a; Marsh, et al. 2007b].

As to possible human health hazards other than cancer, the two medical studies at the Louisville plant [Gooch and Hawn 1981; McGlothlin, et al. 1984] and the recent study of chromosomal aberrations [Musak, et al. 2008] should be included. Apparently there are no studies of possible human reproductive effects more recent than Sanotskii's in 1976.

Richard B. Schlesinger

In general, the Review is well written and the toxicology of chloroprene is well synthesized.

General Charge Questions:

2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of chloroprene.

Herman J. Gibb

The NIOSH reports by Fajen and Ungers (1985) and by McGlothin et al (1984) should be included as background on the Pontchartrain and Louisville plants, respectively. Copies were provided to the peer reviewers by Avima Ruder subsequent to the peer review meeting on January 6, 2010 and are attached. Dr. Ruder also described references of Jones et al. (1975), Gooch and Hawn (1981), and Leonard et al. (2007) in her comments. Jones et al. (1975) and Gooch and Hawn (1981) describe conditions and the population at the Louisville plant and should be added as background information on that facility. The Leonard et al. paper apparently presents mortality analyses of the Louisville cohort using a Dupont worker mortality database. These papers should be reviewed to determine what insights they may offer to the mortality analyses by Pell (1978), Leet and Selevan (1982) and Marsh et al. (2007a, 2007b).

I am not aware of any additional original studies or reports that should be considered. The following reviews by Acquavella and Leonard (2001) and Bukowski (2009) should at least be given consideration although they need not necessarily be referenced. The review by Acquavella and Leonard (2001) appeared in the same journal as the review by Rice and Boffetta (2001) which is cited in the current Toxicological Review.

Acquavella JF, Leonard RC. 2001. A review of the epidemiology of 1,3-butadiene and chloroprene. Chemico-Biological Interactions 135–136 (2001) 43-52.

Bukowski JA. 2009. Epidemiologic evidence for chloroprene carcinogenicity: review of study quality and its application to risk assessment. Risk Analysis 29(9):1203-16.

Dale Hattis

Probably the most significant omission is an analysis by Dr. DeWoskin of EPA of the potential to use a PBPK model for estimation of human vs. mouse and rat delivered doses in modeling cancer dose response relationships for chloroprene. Its omission from the list of references is surprising. The abstract of this paper I retrieved from a MEDLINE search is:

PBPK models in risk assessment--A focus on chloroprene.

DeWoskin RS.

Chem Biol Interact. 2007 Mar 20;166(1-3):352-9. Epub 2007 Feb 8.

US EPA/NCEA (National Center for Environmental Assessment), Mail Drop B243-01, Research Triangle Park, NC 27711, USA. dewoskin.rob@epa.gov

Mathematical models are increasingly being used to simulate events in the exposure-response continuum, and to support quantitative predictions of risks to human health. Physiologically based pharmacokinetic (PBPK) models address that portion of the continuum from an external chemical exposure to an internal dose at a target site. Essential data needed to develop a PBPK model include values of key physiological parameters (e.g., tissue volumes, blood flow rates) and chemical specific parameters (rate of chemical absorption, distribution, metabolism, and elimination) for the species of interest. PBPK models are commonly used to: (1) predict concentrations of an internal dose over time at a target site following external exposure via different routes and/or durations; (2) predict human internal concentration at a target site based on animal data by accounting for toxicokinetic and physiological differences; and (3) estimate variability in the internal dose within a human population resulting from differences in individual pharmacokinetics. Himmelstein et al. [M.W. Himmelstein, S.C. Carpenter, P.M. Hinderliter, Kinetic modeling of beta-chloroprene metabolism. I. In vitro rates in liver and lung tissue fractions from mice, rats, hamsters, and humans, Toxicol. Sci. 79 (1) (2004) 18-27; M.W. Himmelstein, S.C. Carpenter, M.V. Evans, P.M. Hinderliter, E.M. Kenyon, Kinetic modeling of beta-chloroprene metabolism. II. The application of physiologically based modeling for cancer dose response analysis, Toxicol. Sci. 79 (1) (2004) 28-37] developed a PBPK model for chloroprene (2-chloro-1,3-butadiene; CD) that simulates chloroprene disposition in rats, mice, hamsters, or humans following an inhalation exposure. **Values for the CD-PBPK model metabolic parameters were obtained from in vitro studies, and model simulations compared to data from in vivo gas uptake studies in rats, hamsters, and mice. The model estimate for total amount of metabolite in lung correlated better with rodent tumor incidence than did the external dose.** Based on this PBPK model analytical approach, Himmelstein et al. [M.W. Himmelstein, S.C. Carpenter, M.V. Evans, P.M. Hinderliter, E.M. Kenyon, Kinetic modeling of beta-chloroprene metabolism. II. The application of physiologically based modeling for cancer dose response analysis, Toxicol. Sci. 79 (1) (2004) 28-37; M.W. Himmelstein, R. Leonard, R. Valentine, Kinetic modeling of beta-chloroprene metabolism: default and physiologically-based modeling approaches for cancer dose response, in: IISRP Symposium on Evaluation of Butadiene & Chloroprene Health Effects, September 21, 2005, TBD--reference in this proceedings issue of Chemical-Biological Interactions] propose that observed species differences in the lung tumor dose-response result from differences in CD metabolic rates. The CD-PBPK model has not yet been submitted to EPA for use in developing the IRIS assessment for chloroprene, but is sufficiently developed to be considered. The process that EPA uses to evaluate PBPK models is discussed, as well as potential applications for the CD-PBPK model in an IRIS assessment.

In reading the document, I don't recall coming across an explanation for why the implications of this model for cancer risk were not explored. It seems to me that the high dose saturation effects that are apparent in the tumor data could be explained in part by even a basic application of this kind of model. Explaining the high dose saturation of the

metabolic activation would, I think, (1) avoid the need to eliminate the high dose for some data sets and (2) lead to an increase in the estimate of the linear coefficients for the cancer dose response model. The PBPK model may well be considered not sufficiently tested against human data for un-caveated application to human risk projection, but I think its implications should at least be explored for sensitivity analyses.

Ronald L. Melnick

No additional studies were found that would significantly impact the overall assessment.

John B. Morris

I am aware of no additional toxicity studies relative to chloroprene. The mouse bronchiolar airway lesions are reminiscent of those induced by naphthalene and styrene. In this regard, comparisons to these compounds might provide some useful perspectives.

Avima M. Ruder

Two recent studies of genetic damage in workers exposed to chloroprene are relevant to this review.

Heuser VD, de Andrade VM, da Silva J, Erdtmann B. 2005. Comparison of genetic damage in Brazilian footwear-workers exposed to solvent-based or water-based adhesive. Genet Tox Environ Mutat/Mutat Res 583(1):85-94.

This study compared Comet assay results for unexposed workers, workers using water-based adhesives, and workers using solvent-based adhesives containing polychloroprene (and, presumably, some chloroprene as a contaminant), with a significantly higher damage index among the solvent-based adhesive users than either the unexposed or workers using water-based adhesives.

It was not entirely clear from the article whether the solvent-based adhesive group used adhesives (and other compounds), as stated on page 90, or produced the polychloroprene (page 91). In either case, there are a number of additional exposures which might have been associated with the chromosome damage. Other than the chromosome results no health effects were reported.

Musak L, Soucek P, Vodickova L, Naccarati A, Halasova E, Polakova V, Slyskova J, Susova S, Buchancova J, Smerhovsky Z and others. 2008. Chromosomal aberrations in tire plant workers and interaction with polymorphisms of biotransformation and DNA repair genes. Mutat Res 641(1-2):36-42.

This study compared lymphocyte chromosome aberrations among smoking and nonsmoking tire workers (exposed to butadiene) and controls. In addition, participants were genotyped for polymorphisms in genes encoding metabolic enzymes. "Chromosomal aberrations were higher in subjects with GSTT1-null (2.4 ± 1.7%) than in

those with GSTT1-plus genotype (1.8 ± 1.4%; F = 7.2, P = 0.008)." In light of the papers on diene (butadiene, chloroprene, isoprene) metabolism that indicate that the detoxification of a mutagenic metabolite goes through the GST pathway [Himmelstein, et al. 2004a; Himmelstein, et al. 2004b; Munter, et al. 2007; Munter, et al. 2003], this result is significant. It means that the fifty percent of the human population that is GST-null may be at higher risk from exposure; any exposure-associated carcinogenicity could be higher in this susceptible subpopulation.

Other studies to consider:

Fajen JM, Ungers LJ. 1985. DuPont de Nemours and company, Pontchartrain Works, LaPlace, LA, IWS-147-31. LA, LaPlace: NIOSH, Cincinnati, OH. 1-18 p.

Jones JH, Young RJ, Selevan S. 1975. du Pont de Nemours and Company, Inc., Louisville, Kentucky, IWS-87-10. KY, Louisville: NIOSH, Cincinnati, OH. 1-9 p.

McGlothlin JD, Meyer C, Leet TL. 1984. E.I. DuPont De Nemours And Company, Louisville, KY, HETA-79-027-1459. KY, Louisville: NIOSH, Cincinnati, OH. 1-28 p.

These NIOSH site visits provide concise histories of processes and chemicals at the plants, as well as descriptions of records and medical monitoring (Fajen and Jones reports) and a Health Hazard Evaluation (McGlothlin).

Leonard RC, Kreckmann KH, Lineker GA, Marsh G, Buchanich J, Youk A. 2007. Comparison of standardized mortality ratios (SMRs) obtained from use of reference populations based on a company-wide registry cohort to SMRs calculated against local and national rates. Chem Biol Interact 166(1-3):317-22.

This study calculated SMRs for the Louisville and Pontchartrain chloroprene plants using the DuPont employee database as a reference population, rather than the U.S. national or local population. For the Louisville plant, "…the SMRs based on the total U.S. DuPont worker mortality rates for all causes of death (1.13), all cancers (1.11), and respiratory cancers (1.37) are statistically significantly increased. The SMR for liver cancer (1.27), although elevated, is not statistically significant."

Richard B. Schlesinger

There are none that I am aware of.

Chemical-Specific Charge Questions:

(A) Oral Reference Dose (RfD) for Chloroprene

1. An RfD was not derived for chloroprene. Has the scientific justification for not deriving an RfD been clearly described in the document? Please identify and provide the rationale for any studies that should be selected as the principal study.

Herman J. Gibb

The scientific rationale for not deriving an RfD has been clearly described.

Dale Hattis

Yes. But such a derivation would be possible if the PBPK model (or some suitable range of models derived from sensitivity analyses) were used.

The principal study selected for analysis is fine.

Ronald L. Melnick

Yes, the lack of an adequate multiple-dose oral toxicity study on chloroprene that could be used for a dose-response analysis and the lack of information on the disposition of chloroprene after inhalation or oral exposure that would enable a reliable route-to-route extrapolation justify not deriving an RfD for this chemical. Because of a likely large first-pass liver effect after oral exposure, the systemic distribution of parent compound and reactive metabolites could be very different after oral or inhalation exposures.

John B. Morris

An oral RfD was not derived for chloroprene. The current database is clearly described. The rationale for the decision to not derive an oral RfD is clearly and concisely described. The scientific justification is appropriate and the decision is well founded.

Avima M. Ruder

As the document states, there are no human data on oral exposure and only one lifetime animal study, so clearly the justification for not deriving an RfD exists.

Richard B. Schlesinger

The decision not to derive an RfD is clearly justified in the document as based upon the lack of appropriate datasets for oral exposure.

(B) Inhalation Reference Concentration (RfC) for Chloroprene

1. A chronic RfC for chloroprene has been derived from an inhalation toxicity study (NTP, 1998) investigating non-cancer effects in multiple organ systems. Please comment on whether the selection of this study as the principal study is scientifically justified. Please identify and provide the rationale for any other studies that should be selected as the principal study.

Herman J. Gibb

The selection of this study is justified. The document states that the Trochimowicz et al. study was not chosen as the principal study "primarily due to the lack of observed effects at similar exposure levels as the NTP (1998) study"(page 4-39, lines 19-20; page 5-2, lines 26-29). That doesn't seem as strong an argument as the high mortality in the low dose animals which were suffocated by the ventilation system (page 5-2, lines 13-16, 29-31).

Dale Hattis

The principal study selected for analysis is fine.

Ronald L. Melnick

The selection of the NTP chronic inhalation toxicity study as the principal study for the derivation of an RfC for chloroprene is scientifically justified. This was a well designed and conducted study, which identified several non-cancer effects in multiple organs of rats and mice exposed to a wide range of concentrations of chloroprene. A major strength of this study is the multiple histopathological reviews of lesions identified in rats and mice. The study clearly demonstrates the toxicity of chloroprene in multiple species and the data are suitable for dose-response analyses.

John B. Morris

The selection of the NTP inhalation study as the principal study is scientifically justified. It was well conducted and subject to peer review.

Avima M. Ruder

The data files for two human studies conducted at the Louisville plant [Gooch and Hawn 1981; McGlothlin, et al. 1984] might have some information on subchronic effects. Gooch and Hawn did biochemical and hematological assays on blood specimens from workers characterized by their duration of chloroprene exposure. McGlothlin and colleagues conducted medical interviews with workers who had been monitored for chloroprene exposure (personal zone air samples). The report does not present any tabular data on health effects. However, the lack of quantitative exposure data for Gooch

and Hawn and of quantitative medical data for McGlothlin et al. rule out their use as a principal study. Selection of the NTP study is justified.

Richard B. Schlesinger

This study is clearly the best one to use for derivation of the RfC. It has a range of exposure concentrations and examined two species and multiple organ systems. The other chronic bioassay of Trochimowicz et al. has a number of problems associated with it that in my mind preclude its use as the key study.

(B) Inhalation Reference Concentration (RfC) for Chloroprene

2. An increase in the incidence of degenerative nasal lesions in male rats, characterized by olfactory epithelial atrophy and/or necrosis with increasing severity, was selected as the critical effect. Please comment on the scientific justification for combining the incidence of atrophy and necrosis and for selecting this endpoint as the critical effect. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

Herman J. Gibb

It seems reasonable to combine the incidence of epithelial atrophy and necrosis. The rationale for choosing degenerative nasal lesions over epithelial hyperplasia or splenic hematopoietic proliferation (page 5-10, lines 4-10) is reasonable.

Dale Hattis

I think there is no problem with the selection of these endpoints for RfC derivation.

Ronald L. Melnick

Combining the incidences of the degenerative nasal lesions, atrophy and necrosis, seems reasonable, but does not make much difference on the overall determination – the incidence of atrophy alone in the control and three dose groups of male rats was 6, 24, 94, and 98%, while the combined incidence of atrophy and necrosis was 6, 26, 96, and 98%; and the derived human equivalent POD values were essentially the same (1.1 mg/m^3 for atrophy and 1.0 mg/m^3 for the combined lesions, respectively).

Nasal degeneration is the appropriate effect for determination of the POD, because this was the most sensitive endpoint producing the lowest human equivalent POD. The document notes that candidate endpoints considered for the critical effect were those that were statistically increased in the lowest exposure concentration group. This limitation should not be imposed because it could result in exclusion of sensitive endpoints depending on the nature of the dose-response relationship. Other endpoints that should also be considered are renal tubule hyperplasia in male rats (single and step section data) and renal tubule hyperplasia in male mice. RfCs should also be derived and presented in Figure 5-1 for other endpoints, including olfactory effects in female rats, male mice, and female mice, and renal tubule hyperplasia in male rats, female rats, and male mice.

John B. Morris

Nasal degenerative lesions in the rat were selected as the critical response because the POD-HEC derived from these data was the most protective. Several concerns could be raised relative to this recommendation. First, the rationale for combining lesions and the precise way in which the data were combined is poorly described. In my view, the concept that necrosis may precede atrophy is quite straightforward. Numerous agents

induce nasal olfactory necrosis and atrophy (esters, styrene, and naphthalene to name a few); critical evaluation of this database will provide insights into the typical progression of lesions. The concept that atrophy precedes necrosis, however, is bewildering to me. I am not aware of a nasal toxicant in which it has been shown that atrophy results in subsequent necrosis. Such an example should be provided to support this concept. In the absence of such information, it is not reasonable, in my view, to assert that atrophy causes necrosis. I, therefore, do not concur with combining the lesions. I note that the difference in POD-HEC between combined and uncombined data is quite small; why invoke a poorly substantiated approach when it results in little difference? My other concerns focus on POD issues and are provided below. In my view, the POD should not be based on nasal lesions, making the issue of combination of lesions moot.

Avima M. Ruder

Combining the effects of atrophy and necrosis appears justified. Table 5-1 does not provide the p-values for trend in dose response for various endpoints. However, it appears that the trend might be stronger for the atrophy or necrosis, with percentages affected ranging from 6 to 98% with increasing doses, than for hematopoietic cell proliferation in the spleens of female mice, with percentages affected ranging from 26 to 78% with increasing doses.

Richard B. Schlesinger

A portal of entry effect was used as the critical effect, which is appropriate for this chemical. The justification provided for combining these two degenerative changes as the overall effect of interest is appropriate, even though it would be assumed that necrosis would precede atrophy. While it appears that the chloroprene while non reactive is metabolized in the upper respiratory tract to a reactive epoxide, there needs to be some explanation as to why the nasal changes themselves were selected over effects in the bronchial tree or alveolar region that were observed at the 12 ppm exposure level as well. An explanation does appear on page 5-7 following results of modeling, but there should have been some indication earlier on as to why the upper respiratory rather than the lower respiratory tract endpoint was selected in the first place.

(B) Inhalation Reference Concentration (RfC) for Chloroprene

3. Benchmark dose (BMD) modeling was used to define the point of departure (POD) for the derivation of the RfC. The POD was based on increased incidence of degenerative nasal lesions in male rats at a benchmark response (BMR) of 10% extra risk. Has the BMD approach been appropriately conducted? Is the BMR selected for use in deriving the POD (i.e., 10% extra risk of degenerative nasal lesions of less than moderate severity) scientifically justified? Please identify and provide the rationale for any alternative approaches (including the selection of the BMR, model, etc.) for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.

Herman J. Gibb

The BMD approach is preferred to other approaches for the given data. The arguments made by one of the peer reviewers, Dr. Morris, to reconsider the calculation of the RfC with regard to blood borne delivery versus airborne delivery are reasonable, and I would recommend that the Agency evaluate both approaches prior to performing dosimetric adjustment. If atrophy/necrosis is eventually selected as the endpoint, a BMR of 10% extra risk is reasonable given the arguments on page 5-4 of the document.

Dale Hattis

The saturation of metabolism to the active metabolites could be clarified with the use of the PBPK model mentioned earlier. This could facilitate dose response modeling and perhaps lead to a somewhat lower point of departure for application of uncertainty factors.

At the peer review meeting an issue arose as to whether the 10% benchmark response level was appropriate in the light of the severity of the nasal lesions in some of the animals. If counts are available on the numbers of animals showing different levels of severity in relation to dose than this would seem to be a good case for the use of the EPA's categorical regression software. With that system it would be possible to take the severity information into account and estimate a somewhat lower BMDs and BMDLs corresponding to a 10% extra risk of mildly adverse effects.

In addition, EPA might consider a modifying the benchmark dose estimation to take into account the approach to saturation of metabolic activation derived from the cancer dose response information (see below).

Finally I agree with some of the other reviewers that the RfC should be derived using the procedures for a category 3 rather than a category 1 vapor.

Ronald L. Melnick

BMD modeling is the preferred approach to derive the POD because it uses all of the dose response data and is less impacted by the group size. Some discussion is needed on why a particular dose response model was selected for the determination of the POD in situations where more than one model provided an adequate fit to the data. If it is EPA's policy to select the model that yielded the lowest AIC value, then that rationale should be explicitly noted. The characterization of chloroprene as a Category 1 gas and the application of a dosimetric adjustment factor for portal-of-entry effects have not been adequately justified.

The NTP study that was used to derive the RfC did not achieve a NOAEL, and the severity of the nasal lesions was greater than minimal in the lowest exposure concentration group. In fact, several male rats in the low exposure group (12.8 ppm) were graded with moderate severity for olfactory atrophy and necrosis. The benchmark response of 10% extra risk is not a NOAEL and the estimated BMD_{10} used to derive the RfC is approximately 60% of the lowest concentration used in the chronic toxicity study of chloroprene. Because the NTP study included 50 animals per group, a BMR of 2% or 5% extra risk would likely provide a reliable estimate for the derivation of the POD without substantially increasing statistical uncertainty at the POD. Thus, I strongly recommend BMD modeling and derivation of the POD from the 2% or 5% extra risk response; if that is not done then an additional uncertainty factor of 3 to 10X would need to be applied to the human equivalent POD.

John B. Morris

I do not concur with the approach used to derive the POD-HEC. Multiple POD-HEC values were derived for differing lesions and the most sensitive was then selected. I note that the POD values (prior to DAF correction) for all the lesions are virtually identical, spanning 2.1-8.3 mg/m3 range. The only reason the POD-HEC is lower for the nasal lesions is that the DAF is so low. Thus, the selection of the nasal lesions as the most sensitive response is simply an artifact of the DAF (RGDR) calculation and not based on the primary experimental observations.

My concerns relative to the RGDR are described below. Essentially they are: 1) the RGDR calculation is theoretically flawed and discordant with the inhalation dosimetry database, and 2) there is no basis to conclude that airborne rather than blood-borne chloroprene induces nasal olfactory lesions. The absence to consider blood-borne delivery is particularly confusing in light of the fact that the possibility of blood-borne delivery relative to pulmonary lesions received much attention. Why this was ignored for the nose is perplexing. The distribution of lesions (olfactory, but no respiratory mucosal damage) could certainly be reflective of a critical role for blood borne delivery and/or in situ metabolic activation. The absence of nasal respiratory injury suggests the parent compound and/or direct reactivity of the parent compound are not likely involved. Commonly a strong anterior/posterior gradient in respiratory mucosal injury is seen for vapors which are directly reactive. This is not the case for chloroprene, in fact, no respiratory mucosal lesions were seen. Were blood borne delivery considered I believe

the RDGR would be 1. In my view, the assumption that chloroprene is a category 1 gas is also flawed (see below). Given that numerous compounds produce nasal olfactory injury following parenteral administration, the observation of nasal olfactory injury cannot be used in support of a category 1 assignment. The partition coefficient of chloroprene is quite small (10) from a nasal dosimetric view. It is difficult, if not impossible, to envision a scenario in which nasal backpressure does not influence dosimetry and/or that nasal deposited chloroprene does not penetrate to the depth of the blood. In my view, chloroprene is a category 3 gas.

At best, the assignment of category 1 status and the exclusion of blood-borne delivery mechanisms represent a weakness of the RfC derivation. An alternate approach would be to select the POD on a parameter closely associated with the collected data rather than to pick a value subject to artifact from the RGDR approach. Were this done, a differing critical lesion would be selected – likely alveolar epithelial hyperplasia and/or hematopoietic proliferation. Given that the subsequent text includes considerable discussion of the possibility of blood borne delivery relative to pulmonary injury, the selection of an inhalation based DAF of 2.3-4.1 would need to be critically discussed and supported were lung lesions selected as the critical effect. For the cancer risk extrapolation both inhalation based and blood-borne based DAF values were used. Why not use both approaches for the non cancer endpoints as well? The lack of consistency is striking.

I am supportive of using a BMD approach as the database appears sufficiently robust to allow for this calculation. An extra risk of 10% of mild lesions is an appropriate endpoint in my view. However, if moderate grade lesions were observed at exposure concentrations approximating the calculated BMD10, it would suggest the calculated value is too permissive. As noted above, I would recommend selecting the endpoint based on the observed data and then performing a single DAF-based calculation based on those data. Such an approach would minimize artifacts due to complexities associated with selection of the most appropriate DAF.

Avima M. Ruder

I don’t have the expertise in risk assessment to comment on whether the modeling and extrapolation from animal to human was appropriately conducted. However, a 10% increase in an effect appears to be a significant enough departure from good health to justify the calculation. Upon reflection, I agree with the argument made by Dr. Melnick that the proposed benchmark dose does not represent a NOAEL and that it might be better to look at a lower response level (2-5%). From the responses from EPA staff at the review meeting it appears that a 2-5% extra risk response level was considered in internal EPA discussions. I also think that the issues raised by Dr. Morris as to whether chloroprene is a category 1 gas or not need to be clarified.

Richard B. Schlesinger

The BMD approach is very well suited for the large data set of the principal study being used in this document and using chronic toxicity and carcinogenicity as endpoints. In general when using the BMD, a 10% level of acceptable risk is used. Thus, this document follows relatively standard procedures in this regard. However, based upon the data, this level may be too high and it is suggested that a lower level, perhaps 5%, be used in this case. The document could be clearer in showing the different stages in the development of the RfC. It does provide a formula on page 5-4 but does not show the use of the formula with actual numbers from the principal study. It would be helpful to the reader if such a step by step actual derivation was provided. For example, it would help to see the actual value for the PODadj (mg/m^3) that was used to derive the HEC.

(B) Inhalation Reference Concentration (RfC) for Chloroprene

4. Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD for the derivation of the RfC. If changes to the selected UFs are proposed, please identify and provide a rationale(s

Herman J. Gibb

The uncertainty factors seem reasonable.

Dale Hattis

I have no quarrel with the selection of uncertainty factors made in the document. The analysis seems very standard. The only area of modest controversy might be the choice of a database uncertainty factor of 3. This seems adequately justified by the absence of a two-generation reproductive study, although the negative findings for teratogenesis and dominant lethal effects could have been considered an adequate substitute.

Ronald L. Melnick

The selection of uncertainty factors of 10X for human variation, 3X for animal-to-human toxicodynamic uncertainty, and 3X for database insufficiencies are reasonable and consistent with EPA policy. However, it is not possible to know if the UFs selected for human variability and interspecies uncertainty adequately account for the extent of these variations. For example, human variability is greater than 10X for the activities of the enzymes involved in chloroprene metabolism (both activation of chloroprene and detoxification of the reactive epoxide intermediate). As noted in response #3 above, the BMD_{10} is a true effect level with several animals diagnosed with moderate lesion severity (i.e., the severity level just below marked). The EPA assumption that the BMD_{10} represents a minimal biologically significant change that was less than moderate severity is not correct. Thus, an additional uncertainty factor of 3-10X should be applied to the RfC derived from a BMD_{10}; alternatively, the POD should be derived from a BMR or 2% or 5% extra risk. An additional deficiency in the database includes lack of data on potential neurodevelopmental toxicity, or other long-term effects following perinatal exposure.

John B. Morris

The rationale for UF selection is clear and appears consistent with typical procedures. The discussion would be greatly enhanced by inclusion of discussion of the impact and uncertainty of selecting DAF factors based on airborne delivery. My concerns, in this regard, are provided above. In my view, it is important to recognize that the DAF calculation is subject to considerable uncertainty and, as such, should not be accepted as factually based. Discussion should also be included on the basis for inclusion of a database limitation uncertainty factor as a multi-generation study is available. It should be stated if this is policy-based rather than scientifically-based decision.

Avima M. Ruder

The uncertainty factors appear justified. As I commented above, there is probably considerable human variation in the metabolism of chloroprene, due to polymorphisms in the genes coding metabolic enzymes. However, as Drs. Schlesinger, Hattis, and Melnick suggested during the review (or as I understood them to suggest), it might be more appropriate to change the benchmark dose response, rather than the uncertainty factors. Their arguments should be considered.

Richard B. Schlesinger

The specific UFs chosen are well justified and appropriate for the data set used and follow standard USEPA guidelines.

(C) Carcinogenicity of Chloroprene

1. Under the EPA's 2005 Guidelines for Carcinogen Risk Assessment (www.epa.gov/iris/backgr-d.htm), the Agency concluded that chloroprene is likely to be carcinogenic to humans by all routes of exposure. Please comment on the cancer weight of evidence characterization. Is the cancer weight of evidence characterization scientifically justified?

Herman J. Gibb

The characterization is clearly justified based on the animal and genotoxicity data, but the argument for the epidemiologic data has been overstated.

The reported evidence of a liver cancer risk in the Louisville cohort studied by Marsh et al. (2007a, 2007b) summarized on page 4-18, lines 3-5 relies heavily on a purported dose response in 4 cumulative exposure categories. The document does not describe what the relative risks (and confidence limits) are in each of the four exposure categories but states that the probability of the trend is 0.09 (page 4-13, lines 13-17; page 4-71, lines 4-7)[1,2]. Furthermore, the document neglects to report what the overall SMR for liver cancer is in the Louisville cohort. Interestingly, the document concludes that there is no evidence of a dose response relationship for respiratory cancer yet describes the relative risks and confidence limits for respiratory cancer by all four cumulative exposure levels for all four facilities in the Marsh et al. study (page 4-14, Table 4-9). Why isn't the reader given that information for the liver cancer relative risks, at least for the Louisville cohort, since the document has gone to the point of suggesting that the data indicates that there is a liver cancer dose response? Furthermore, in the discussion of "biological gradient" on page 4-71, no mention is made of Table 4-11 on page 4-17 showing that two studies demonstrate evidence of a dose response for liver cancer, and two demonstrate *no* evidence of a dose response. The dose response in one of the studies (Leet and Selevan 1982) would not even exist if only deaths from liver cancer were included in the analysis since two of the three deaths from cancer of the liver and biliary passage in the high exposure category were due to gall bladder cancer. The other study in Table 4-11 that suggests a dose response is Bulbulyan (1999), but the relative risks in the high and low dose are not statistically different. The statement at the bottom of page 4-18 that there is evidence of a dose-response relationship in different cohorts in different continents (U.S., China, Russia, and Armenia) grossly misrepresents the evidence.

Known risk factors for liver cancer include Hepatitis B and C infection, aflatoxin ingestion, certain inherited metabolic diseases, cirrhosis due to alcohol abuse, obesity, and certain inherited metabolic diseases (American Cancer Society). None of these factors with the exception of alcohol consumption (page 4-69, lines 28-29) have been

[1] The document states on page 4-13, lines15-17, and page 4-13, lines 4-71, lines 5-6 the *range* for the *three* highest exposure levels was from 1.9-5.1 but doesn't state what the RR's for each of the four exposure levels are nor does it provide confidence limits on the RRs.

[2] If the p = 0.09 is calculated by the authors of the EPA document (as opposed to Marsh et al.), that should be indicated.

discussed in the review. It is interesting that in the Major Conclusions on page 6-2, lines 27-29, the document notes that "These associations (respiratory cancer) are not considered as strong as those with liver cancer due to the inability to control for confounding by smoking status, a strong indicator of lung cancer." What about the well-known risk factors for liver cancer? Were they considered in the various studies? On page 4-69, lines 28-29, the document indicates that the lack of data on alcohol consumption is a "key limitation." On lines 31-32, the document states that there is also a "high likelihood of co-exposures which may be confounders." Nonetheless, the document goes on to blithely state that "Despite this potential, there is little evidence of substantial exposure to liver carcinogens in these populations." How can such a statement be made if the study authors never considered the major risk factors?

Of particular note with respect to the Li et al. study is that the highest liver cancer rate in the world is China (as much as 10X that in the U.S.), primarily the result of Hepatitis B infection and aflatoxin ingestion. Given the considerable risk posed by these risk factors in a Chinese population and that there were only 6 liver cancer deaths in the entire cohort working in a facility where there were multiple chemical exposures, it is impossible to conclude that the study indicates an association between chloroprene and liver cancer.

The document indicates on page 4-8 that Bulbulyan et al. (1998) found 11 deaths due to cirrhosis. It is possible that these deaths could have been caused by chloroprene, but alcohol and hepatitis B/C infections are the most common causes of cirrhosis which should say something about the cohort. Liver cancer is about 50% higher in Eastern Europe than it is in North America, and alcohol consumption in Russia is reported to be almost double that of the U.S.

The analysis of the Bulbulyan (1999) study indicates that there was increasing incidence of liver cancer by duration of employment and by cumulative exposure. Presumably duration of exposure and cumulative exposure were not evaluated together in a multiple regression by the study authors (I do not have the original paper). Given that there was an increasing risk by duration of exposure, one cannot rule out that the increasing risk with cumulative exposure was not due to other exposures at the facility. Presumably, there was no analysis by intensity of exposure? If there was, what did it show?

The document should be more transparent in the presentation of the human data on liver cancer. For example:

- The liver cancer relative risks for all four exposure categories in the Louisville cohort studied by Marsh et al. should be reported.
- The SMR for liver cancer should be reported for the Louisville cohort studied by Marsh et al.
- Whether Marsh et al. (2007a, 2007b) and Leet and Selevan (1982) Louisville cohorts are independent should be addressed. If Leet and Selevan (1982) is a part of or the same as the Marsh et al. cohort (or even very similar), then use of the Leet and Selevan (1982) should not be described as providing independent results of dose

response, consistency, etc. The same is true of the Colonna and Leydavant (2011) and the Marsh et al. studies of the Pontchartrain facility.

- The confounding factors for liver cancer and whether studies addressed these risk factors should be discussed.
- The statement in the Major Conclusions on page 6-2, lines 19-20 that there was "some evidence" of liver/biliary passage cancer risk being associated with chloroprene exposure is followed by the statement on lines 22-23 that these measures of association were "strong, especially in the presence of healthy worker bias" is inconsistent.
- An association between liver cancer and chloroprene exposure being strengthened by the healthy worker effect as indicated in the Major Conclusions is not evident in the summary of the overall weight of evidence (some mention of HWE is made on page 4-69, lines 21-25 but does not indicate that the evidence is strengthened). Furthermore, a healthy worker effect for liver cancer? With such a short life expectancy following diagnosis, I would expect the healthy worker effect for liver cancer to be minimal if it even exists.
- The small number of liver cancer deaths/cases in the studies by Li et al., Bulbulyan (1998, 1999) and Leet and Selevan (1982) and the variability about such small numbers should be better described, particularly in light of the limitations of those studies with respect to calculation of the expected deaths, follow-up, etc.

As the document acknowledges on page 4-17, there is little if any evidence that chloroprene increases the risk of respiratory cancer. The limitations of the earlier studies (Li et al. 1989, Bulbulyan 1998, 1999) are significant with regard to whether or not they indicate an increased risk of liver cancer from chloroprene exposure. The largest and what appears from the document to be the best conducted study (Marsh et al., Louisville cohort) provides little if any evidence that a liver cancer risk exists. Furthermore, the document has not been transparent in its reasoning that there is a risk of liver cancer.

In summary, the descriptor of "likely to be carcinogenic to humans" is supported by the animal and genotoxicity data, but not by the human data. While the descriptor is appropriate, the document should not try to make more of the epidemiologic studies than is warranted.

Dale Hattis

Yes. The ample information on carcinogenesis in many sites in animals, the clear metabolism information to mutagenic metabolites, and the analogies to related chemical carcinogens with analogous metabolic pathways to DNA-reactive metabolites all combine to make this conclusion unequivocal. As suggested by Dr. Melnick, the final document should consider whether the available evidence warrants an upgrade of the classification to "carcinogenic to humans.

Ronald L. Melnick

Results from the NTP study demonstrating multiple organ carcinogenicity of inhaled chloroprene in both sexes of rats and mice are consistent with the EPA descriptor "likely to be carcinogenic to humans." Because the carcinogenicity of chloroprene is likely due to its epoxide metabolites, and because cytochrome P450-mediated epoxidation of chloroprene can occur in several organs including the liver, kidney, and lung, metabolism of absorbed chloroprene to a mutagenic intermediate can occur by any route of exposure. The systemic distribution of tumors in the NTP studies demonstrates that chloroprene can induce tumors beyond the sites of initial contact. Liver toxicity of chloroprene in rats after oral exposure (stomach tube) indicates the occurrence of oral absorption of this chemical. Chloroprene is absorbed by the skin (Hazardous Substances Data Bank; see page 3-1).

However, the descriptor "carcinogenic to humans" may be more appropriate based on the multiple tumor response in two species, the fact that chloroprene is activated by CYP2E1 to a DNA reactive intermediate (chloroethenyl oxirane) by rat, mouse, or human liver microsomes, the finding of a unique K-ras mutation (A→T at codon 61) in chloroprene-induced lung neoplasms in mice, and the relatively consistent evidence of an association between increased liver cancer mortality risk and occupational exposure to chloroprene. The EPA document does not adequately justify the characterization of chloroprene as "likely to be carcinogenic to humans" rather than "carcinogenic to humans," especially since many of the identified methodological limitations in the epidemiologic studies (e.g., exposure misclassifications, healthy worker effect) would result in an underestimate of risk. According to EPA's cancer risk assessment guidelines, the descriptor "carcinogenic to humans" may be applied when there is less than convincing epidemiologic evidence of a causal association between human exposure and cancer if there is strong evidence of carcinogenicity in animals, the MOA and precursor events have been identified in animals, and key precursor events in animals are anticipated to occur in humans and progress to tumors. These conditions have been demonstrated for chloroprene.

John B. Morris

I concur that the weight of evidence supports the concept that chloroprene may be carcinogenic by all routes of exposure. Multiple tumors were seen in two species in inhalation bioassays. Additionally some data suggesting increased tumor risks in humans is available. Tumors were seen in non-site of contact sites in the rodent studies. (In this regard respiratory tract as well as gastrointestinal tract tumors may be considered as site of contact because of preening activity.) Moreover, there is discussion of the possibility of a critical role blood-borne chloroprene relative to nasal and pulmonary lesions. If there is, indeed, a role for blood borne chloroprene, then the possibility of carcinogenicity after multiple routes of exposure is elevated because systemic absorption and blood-borne delivery to multiple targets is possible. (The document indicates dermal absorption may occur.) Importantly, a potential increase in liver tumors was noted in some occupationally exposed cohorts. In my view, these epidemiological data support the concept that chloroprene may represent a carcinogenic hazard to man.

Avima M. Ruder

The literature supports the likely carcinogenicity of chloroprene and the mutagenicity of its epoxide metabolites. The need for regulation of environmental (in addition to occupational) exposure to chloroprene is justified by a report on public health in the area where the Louisville DuPont plant and other industrial facilities, as well as residences, are co-located. In that report, the Agency for Toxic Substances and Disease Registry (ATSDR) stated that the volume of release of chemicals from the plants made it likely that soil and water (groundwater and the Ohio River) had been contaminated in the past; chloroprene air contamination was measured as 218 ppb or 789 $\mu g/m^3$ in 1956-7 downwind of the plants and 6 ppb or 2.68 $\mu g/m^3$ in 1988 at a monitoring station in downtown Louisville not downwind of the plants [Agency for Toxic Substances and Disease Registry 1998].

ATSDR provided a rationale for the greater vulnerability of children to toxic exposures: they are more likely to play outdoors and bring food into contaminated areas; are shorter and therefore closer to dust, soil, and contaminants; weigh less, resulting in higher doses per unit body weight; and are developing rapidly [Agency for Toxic Substances and Disease Registry 1998]. The EPA's use of age-adjustment factors seems appropriate.

Richard B. Schlesinger

While the *Guidelines for Carcinogen Risk Assessment* are being followed in the chloroprene assessment, even though there are limited to no data on exposure other than inhalation, it seems that the mode of action of the chemical is such that it may not be carcinogenic via all routes, e.g., dermal exposure. It is nonreactive chemically and relatively insoluble in water. The weight of evidence characterization is clear and justified. The animal toxicological data support the conclusion that it may likely be carcinogenic to humans. While the epidemiological evidence in this regard is equivocal, the conclusion is also supported by the fact that the MOA involves conversion to epoxides.

(C) Carcinogenicity of Chloroprene

2. A two-year inhalation cancer bioassay in B6C3F1 mice (NTP, 1998) was selected as the basis for derivation of an inhalation unit risk (IUR). Please comment on whether the selection of this study for quantification is scientifically justified. Please identify and provide the rationale for any other studies that should be selected as the basis for quantification.

Herman J. Gibb

The selection of this study is justified. The document states that the Trochimowicz et al. study was not chosen as the principal study "primarily due to the lack of observed neoplastic effects at similar exposure levels as the NTP (1998) study"(page 5-12, lines 5-8). As with the response to Question 1 for the RfC above, high mortality in the low dose animals (page 4-39, lines 19-20; page 5-2, lines 13-16, 29-31) would be a stronger argument for not choosing the Trochimowicz study than would differences in observed effects between studies. Differences in study results can occur regardless of how well the individual studies are conducted.

Dale Hattis

Choice of the two-year inhalation bioassay is beyond dispute. However, as indicated earlier, the dosimetry, in terms of active metabolite concentration AUC, could have been informed by application of a preliminary PBPK model.

Ronald L. Melnick

The selection of the NTP 2-year inhalation carcinogenicity study of chloroprene in B6C3F1 mice for derivation of an inhalation unit risk is scientifically justified. The NTP study was well designed and conducted, and identified carcinogenic effects in multiple organs of rats and mice exposed to a wide range of concentrations of chloroprene. A major strength of this study is the multiple histopathological reviews of lesions identified in rats and mice. As with the related human carcinogen, 1,3-butadiene, the carcinogenic potency of chloroprene was greater in mice than in rats.

John B. Morris

In my view, the selection of the two-year inhalation bioassay done by NTP as the critical study is appropriate. This study was well performed and peer reviewed. It is true that the Trochimowicz study provided contradictory results, but without substantive rationale the NTP study cannot be ignored. Inclusion of the mouse lung tumor data for dose-response evaluation may be scientifically problematic. As is commonly observed, the mouse metabolic activity for chloroprene is 50-fold higher (Table 3-4) than that in the human or the rat (in which lung tumors were not increased). This fact should be discussed. It is my view that the mouse lung data may overestimate the risk to humans. It is recognized that exclusion of these data may be problematic, but at a minimum a discussion of this

weakness should be provided. Because the metabolism rates in the rat appear similar to the human, the rat may offer a better species for prediction of human health risks. Certainly the document would be improved by an explicit discussion of the relevance of the mouse response considering its high metabolic capacity.

Avima M. Ruder

The text in section 5.4.4 explains the derivation of the inhalation risk but does not explain why inhalation in mice was chosen over inhalation in rats from the same study. I assume there are physiological differences which make mice a more suitable choice, but none were provided here.

Richard B. Schlesinger

The study selected for derivation of the IUR is well justified based upon the standard procedure used by USEPA in selecting the most sensitive animal model. However, they may want to consider the fact that metabolic activation rate in the rat is closer to that occurring in humans than is the situation in mice.

(C) Carcinogenicity of Chloroprene

3. A mutagenic mode of carcinogenic action is proposed for chloroprene. Please comment on whether the weight of evidence supports this conclusion. Please comment on whether this determination is scientifically justified. Please comment on data available for chloroprene that may support an alternative mode(s) of action.

Herman J. Gibb

The hypothesized epoxide metabolite mode of action is reasonable.

Dale Hattis

Yes. The ample information on carcinogenesis in many sites in animals, the clear metabolism information to mutagenic metabolites, and the analogies to related chemical carcinogens with analogous metabolic pathways to DNA-reactive metabolites all combine to make this conclusion unequivocal. I am not aware of any evidence that comparably supports any other mode of action.

Ronald L. Melnick

Based on the fact that the predominant pathway of chloroprene metabolism is via cytochrome P450-mediated oxidation to a DNA-reactive epoxide intermediate (chloroethenyl oxirane), which is mutagenic in multiple strains of Salmonella, and the finding of activating K-ras and H-ras mutations mutations in tumor tissues obtained from mice exposed to chloroprene, including unique K-ras mutations (A→T transversions in codon 61) in lung tumors, the proposed mutagenic mode of carcinogenic action is scientifically justified. This MOA is consistent with that of other epoxide-forming carcinogens, e.g., 1,3-butadiene and vinyl chloride. There is no scientific data supportive of any alternative mode of action. Recent experimental results presented to the Peer Review Panel by DuPont demonstrated the induction of changes in gene expression related to DNA damage in the lungs of mice exposed to 2.5 ppm or higher concentrations of chloroprene (Figure 8, page 79). These data also support a mutagenic mode of carcinogenic action for chloroprene.

John B. Morris

It should be stated that detailed assessment of mutagenic versus non-mutagenic modes of action is somewhat beyond my expertise. With this qualification, I concur with the proposed mutagenic mode of action of chloroprene. Chloroprene metabolite(s) are DNA reactive and mutagenic in some bacterial strains. Data presented by DuPont suggests the induction of DNA repair responses in chloroprene exposed animals. Mutations were observed in vivo in lung tumors of animals exposed to chloroprene. Were a purely cytotoxic mode of action proposed it would be important to show appropriate temporal and dose-response data supportive of this mode. I am aware of no such data. In my view there are insufficient data to exclude the possibility of a mutagenic mode of action. There

appears to be multiple lines of evidence in support of this mode of action and it, therefore, appears scientifically justified. If, however, it is concluded that a metabolite represents the ultimate toxic species, then the quantitative risk assessment should be discussed/validated in light of the large species differences in metabolism rate.

Avima M. Ruder

The metabolic pathways detailed in figure 3-1 (and in the toxicological literature from which this section is drawn) appear to justify this conclusion. The finding of increased chromosome aberrations among humans with variant metabolic enzymes that clear the epoxide metabolite more slowly [Musak, et al. 2008] also supports this conclusion.

Richard B. Schlesinger

There is much compelling evidence that chloroprene has a mutagenic mode of action due to metabolism into reactive epoxides. While this may not be the only MOA, it clearly is one of them.

(C) Carcinogenicity of Chloroprene

4. Data on hemangiomas/hemangiosarcomas (in all organs) and tumors of the lung (bronchiolar/alveolar adenomas and carcinomas), forestomach, Harderian gland (adenomas and carcinomas), kidney (adenomas), skin and mesentery, mammary gland and liver in B6C3F1 mice were used to estimate the inhalation unit risk. Please comment on the scientific justification and transparency of this analysis. Has the modeling approach been appropriately conducted? Please identify and provide the rationale for any alternative approaches for the determination of the inhalation unit risk and discuss whether such approaches are preferred to EPA's approach.

Herman J. Gibb

The rationale for combining risks from different tumor sites is reasonable given a mutagenic mode of action. It is interesting, however, that the inhalation unit risk estimate for chloroprene is an order of magnitude higher than the inhalation unit risk estimate for butadiene which is considered a structural analog and characterized by EPA as "carcinogenic to humans". A reality check on the unit risk for chloroprene by comparing it with an upper bound on the cancer risk in the Louisville cohort studied by Marsh et al. should be performed. The Louisville cohort has the best exposure information for this purpose. From the resulting comparison, it may be necessary to adjust the unit risk estimate.

Dale Hattis

The approach is transparent and reasonable as far as it goes. However, I think it is not ideal in that it fails to make explicit use of the information that there is likely to be high dose saturation of metabolic activation.

As an alternative, at the peer review meeting I presented a series of model fits using a dose response form that incorporates an assumption of saturating metabolism on a systemic level (applicable to all tumors in the same way) but different effective background rates and potencies for the causation of tumors at low doses:

$$P(d)_i = 1 - e^{-(q0_i + \frac{Vmax_i * d}{Km + d})}$$

where:

d is the external experimental concentration in ppm

$P(d)_i$ is the fraction of animals with at least one tumor for a specific tissue (i)

$q0_i$ is a parameter estimated from data that is related to the background (control group) lifetime incidence of tumors in that tissue

V_{max} is related to the maximum tumor yield over background for the specific tissue (i)

K_m is the external dose that produces half the maximal tumor yield over all tissues (based on an assumption that metabolic activation is systemic, rather than being effective for only one tissue due to local metabolism).

This is essentially a quick and easy but approximate substitute for doing a full PBPK model, but instead uses the tumor response nonlinearity at high doses for all the tumor sites to quantify the approach toward saturation of the activating metabolism. Compared to a PBPK modeling approach, this is not informative for the issue of interspecies projection, but it does provide information about the high-dose-to-low dose projection, assuming that the saturable activating metabolism is systemic and affects the tumor frequency in all tissues in the same way. This sort of treatment is warranted by the fact that, in nearly all tissues with an appreciable tumor yield in both male and female mice, the tumor incidence over background at the highest (80 ppm) chloroprene concentration is much less than double the tumor incidence at the next highest (32 ppm) concentration (see plots below). Contrasting the results for the high-dose saturable metabolic activation model with those for a straight linear model allows us to assess how large the change in estimated low dose cancer slope might be relative to a case where there is only a term that is linear in dose:

$$P(d)_i = 1 - e^{-(q0_i + q1_i\, d)}$$

To maintain parallelism with the EPA analysis as much as possible, I made this comparison excluding the anomalous high-dose point for hemangiosarcomas in female mice. Because of this same anomaly, I choose to begin the discussion of the modeling and the model results with the observations in male mice.

Figure 1 is a raw plot of the end of life tumor data for male mice used by EPA in its analysis (from a comment by Dr. Melnick, I understand that tumor results adjusted for mortality are also available in one of his papers; EPA should probably used those results for a more refined analysis.)

A difficulty with the raw plot the tumor data is that one might object that of course there is a flattening of the curve at higher doses and tumor incidences because no more than one tumor can be effectively detected and recorded in any specific tissue. Thus a more appropriate interpretation of the data is to say that each data point represents the fraction of animals that showed <u>at least</u> one tumor in each specific tissue studied. A more appropriate plot without the potential distortion due to multiple tumors per organ can be made by using a Poisson distribution formula

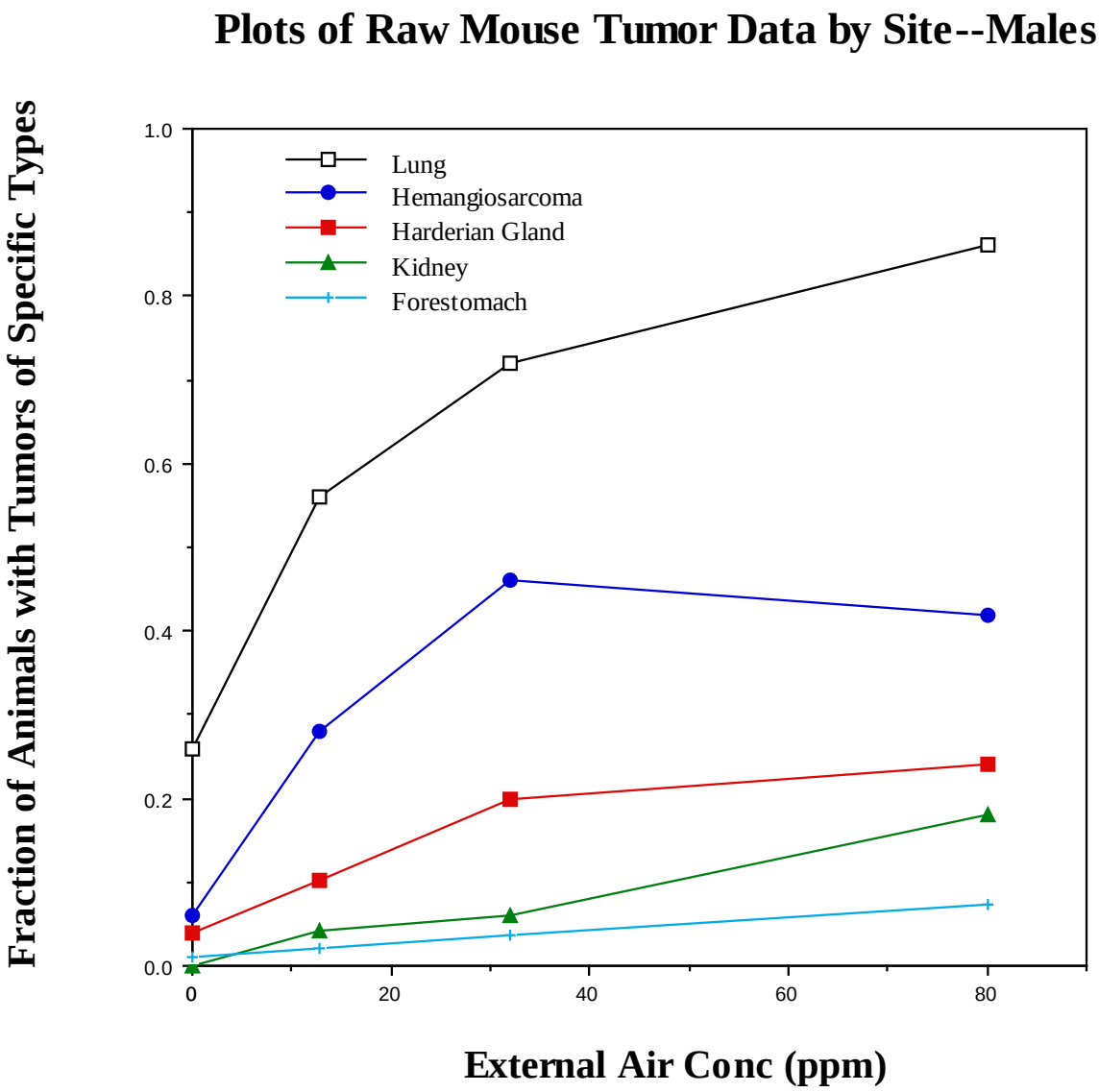
$$P_{0 \text{ tumors in an organ}} = 1 - \text{Fraction of Animals with at Least 1 tumor} = e^{-m}$$

where m = the mean number of tumor transformations per animal

:

.

Figure 1


Plots of Raw Mouse Tumor Data by Site--Males
Fraction of Animals with Tumors of Specific Types
1.0
0.8
0.6
0.4
0.2
0.0
Lung
Hemangiosarcoma
Harderian Gland
Kidney
Forestomach
0
20
40
60
80
External Air Conc (ppm)

Given this, we can solve for m to find

$$\text{mean number of tumor transformations per animal} = -\ln(1 - \text{fraction of animals with at least 1 tumor})$$

Figure 2 is a plot of the male mouse tumor data using this transformations/animal parameter as the dependent variable. It can be seen that even after removing the truncation of the tumors/animal results at 1 in this way, there is still a pronounced flattening of the curves at the higher dose levels, indicating some approach to saturation. This is reminiscent of the vinyl chloride angiosarcoma case where there was saturation of metabolic activation at the higher exposure levels.

One other advantage of the transformations/animal dependent variable is that we can add up the results for the different tumor sites. Figure 3 shows a revised plot of the male tumor data showing the sum of tumor transformations/animal at all five tumor sites. It can be seen that the sum of tumor transformations at all five sites still shows a pronounced convexity as one proceeds to the highest exposure levels.

The fitting of the saturable and linear models was accomplished in Microsoft Excel workbooks designed to incorporate likelihood calculations according to the basic structure published by Haas (1994).[*] Copies of the final workbooks themselves will be submitted to accompany this comment. I would be pleased to explain the detailed features and operation of the modeling system if any EPA personnel would like to pursue this. Basically, each workbook consists of 3 sheets: one for optimization of the maximum likelihood estimates and two for estimation of upper and lower confidence limits on the sum of transformations/animal at all tumor sites. The optimizations were all done with the Excel solver tool, generally with multiple runs of hundreds to thousands of iterations each. Because the maximum likelihood and confidence limit estimates are done on the sum of tumor transformations per animal for all tumor sites, no Monte Carlo post-processing analysis is needed to derive confidence limits on the total tumor risk, as was needed for the separate Weibull model analyses done by/for EPA for the individual tumor sites. On the other hand, a disadvantage of this modeling system is that it only incorporated total tumor incidences observed by the end of the bioassays; not the more detailed time-to-tumor information used in the Weibull model analysis.

Figure 4 shows the overall results of this fitting for both the saturable and linear models. In the case of the saturable model, the parameters estimated are a Vmax and background (zero dose) tumor risk for each organ, and a Km (external ppm needed to achieve half of the total saturated tumor yield) common to all organs—following the hypothesis of saturable metabolism at a systemic level followed by common exposure of all organs to the activated metabolite(s). It can be seen that the saturable model fit corresponds very well with the observations of total tumors per animal (the P value is 0.51, meaning that a difference between data and model predictions as large as that observed would be expected to be produced about half the time from chance sampling-error fluctuations).

[*] Haas, C. N. "Dose Response Analysis Using Spreadsheets" Risk Analysis 14:1097-1100 (1994).

Figure 2

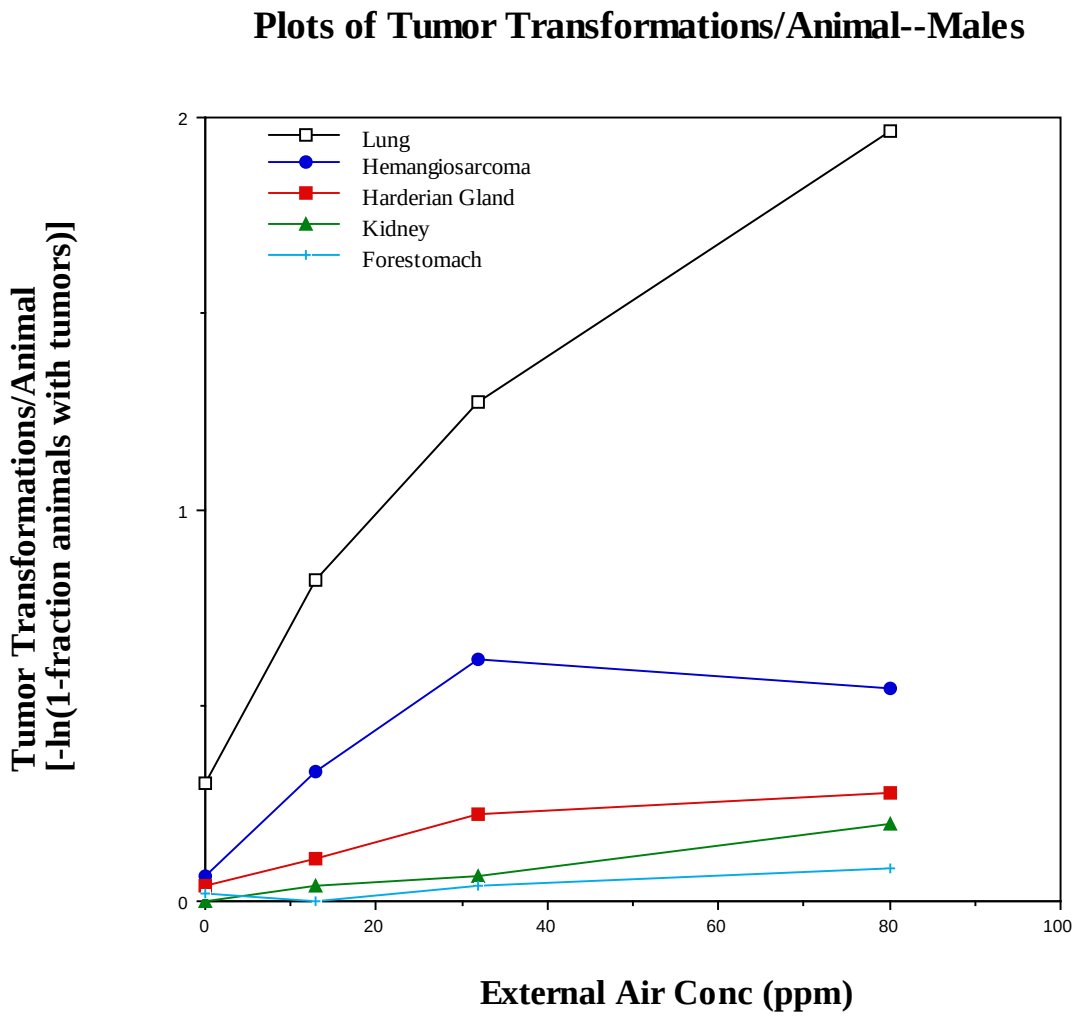

Plots of Tumor Transformations/Animal--Males
Tumor Transformations/Animal
[-ln(1-fraction animals with tumors)]
Lung
Hemangiosarcoma
Harderian Gland
Kidney
Forestomach
0
1
2
0
20
40
60
80
100
External Air Conc (ppm)

Figure 3

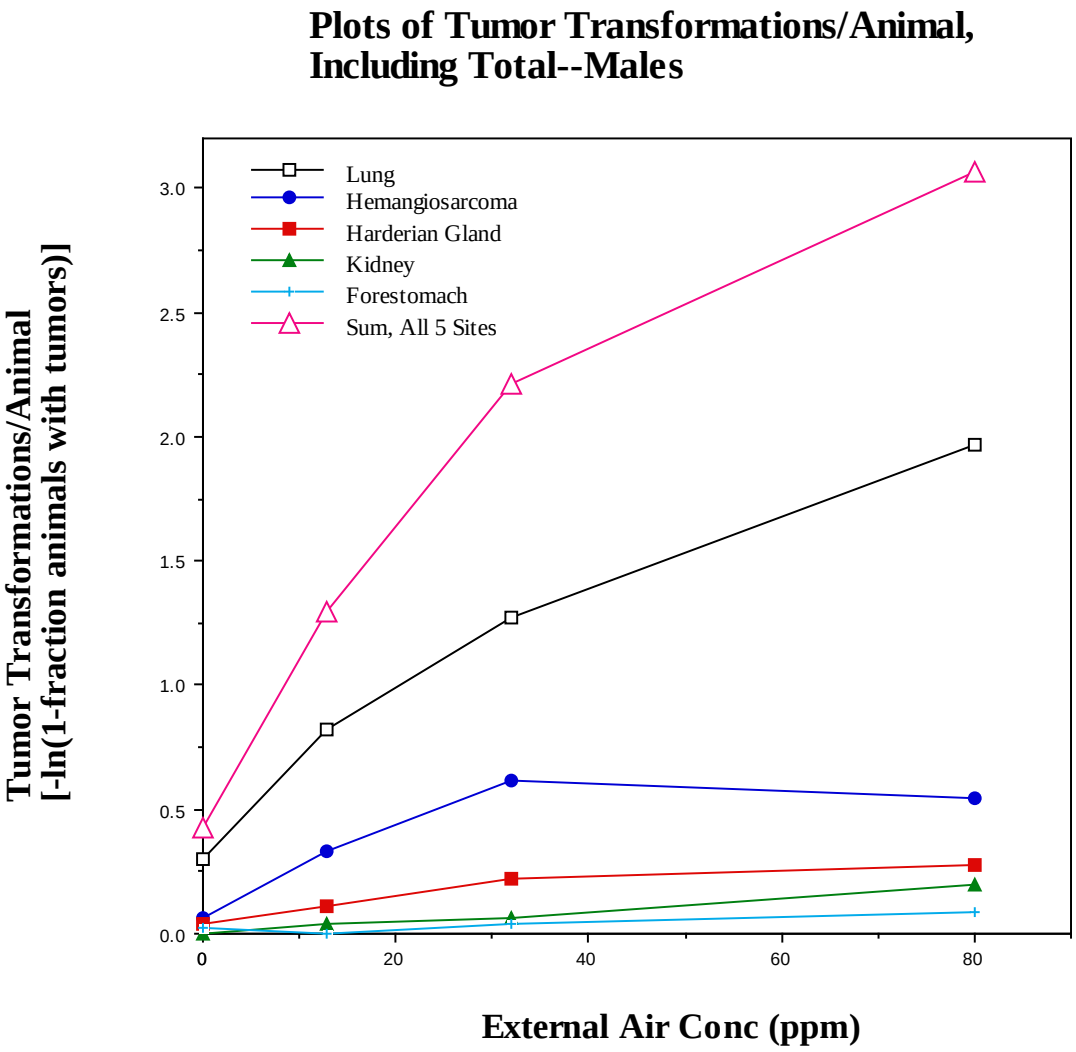
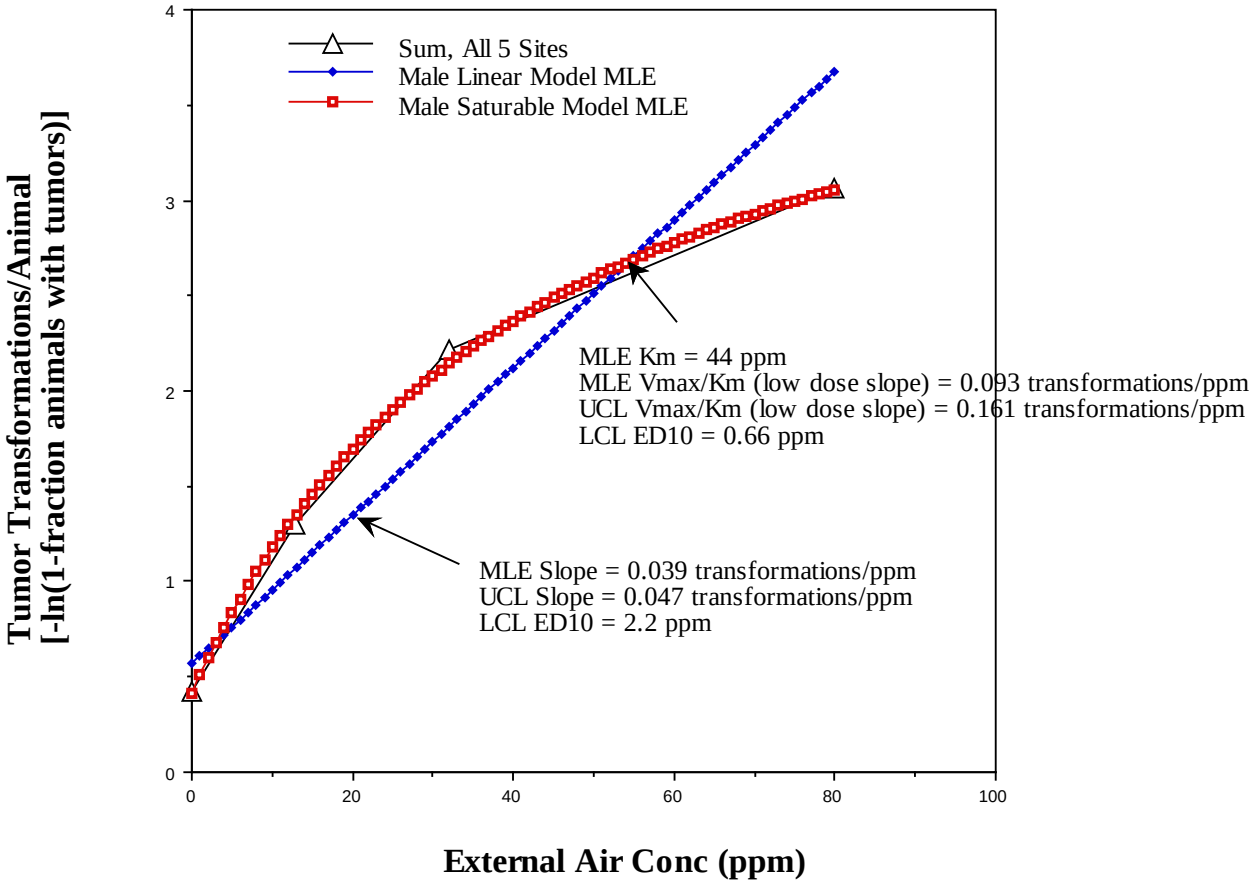

Plots of Tumor Transformations/Animal, Including Total--Males
Tumor Transformations/Animal [-ln(1-fraction animals with tumors)]
Lung
Hemangiosarcoma
Harderian Gland
Kidney
Forestomach
Sum, All 5 Sites
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
20
40
60
80
External Air Conc (ppm)

Figure 4

Comparison of Observed Tumor Transformations/Animal For All 5 Sites in Males with Maximum Likelihood Expectations for Linear and Saturable Models



The linear model fit somewhat less well at P = 0.06, although still barely within the conventional P = 0.05 criterion based on estimation of one fewer parameter (10, rather than 11, corresponding to a background rate and a transformations/ppm parameter for each tumor site).

The results in Figure 4 indicate a half saturation point (Km) of about 44 ppm, and an approximately 2-3 fold greater cancer potency at low doses for the saturable, compared to the linear model, depending on whether one makes the comparison based on MLE slopes or lower confidence limit ED10's. Thus the indication is that a simple linear formulation, as incorporated into EPA's Weibull model is likely to considerably understate the low dose potency indicated by the data for males.

Figure 5 shows a plot of the female tumor data comparable to Figure 2. The same tendency for flattening at high exposure levels is apparent. Figure 6 shows the results a similar comparison of saturable and linear model fits for the female tumor data (excluding, as did EPA, the high dose point for the hemangiosarcomas). The overall fit in this case is less successful than for the male tumor data, with a P value of about 0.02, but the saturable model still fits a great deal better than the linear model with a P value of about 9×10^{-5}. In this case the indicated Km is slightly lower (30 ppm) indicating a slightly greater effect of the indicated saturation of metabolic activation, and the saturable model again suggests a low dose cancer potency a few fold greater than expected with the linear model formulation.

In summary results lead me to five conclusions/recommendations:

- The tumor data are better fit by models incorporating systemic saturable metabolism.
- Saturable models lead to 2-3 fold increases in expected low dose risks compared to simple linear models.
- However, the current saturable models do not incorporate available time-to-tumor information.
- The best way forward would therefore be to add a saturable component to the Weibull time-to-tumor model.
- A second-best approach would be to multiply the expected ratio of saturable vs. linear model-predicted low dose risk by the existing Weibull linear model coefficient (or make a similar adjustment downward in the Weibull model estimated ED10 or LED10).

Figure 5

Plots of Tumor Transformations/Animal
Excluding Hemangiosarcomas--Females

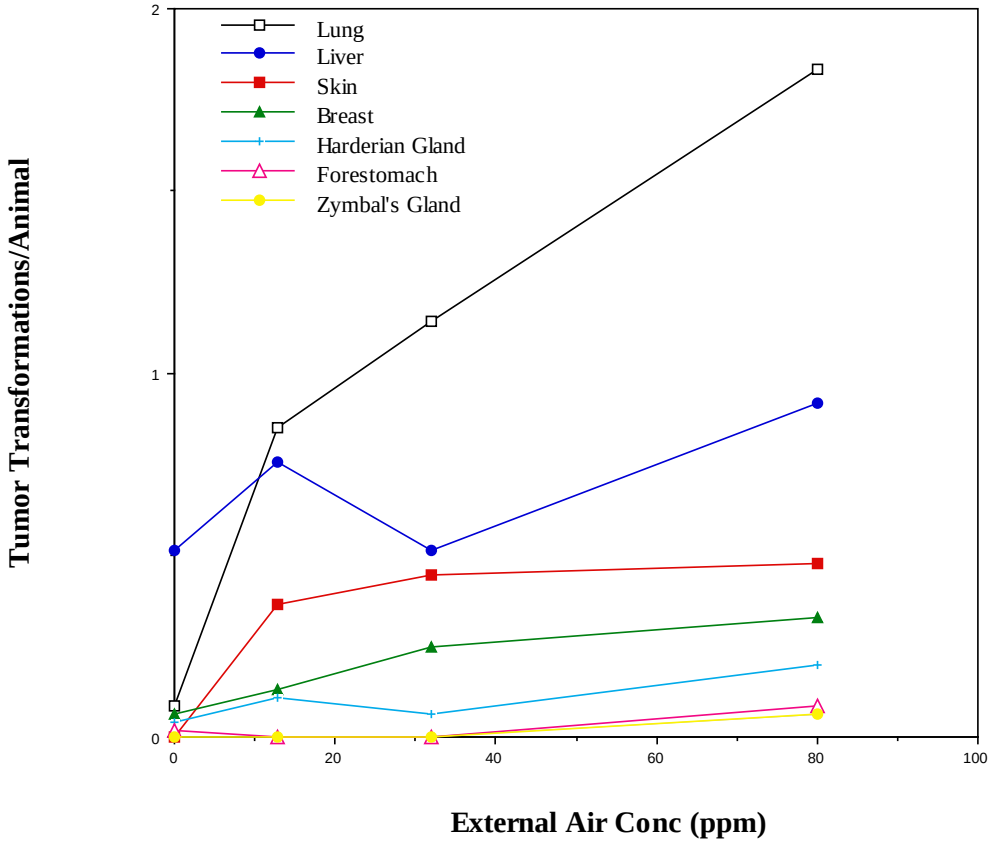
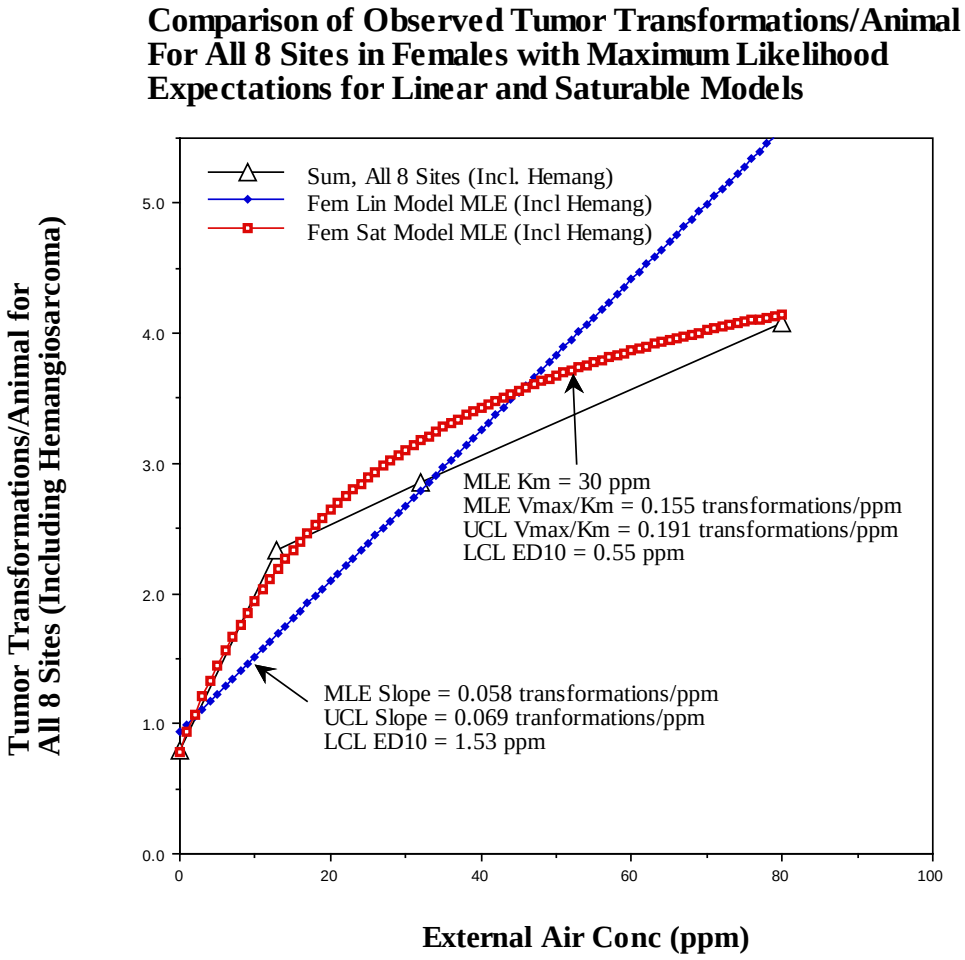


Figure 6


Comparison of Observed Tumor Transformations/Animal For All 8 Sites in Females with Maximum Likelihood Expectations for Linear and Saturable Models
Sum, All 8 Sites (Incl. Hemang)
Fem Lin Model MLE (Incl Hemang)
Fem Sat Model MLE (Incl Hemang)
MLE Km = 30 ppm
MLE Vmax/Km = 0.155 transformations/ppm
UCL Vmax/Km = 0.191 transformations/ppm
LCL ED10 = 0.55 ppm
MLE Slope = 0.058 transformations/ppm
UCL Slope = 0.069 tranformations/ppm
LCL ED10 = 1.53 ppm
Tumor Transformations/Animal for All 8 Sites (Including Hemangiosarcoma)
External Air Conc (ppm)
0.0
1.0
2.0
3.0
4.0
5.0
0
20
40
60
80
100

Ronald L. Melnick

Yes, all of the induced tumor sites in mice should be used to estimate the inhalation cancer unit risk; an assessment based on separate modeling of each tumor type would underestimate the carcinogenic potency of chloroprene. Cancer potency estimates are increased only about 2-fold by combining all sites in the assessment compared to estimates based on only the most potent response in either male or female mice. Because of the reduced mortality of exposed mice due to induction of malignant tumors, a multistage Weibull time-to-tumor model that accounts for differences in survival among groups is most appropriate. The chloroprene document should provide discussion on why no uncertainty factor (other than early-life susceptibility) for human variability was applied to the cancer unit risk estimate. There are certainly substantial differences in human metabolism of chloroprene and its reactive epoxide metabolite and in human susceptibility to chloroprene-induced cancer.

The suggestion by Dale Hattis to apply a model that accounts for saturable metabolism of chloroprene to its epoxide intermediate should be pursued and incorporated into the estimate of the inhalation cancer unit risk. This analysis should use survival-adjusted tumor incidence values. The blood time-course data for chloroprene presented by DuPont (Figure B-1, page 99) to the Peer Review Panel clearly demonstrates saturable metabolism of chloroprene in mice at exposures between 13 and 90 ppm.

John B. Morris

The modeling approaches for the quantitative risk evaluation of chloroprene carcinogenicity were transparently described. Cancer unit risks are calculated individually for specific tumor types and an overall unit risk was calculated. Presumably the overall unit risk was calculated in concordance with accepted EPA procedures. It is beyond my expertise to comment on the generalized appropriateness of combining tumors in this way relative to overall cancer unit risk calculation. If tumors are to be combined then the human relevance of each tumor type must be considered. As noted above, in my view, some skepticism is appropriate relative to the quantitative importance of mouse bronchiolar tumors. The mode of action includes metabolic activation as the first step. The metabolic activation rates in the mouse exceed those in other species by 50-fold (Table 3-4). Clearly this is a critical observation relative to quantitative risk extrapolation. This pattern of mouse vs. human bronchiolar metabolism is certainly not unique to chloroprene. The large differences in mouse vs. human relative to pulmonary activation raise questions as to the relevance of the mouse lesions. At the very least, this issue needs to be discussed. Exclusion of the mouse lung tumors would influence the final overall unit risk estimate indicating this is not a trivial concern.

It should be noted that the epidemiological data suggests the liver at the primary target, although this may be the result of statistical issues related to the high incidence of lung tumors in humans obscuring a response. Nonetheless, a discussion of the site discordance would strengthen clarity of the text. I don’t know if it is possible, but some comparison of the unit risk versus the observed tumor risks in the worker populations would seem warranted. Is it possible to estimate an upper bound risk from the human data?

Alternatively, is it possible to project human occupational risks from the unit risk factor to determine if the unit risk factors are consistent with epidemiologic observations? I recognize that only crude comparisons could be made, but a large discordance would be a cause of concern.

Avima M. Ruder

The assumption of tumor independence (p 5-20), based on the National Research Council risk assessment document, appears justified. However, the results of the animal studies should be evaluated to determine if there is a distinction (genetic, epigenetic, or other) between animals which get one tumor versus those which get more than one.

Richard B. Schlesinger

The derivation of the IUR could be made somewhat clearer in the text.

(C) Carcinogenicity of Chloroprene

5. Lung tumors have been alternatively treated as systemic or portal-of-entry effects in the modeling of cancer endpoints. Please comment on the scientific justification for this modeling approach. Please comment on whether the rationale for this decision has been transparently and objectively described. Please comment on data available for chloroprene that may support an alternative method for modeling the observed lung tumors in mice.

Herman J. Gibb

It makes sense that lung tumors could develop from a systemic as well as a portal-of-entry effect. The extent that the lung tumors occur by systemic vs. portal of entry effects may not be possible to determine, but the text should provide more elaboration for the reader so that they can better understand the approach.

Dale Hattis

The early results for the saturation modeling described in section 4 above strongly suggest that the lung tumors for both male and female mice are completely compatible with the systemic saturable metabolic activation model with a half-saturation point similar to that derived with data for other tumor locations. Therefore, I think the lung tumors should not be treated as if they depended on local metabolism and other portal-of-entry specific processes.

Ronald L. Melnick

Both treatments of the lung tumor data are appropriate because these tumors may have arisen from metabolites formed in the lung, or in other organs, particularly the liver, and subsequently distributed to the lung. No data are available to distinguish the extent of these possibilities. The EPA document did note that the induction of tumors in multiple organs after inhalation exposure to chloroprene demonstrates the systemic distribution of carcinogenic metabolites by this route of exposure.

John B. Morris

The importance of portal of entry versus systemic delivery of chloroprene is not known. A reasonable approach would be to make estimates using both approaches and then make a determination of whether or not it is of quantitative importance. Naturally, the default approach would be to select the more health protective approach. In my view, the fundamental issue in this regard is actually based on the assignment of category 1 status to chloroprene. This assignment is not appropriate (see my other comments), and at the very least needs to justified. Chloroprene should be determined to be a category 3 vapor in my view. It is a low partition coefficient vapor that does not appear to be highly reactive. Indeed, were it highly reactive it would be impossible to measure a partition coefficient. Moreover, the pattern of nasal injury (olfactory but not respiratory mucosal

damage) is inconsistent with a highly reactive vapor. Finally the modeling efforts of Himmelstein would not have been successful were chloroprene highly reactive in tissues. True it is metabolized, but the provided data do not indicate it is metabolized to such an extent that it should behave as a category 1 vapor. If category 1 vapors do not penetrate to the blood in any sufficient degree and if they should be scrubbed very efficiently in the nose, then why are distal lung tumors and non-respiratory tract tumors observed? Were chloroprene to be determined to be a category 3 vapor, then I believe the whole issue of portal of entry versus system delivery will be moot because a DAF=1 would be assumed for both cases. The regional injury pattern in the respiratory tract (olfactory and bronchiolar injury) is suggestive for a critical role of local metabolic activation. It is possible however that active metabolite is formed in and then escapes from the liver.

Avima M. Ruder

If chloroprene is indeed rapidly absorbed in mice, it makes sense that a systemic effect from the metabolite as well as a portal-of-entry effect could occur. From the text (p 5-21) I could not determine whether it is postulated that the portal-of-entry effect is from the parent compound or the metabolite; this could be made clearer.

Richard B. Schlesinger

Since it is not clear, as noted in the Document, the extent to which chloroprene induces cancer via direct contact with the lungs or via systemic contact of lungs with metabolites, the approach used is valid. However, the application of this approach is not clear from the discussion in the document.

(C) Carcinogenicity of Chloroprene

6. An oral slope factor (OSF) for cancer was not derived for chloroprene. Is the determination that the available data for chloroprene do not support derivation of an OSF scientifically justified?

Herman J. Gibb

The determination is justified. There were no data on which to base an OSF and the PBPK model developed by Himmelstein (2004) (description on page 3-7) did not seem adequate to allow route-to-route extrapolation.

Dale Hattis

Not completely. With a PBPK model formulation, an oral slope factor could be estimated.

Ronald L. Melnick

Yes, the lack of an adequate multiple-dose oral carcinogenicity study on chloroprene and the lack of information on the disposition of chloroprene, including the AUC for the DNA-reactive epoxide intermediate, after inhalation or oral exposure that might enable reliable route-to-route extrapolation justify not deriving an oral slope factor for this chemical. Because of a likely large first-pass liver effect after oral exposure, the systemic distribution of parent compound and reactive metabolites could be very different after oral versus inhalation exposures.

John B. Morris

I concur with the determination that the available data do not support derivation of an oral slope factor.

Avima M. Ruder

As there are no quantitative data on effects of oral administration (p 5-1), the determination appears justified.

Richard B. Schlesinger

The lack of oral exposure data clearly justifies not deriving an OSF.

V. SPECIFIC OBSERVATIONS

Herman J. Gibb

Page 4-1, line 8: Delete “and”

Page 4-3, line 1: Delete “also”

Page 4-3, line 8: Delete “number”

Page 4-3, lines 8-9: Delete “of these”

Page 4-3, line 14: Delete the second “were”

Page 4-5, lines 1-2: The document indicates that a limitation of Li et al. is that only three years of local area data were used to estimate the expected numbers of deaths which may not be representative with regard to the period of follow-up of the cohort. An issue not considered is the stability of the expected rates based on local data.

Page 4-5, line 5: This discussion is unclear. If the general population had a higher mortality for a given disease during the periods not examined, then there would have been a higher number of expected deaths and the SMR for that disease would have been overestimated for the period of time that was considered, not underestimated. If the mortality was lower, then the SMRs would have been overestimated. In any case, the discussion is not clear.

Page 4-6, line 18: Change “1979-1993” to “1979 to 1993”.

Page 4-6, line 22: Insert “the” before “general”.

Page 4-8, line 19: Change “1979-1988” to “1979 to 1988”.

Page 4-9, line 12: There is an inconsistency in how the SIR is reported on line 12 and in Table 4-6. Line 12 reports as 327 with 95% CI of 147 and 727; Table 4-6 reports as 3.27 with 95% CI of 1.47 and 7.27. The epidemiology section has several examples of changing back and forth between the convention of using the convention of multiplying by 100 and the ratio. Need to make consistent.

Page 4-9, line 23: Change “suggested” to “suggest”

Page 4-9, line 23: What are “highly exposed operators”? High cumulative exposure? Intensity of exposure? Duration of exposure? It makes a difference in the interpretation.

Page 4-10, line 29: Insert “in the group employed” before “prior”. Presumably the author is describing those employed prior to 1977 and not those who developed cancer prior to 1977.

Page 4-10, line 33: The document states that "all of the SIRs exceeded 100" yet Table 4-7 indicates no SIR is over 100. Again, the authors need to use a consistent convention (report as a multiple of 100 or not report as a multiple of 100).

Page 4-11, line 10: Change "cancers" to "cancer"

Page 4-11, line 15: Is there any indication of how many workers died or left the area prior to 1979? Does the author have an idea of how much impact this would have on results or is it part of a laundry list of study faults? The power of the study was low regardless of whether workers died or left.

Page 4-14, lines 16-24 and Page 4-15, lines 1-3: It is not difficult to understand why Marsh et al. would conclude that their study provided no evidence of cancer risk associated with chloroprene exposures. Table 4-9 on page 4-14 shows little evidence of a dose response. It is inappropriate to conclude as is done in lines 1-3 on page 4-15 that Marsh et al.'s explanations are "not entirely consistent with the data presented". The authors of this document have chosen one interpretation; the authors of the study have chosen another interpretation.

Page 4-15, lines 24-35: Some of the criticisms are too harsh. For example, how often are causes of death verified by histological confirmation or review of medical records? Nice if it can be done, but the vast majority of mortality studies would fall in the same boat. Incomplete enumeration of incident cases is a criticism that could be leveled at many incident studies. The statement that despite the lack of quantitative exposure information, occupational studies are still able to contribute to the overall qualitative weight of the evidence considerations (lines 31-33) states the obvious, but the statement should not be used as license to draw conclusions on studies that have serious limitations.

Page 4-16, Table 4-10: All SMRs are reported as the multiple of 100 except for Bulbulyan et al. (1998). "Sullivan" should be "Selevan". It would be more logical to have the intermediate exposure column first, followed by the high exposure column, followed by the total cohort column.

Page 4-17, Table 4-11: The relative risk is reported as a multiple of 100 for the high and intermediate exposures in the Leet and Selevan (1982) study but not for the other studies. "Sullivan" should be "Selevan." It would be more logical to have the intermediate exposure column first, followed by the high exposure column, followed by the total cohort column.

Page 4-18, lines 7-8: The limited number of cases (one in each cohort) "precluding meaningful examination" states the obvious.

Page 4-18, line 19: "these cancers"? Should this be "an increased liver cancer risk"?

Page 4-19, line 8: "No workers experienced loss of hair." This is the first place where loss of hair is mentioned. Since that is an unusual effect, it would be better to report the results of the distillation workers after the results of the polymerization workers.

Page 4-63, line 13: What is "horizontal activity"?

Page 4-66, line 30: Delete "based on available data".

Page 4-67, Table 4-38: "Sullivan" should be "Selevan"

Page 4-69, lines 6-8: "Although not statistically significant, these findings were comparable to results (RR range 2.9-7.1) detected in two other studies for high and intermediate cumulative exposures (Bulbulyan et al., 1999, 1998)." Given that there could have been considerable differences in exposure, follow-up, duration of exposure, etc. between the studies, such a statement is not justified.

Page 4-69, lines 23-26: "only Bulbulyan et al. (1999) observed a statistically significant association between chloroprene exposure and liver cancer mortality." The preceding sentence suggests that this was done by an internal analysis, but the increase in liver cancer mortality was observed from an external analysis.

Page 4-69, lines 29-30: "….although there is no direct evidence that alcohol is related to the exposure of interest (i.e., chloroprene)." There may be no "direct evidence that alcohol is related to the exposure of interest"; there is no direct evidence that is not either. More convincing that alcohol did not play a confounding role would have been clear evidence of a dose response to chloroprene since it would be unlikely that alcohol consumption would correlate with chloroprene exposure. Evidence of a dose response, however, is equivocal (see Table 4-11 on page 4-17).

Page 4-70, lines 7-10: Criticizing mortality studies for not doing a medical record review or histological examination to confirm cause of death is extreme. Almost all mortality studies could be faulted for not doing that.

Page 4-71, lines 21-24: What "current understanding" allows one to state that specificity is "one of the weaker guidelines"? Reference?

Page 6-1, line 22: Replace "th" with "the".

Dale Hattis

1. Table 3.2 should express results in fraction of total metabolites rather than relative to butanol standard. Or it could be expressed in terms of absolute rates per unit time per unit microsomal protein. Recalculate?

2. p. 3-5, lines 5-7: "Estimates for V_{max} and K_m for oxidation of chloroprene in liver microsomes ranged from 0.068–0.29 μmol/hour/mg protein and 0.53–1.33 μM, respectively."

The meaning of the ranges should be described. If these are in fact the ranges of all observations, then the number of observations should be given; also, there should be some way of describing the dependencies of the estimates of V_{max} and K_m values.

3. Presentation of metabolic data in Table 3-4 is inadequate. No error bars or statements of how many animals tested independently (or pooled?), or more crucially, how many humans and how they differ in V_{max}/K_m for various organs (obtain original papers on metabolism).

Source: Himmelstein et al. (2004a).
Himmelstein, MW; Carpenter, SC; Hinderliter, PM. (2004a) Kinetic modeling of beta-chloroprene metabolism: I. In vitro rates in liver and lung tissue fractions from mice, rats, hamsters, and humans. Toxicol Sci 79(1):18–27..

4. Table 3.5: Again, no error bars or description of the number of animals studied or experimental errors.

5. p. 3-7, lines 4-5: "The clearance of these thioethers reached a threshold at 24 hours after dosing, indicating that elimination was rapid."

Use of the word "threshold" here is unclear and ill-advised. If what is meant is that there was no further increase in thioether excretion, then that should be said explicitly.

6. Table 3-6: Why are values not provided for the major physiological parameters (body weight, cardiac output, and alveolar ventilation)?

7. Epi data discussion: The authors do qualify the discussion of the epidemiological data with the healthy worker effect. However, they do not as yet include suitable caveats for the "internal" comparisons by mentioning the distortions expected from the healthy worker survivor" effect — that longer exposed workers with higher cumulative exposures have lower mortality than shorter term workers. This must be incorporated into the analysis. Some language I have adapted from prior work (Hattis and Goble 2007) is:

> "The "healthy worker survivor" effect is a known phenomenon that produces established distortions in relationships between measured risks and measures of cumulative exposure, as shorter term workers suffer greater mortality than workers who work at exposure-producing jobs for longer periods of time (Steenland et al., 1996; Kolstad and Olsen, 1999; Garshick et al. 2004; Siebert et al. 2001; Steenland and Stayner 1991). Adjustments for this effect are at the cutting edge of current practice for the treatment of human epidemiological data, but they are vital for achieving the best possible analysis of those data. Even if the data will not support the more complex analyses [and analyses of this sort are

notoriously complex (Robins 1986; Arrighi and Hertz-Picciotto 1996; Hertz-Picciotto, personal communication)], EPA could provide at least some discussion of how large the distortions might be by citing such previous cases as the cancer risks from diesel particles (Garshick et al. 2004; 2008) and the approach that California risk assessors (and possibly others) have taken to risk analysis where the healthy worker survivor effect is even more prominent than it may be in this case. (For diesel particulates, initial estimates of the relative risk vs. cumulative dose curve even had a negative, rather than a positive slope.)"

8. The discussions of both liver and lung cancer might benefit from some attempt at integrative meta-analysis, combining the effects of multiple studies for reasonably comparable levels of exposure. This, however, likely depends on obtaining some disaggregated data from the individual investigators, and that might not be possible. Even if the combination is somewhat speculative, it might be informative to make some attempt to combine the human evidence for comparison with the projections from animal studies.

9. Chronic NTP exposures: For later modeling, the authors should report integrated average exposures that were measured, rather than the nominal target exposures. The difference may well be small, as indicated in the discussion, but the measurements should be used in preference to the target levels in the dose response modeling which appears later in the document.

10. p. 4-54, lines 16-18: "Estimates for V_{max} and K_m for oxidation of chloroprene (into (1-chloroethenyl)oxirane) in liver microsomes ranged from 0.068–0.29 μmol/hour/mg protein and 0.53–1.33 μM, respectively."

Again, what is the meaning of these ranges? Simple ranges of all best estimates for all species? 5%-95% confidence limits? What is the number of experiments based on how many different individuals in which species, particularly for humans?

Undescribed ranges of this type are absolutely useless for understanding the uncertainty and variability of the data, or for drawing inferences for subsequent steps in the risk analysis.

11. p. 4-61, lines 5-7: "A comparative report of the carcinogenicity of these compounds highlights the qualitative and quantitative concordance of their tumorigenic effects (Melnick and Sills, 2001). The female mouse lung was the most sensitive site of carcinogenicity for both chloroprene and butadiene."

It would be useful to have some quantitative comparison of cancer potency in rodents for these compounds. The full abstract is:

Comparative carcinogenicity of 1,3-butadiene, isoprene, and chloroprene in rats and mice.

Melnick RL, Sills RC.

Chem Biol Interact. 2001 Jun 1;135-136:27-42.

National Institute of Environmental Health Sciences, National Institutes of Health, PO Box 12233, Research Triangle Park, NC 27709, USA. melnickr@niehs.nih.gov

1,3-Butadiene, isoprene (2-methyl-1,3-butadiene), and chloroprene (2-chloro-1,3-butadiene) are high-production-volume chemicals used mainly in the manufacture of synthetic rubber. Inhalation studies have demonstrated multiple organ tumorigenic effects with each of these chemicals in mice and rats. Sites of tumor induction by these epoxide-forming chemicals were compared to each other and to ethylene oxide, a chemical classified by the National Toxicology Program (NTP) and by the International Agency for Research on Cancer (IARC) as carcinogenic to humans. For this group of chemicals, there are substantial species differences in sites of neoplasia; neoplasia of the mammary gland is the only common tumorigenic effect in rats and mice. Within each species, there are several common sites of tumor induction; these include the hematopoietic system, circulatory system, lung, liver, forestomach, Harderian gland, and mammary gland in mice, and the mammary gland and possibly the brain, thyroid, testis, and kidney in rats. For studies in which individual animal data were available, mortality-adjusted tumor rates were calculated, and estimates were made of the shape of the exposure-response curves and ED10 values (i.e. exposure concentrations associated with an excess risk of 10% at each tumor site). Most tumorigenic effects reported here were consistent with linear or supralinear models. For chloroprene and butadiene, the most potent response was for the induction of lung neoplasms in female mice, with ED10 values of 0.3 ppm. Based on animal cancer data, isoprene and chloroprene are listed in the NTP's Report on Carcinogens (RoC) as reasonably anticipated to be a human carcinogen. Butadiene is listed in the RoC as known to be a human carcinogen 'based on sufficient evidence of carcinogenicity from studies in humans, including epidemiological and mechanistic information', with support from experimental studies in laboratory animals. Epidemiology data for isoprene and chloroprene are not considered adequate to evaluate the potential carcinogenicity of these agents in humans.

I believe the similarity of ED10s for lung tumors is potentially helpful for the reader, however, a more comprehensive summary of potencies for other and/or all tumors would provide important background for the quantitative cancer risk analysis. Table 4-37 should be supplemented with a table giving quantification of the indicated potency for multiple- and all sites.

12. p. 4-69, lines 13-19: “One of the strengths of several of the more recent epidemiologic studies was improved exposure assessment data. These studies utilized industrial hygiene information to determine which areas or jobs were most likely to have received higher chloroprene exposures. This allowed for examination of various exposure contrasts and helped reduce the potential for exposure misclassification. As such, valid internal analyses were conducted which were less impacted by bias due to the healthy worker effect. Despite these improvements, several study limitations added to the

uncertainty in addressing the weight of evidence of the epidemiologic data."

The discussion following this paragraph should include the healthy worker survivor effect.

13. Table 5-2: DAFs greater than 1 for lung and less than 1 for nasal epithelium deserve specific discussion.

14. Page 5-20, top: Variability (uncertainty?) in slope factors follows a normal distribution? Try lognormal.

15. Cancer modeling: In view of the saturation of the generation of active metabolite, and the need to drop high doses in some cases, there should be investigation of a Michaelis Menten transformation of dose, in lieu of a full PBPK model. Demonstrate results of this for the incidence of tumors in mice (without the Weibull factor for time dependent tumor observations).

References

Arrighi HM, Hertz-Picciotto I. 1996. Controlling the healthy worker survivor effect: an example of arsenic exposure and respiratory cancer. Occup Environ Med. 53(7):455-462.

Garshick E, Laden F, Hart JE, Rosner B, Smith TJ, Dockery DW, Speizer FE. 2004. Lung cancer in railroad workers exposed to diesel exhaust. Environ Health Perspect. 112(15):1539-43.

Garshick E, Laden F, Hart JE, Rosner B, Davis ME, Eisen EA, Smith TJ. 2008. Lung cancer and vehicle exhaust in trucking industry workers. Environ Health Perspect. 116(10):1327-32.

Hattis, D. and Goble, R. L. "Uncertainties in Risk Assessment for Carcinogenesis: A Road Map Toward Practical Improvements" White paper for the U.S. Environmental Protection Agency, May, 2007.

Kolstad HA, Olsen J. 1999. Why do short term workers have high mortality? Am J Epidemiol. 1999 Feb 15;149(4):347-52.

Neumeyer-Gromen A, Razum O, Kersten N, Seidler A, Zeeb H. 2009. Diesel motor emissions and lung cancer mortality--results of the second follow-up of a cohort study in potash miners.Int J Cancer. 124(8):1900-6.

Robins J. 1986. A new approach to causal inference in mortality studies with a sustained exposure period--application to control of the healthy worker survivor effect. Mathemat Modeling 7:1393-1512.

Siebert U, Rothenbacher D, Daniel U, Brenner H. 2001. Demonstration of the healthy

worker survivor effect in a cohort of workers in the construction industry. Occup Environ Med. 2001 Dec; 58(12):774-779.

Steenland K, Deddens J, Salvan A, Stayner L. 1996. Negative bias in exposure-response trends in occupational studies: modeling the healthy workers survivor effect. Am J Epidemiol. 143(2):202-210.

Steenland K, Stayner L. 1991. The importance of employment status in occupational cohort mortality studies. Epidemiology. 2(6):418-423.

Ronald L. Melnick

Page 3-2 to 3-5. The discussion on chloroprene metabolism is deficient in its consideration of species differences in glutathione conjugation, catalyzed by glutathione-S-transferase, in the detoxification of (chloroethenyl)oxirane.

Page 3-7 to 3-8. Discussion is needed on likely differences in chloroprene clearance among species. Factors influencing the clearance of chloroprene include fat:air partition coefficients, % of body weight as fat (mouse: 5%; rat: 7%; human 21%), metabolic elimination, etc.

Page 4-13. It seems odd that of the 652 cancer cases in the Louisville facility, only 1 case was unexposed (Table 4-8). This might suggest that a large percentage of individuals classified as exposed were essentially unexposed. The document should provide greater emphasis on the potential impact of exposure misclassifications.

Page 4-16 to 4-17. Use common units for SMR and RR values in Tables 4-10 and 4-11. On some cases the actual ratios are given, while in other cases the ratios are expressed as per cent.

Page 4-22. Contrary to the statement on lines 2-6, the data in Table 4-14 show incidences of ovarian or mammary tumors in control female rats.

Page 4-47, lines 5-7. Additional analyses are needed before dismissing the finding of increased resorptions in the 10 and 25 ppm exposure groups.

Page 4-60. Delete lines 12-15. The hypothesis that chloroprene would only produce tumors in directly exposed tissues has been disproved by the NTP studies which demonstrated the multiple organ carcinogenicity of this chemical.

Page 4-63, line a6. Severities were minimal to moderate, not minimal to mild.

Page 4-73, line 7. The document specifies a mutagenic MOA involving the reaction of epoxide metabolites formed at target sites. Until studies are conducted evaluating blood levels of epoxide intermediates it would not be appropriate to impose this target site

limitation. It is not known if epoxide formation occurs in all of the tumor target sites identified in the rodent carcinogenicity studies.

Page 5-19, Table 5-7. The unit risk value for hemangiosarcomas/hemangiomas is incorrect – it should be 2.8×10^{-5}, not 8.3×10^{-5}.

John B. Morris

Pages 3-1 - 3-6

The data on partition coefficient should be discussed more completely. It is true that it is possible to infer information on tissue distribution from such data. It is also possible to make inferences on regional respiratory tract absorption from these numbers. A vapor with a blood:air partition coefficient less than 10 is not likely to be scrubbed efficiently from the airstream in the upper airways. This is an important point because an inhalation cancer potency factor will be derived assuming category 1 status.

More detail should be provided on the metabolism kinetics for chloroprene. The information on elucidation of putative metabolites is clear and concise, but the data on kinetics is incompletely presented data and is very difficult to interpret fully. The information in Table 3-1 needs to be more fully described. Is this table cited in the text? Precisely how were these data obtained, what is the meaning of these data, particularly with respect to rodent-human extrapolations? The relative level of metabolite 1 in the humans was approximately 10-fold lower than the F344 rat and mouse. The level of metabolite in the Wistar rat and hamster was lower as well. Were these quantitative differences synthesized into a coherent explanation of species differences in response?

Similar issues could be related relative to Tables 3-4 and 3-5. The text should precisely indicate how the estimates for V_{max}/K_m for lung metabolism were obtained. The mouse – human comparison for lung metabolism is particularly important, a fact that was not adequately considered in the risk evaluation. The presented data indicate the activity in human lung is 50-fold lower than in mouse lung (Table 3-4). The liver activities in the mouse and man are much more similar. Since metabolic activation is the first step in the mode of action and lung tumors in mice drives the risk extrapolation, this comparison becomes particularly important. Exactly how was the value of 1.3 for V_{max}/K_m in the human obtained? What is the reliability of this number? Can it or can it not but used for quantitative species extrapolations? An explicit rationale for not using these data in the data synthesis sections needs to be provided. It should be noted that this type of species difference (mouse to human pulmonary metabolism) is hardly unique to chloroprene. For example, consider styrene.

Pages 4-1 - 4-18

The section on human exposures to chloroprene appears to be objectively and concisely presented. Epidemiology is not within my area of expertise. My only comment is the thought that it would be useful if as much information as possible on occupational exposure levels would be presented in the text. At least to me, information on exposure concentrations in addition to cumulative (ppm-year) would be of value. If available,

recent published reviews of the epidemiological data relative to chloroprene should be cited.

Page 4-25

Clarity would be enhanced if the table also provided information on the magnitude of injury in Table 4-16 and subsequent tables. A footnote might be adequate. Alternatively, the average injury score might be provided parenthetically in each column. The wording of the text infers there was no observed histopathological damage in the lungs of mice in the 16 day study. Clarity would be enhanced if this were explicitly stated.

Page 4-28

Clarity would be enhanced if it were explicitly stated that lesions were not observed in the nasal respiratory mucosa in the 13-week study. All lesions in Table 4-19 were in olfactory mucosa, the reader must make the inference that respiratory mucosa damage was absent. This is an important issue relative to data interpretation.

Page 4-29

Clarity might be enhanced if it is stated that preening behavior might have lead to direct gastrointestinal exposure to chloroprene. If this is not thought to be the case, then it should be explicitly stated.

Pages 4-30 - 4-43

It is noted that all nasal lesions in Table 4-16 are presented under the heading of "olfactory," implying that no nasal respiratory mucosal lesions were observed. This needs to be explicitly stated. The subsequent text is quite ambiguous in this regard. For example, in the absence of any respiratory mucosal lesions, why include speculation on the relative expression of CYP450 in olfactory versus respiratory mucosa of the rat nose? (I did a quick scan of the NTP report to confirm, at least superficially, the absence of respiratory mucosal lesions.) All subsequent descriptions of these data, e.g. chronic nasal inflammation (p5-2) should be qualified to state chronic nasal olfactory inflammation (if this is, in fact, true). Site specificity of nasal lesions is a critical aspect in the evaluation of nasal response.

Subsequent portions of the text refer to time to tumor data. Where are these data and derivation described? Should some discussion of maximum tolerated dose and whether it was exceeded be included in the text?

Clarity would be enhanced if the text provided more detail on how the survival adjusted neoplasm rates in Table 4-28 were calculated.

The description of the Trochimowicz et al. 1998 study indicates there was less chronic respiratory disease in exposed than controls. Perhaps more information should be

provided on the lesions that were present in control animals. This would seem to be a relevant issue with respect to interpretation.

Page 4-54

The text (line 20) indicates epoxide hydrolysis was faster for the human and hamster than rat or mouse. Where are these data presented?

Page 4-45

The test (lines 27-32) indicates "some activity" was observed in strains TA97A and TA98. Subsequently (p. 4-65), it is stated the epoxide mutagenicity is "positive in all strains." Are these two parts of the text concordant?

Page 4-61, Table 4-37

This table is very confusing. What was the basis for including data from the rat relative to "sites of increased incidence" of neoplasms? Listed are many sites in which statistically significant results were not enumerated in previous portions of the text. Obviously, clarity needs to be improved.

Pages 4-62 - 4-65

In general, this "synthesis" of the inhalation exposure data is not a synthesis but merely a reiteration of the results. Rather than repeat the results study by study, it might be much preferable to organize this section on the basis of target organ. It could, for example, discuss the olfactory lesion data in toto, followed by the liver, etc. On page 4-62 line 15, it is stated that chloroprene is associated with reproductive and developmental effects, yet the earlier portions of the text concluded otherwise.

Table 4-38

Table 4-38 is somewhat confusing. Why was lung cancer mortality listed under "rare tumors?" The table includes a reference to time to tumor, yet such data were not presented earlier in the text.

Page 4-72

Lines 11-12 include a listing of increased incidences of tumors, yet the basis for inclusion in this listing is unclear. Some organs are listed in which the tumor incidence was not significantly increased. The discussion of species differences (lines 27-31) should include reference to possible species differences in epoxide hydrolysis rates. Such data are presented earlier and its absence here is confusing. This section fails to include the most important species difference – the appearance of lung tumors in mice but not rats. An in situ pulmonary metabolic basis might be provided, given that the metabolic activation rate in mice appears to be 50-fold higher than the rat but that in the liver differs by only

2-fold (Table 3-4 and 3-5). This would also serve to emphasize the potential role of metabolism relative to carcinogenicity. Epoxide formation is thought to be important relative to the respiratory tract toxicity/carcinogenicity of naphthalene and styrene and the same species differences (lung tumors in mice but not in rats) is seen for these vapors. Line 32 includes a reference to Dong et al 1989; this study was not described previously.

Page 4-75

The statement that in vivo uptake of chloroprene involved the balance between epoxide formation and detoxification is confusing. Certainly the toxicity depends on the balance, but it is unlikely that uptake does. Uptake rates depend on the blood and tissue concentration of parent, downstream conversion of metabolite is not necessarily important in diffusion-based uptake. Greater clarity is needed.

Page 4-76

It is stated on lines 3-4 that there is remarkable similarities in the potency and shape of the dose response between butadiene and chloroprene. Such data are not presented in earlier portions of the text.

Page 4-77

It is stated that Melnick et al. (line 18) performed a 6 month exposure-6 month follow-up study. Where are these data presented?

Page 5-3, top

The text needs to clearly describe how the atrophy and necrotic data were combined. I am not certain there are any data indicating nasal olfactory atrophy leads to necrosis (as stated on lines 5-6). The concept that necrosis may lead to atrophy is quite straightforward however.

Page 5-5

In my view, chloroprene is not a category 1 gas (see also my comments above). Its partition coefficient is only 10, clearly backpressure in nasal tissues controls the uptake process. The presence of non-respiratory tract tumors clearly indicates it is absorbed into the bloodstream. This vapor does not possess the physical chemical characteristics required of category 1 gases; in my view, it is a category 3 gas. The text needs to rigorously support this conclusion with respect to the physical chemical characteristics of chloroprene relative to those required of category 1 gases. The presence of olfactory lesions is NOT evidence that the toxicant was delivered via the airstream. Numerous compounds produce selective olfactory injury after parenteral administration. Indeed, the presence of olfactory but not respiratory nasal mucosal injury might be considered to provide data in support of a blood-borne mechanism. Naphthalene is one example of this phenomenon. Importantly, the subsequent text describes in great detail how the lung

lesions may be due to blood-delivered rather than air-delivered chloroprene. The text needs to be consistent. Redistribution of chloroprene from fat stores during non-exposure periods is one potential mechanism for a role of blood borne chloroprene in inducing olfactory lesions.

The RfC methodology is fatally flawed with respect to RGDR calculation. The derivations of these equations are based on the faulty assumption that the mass transfer coefficient is uniform throughout the nose. Dosimetry predictions from RGDR-based evaluations are totally discordant with the data. For example, the RGDR-predictions are counter to the theoretically sound modeling and experimental data obtained for formaldehyde and vinyl acetate. The RGDR-based estimates of species differences in dosimetry are discordant with the database on acetaldehyde dosimetry in multiple laboratory animal species. While application of a flawed methodology may be consistent with EPA policy, it certainly is not consistent with the scientific state-of-the-art. Perhaps it is felt that chloroprene is truly a category 2 gas, but it is assigned category 1 status because of difficulty in implementing RGDR calculations for category 2 gases. If so, it should be explicitly stated. As noted above, its low partition coefficient and the existence of distal organ effects indicate chloroprene is likely a category 3 gas.

The mode of action is assumed to include metabolic activation to the epoxide. The RGDR of 0.28 indicates the humans will receive roughly 4-fold more toxicant (1/0.28) than the rat. Is it meant to imply that the metabolic activation rate in the human nose is 4-fold higher than the rat? Is there a single example of this being the case? The use of the RGDR needs to be discussed in light of the metabolically-based mode of action.

Page 5-8

I recognize that it may be policy to include a database limitation factor due to the lack of a two generation study, but I do not feel it is scientifically justified in this case. A multigeneration study does exist. The rationale for the selection of this uncertainty factor should include this study.

Table 5-3

Table 5-3 does not include a row in the consideration column for database limitation.

Table 5-4

This table provides time to tumor data, but such data have not been presented.

Page 5-21

Would it be possible to compare the tumor risk calculations with the human workplace experience? This might provide a useful “reality check.” Even if the occupational exposure levels were only crudely known, it might be possible to determine if the estimated cancer risks were at least somewhat reflective of reality.

Page 5-25

The cross-species scaling section is deficient in that it does not include consideration of metabolism rate. The first step in the mode of action is metabolic activation to an epoxide and the toxicokinetic data indicate the mouse lung activity exceeds that in the human by 50-fold (Table 3-4). Clearly, this is highly relevant. Moreover, magnitude of species difference in metabolism is not unique, consider styrene or naphthalene. One might convincingly argue that the enormous metabolic activation rate in the mouse coupled with the low epoxide hydrolysis rate renders this species inappropriate relative to extrapolation of lung tumors. The authors of the document may not agree, but a critical discussion and rationale for using the mouse data needs to be included.

Page 6-5

The sentence on lines 18-19 is confusing. Lesions were specific to the olfactory mucosa, what is the relevance of cytochrome P450 in respiratory mucosa in this regard?

Avima M. Ruder

Page 2-1 line 12. volume produced or volume used?

Page 2-1 line 18. Is Mg a million grams? Not in List of Abbreviations.

Page 2-1 line 22. Starting material for chloroprene synthesis is butadiene *in the U.S.*

Page 2-2 line 15. Suggest rewording to: The polymerization process has been discussed…

Page 3-2 line 5. Suggest inserting "that of" between "similar to" and "vinyl chloride"

Page 3-4 Figure 3-1 and caption. Why these numbers? Why not consecutive in key/caption? Why no 2, 3, 6, etc.?

Throughout section 4, SMRs and SIRs should consistently use base 1 or base 100, not vary (cf pp 4-10 and 4-11). The adjectives low-exposure and high-exposure are not consistently hyphenated (cf p 4-2 lines 18 and 19 versus line 25, p 4.7 table 4-4 title vs. header for column 3). Deaths can be in excess but cannot be elevated (cf p 4-3 line 13). SMRs can be elevated. Deaths in and of themselves cannot be statistically significant; SMRs can be (cf p 4-3 line 13). Mortality is a rate and therefore "Mortality rate" (cf page 4-6 line 22) is redundant. Check citations! Leet and Selevan becomes Leet and Sullivan in tables 4-10 and 4-11.

Page 4-1 line 2. occupationally exposed should not be hyphenated. "during" not "from" the period …

Page 4-1 line 8. delete "and" at beginning of line

Page 4-1 line 20. Need comma after 1957. Similarly page 4-3 lines 24-25, page 4-4 line 13, etc.

Page 4-2 line 14. Change "both internal…and" to "either internal…or"

Page 4-2 lines 24-25. Needs commas after SMR and liver.

Page 4-2 line 31. Lack of adjustment (data were available) or lack of ability to adjust (data were unavailable)?

Page 4-3 line 8. A total…*was* observed

Page 4-3 line 13. Suggest rewording to: "observed cancer deaths were also in excess (SMR = 140) but the SMR was not statistically significant…"

Page 4-3 line 14. Change last phrase to "and four deaths due to lung cancer"

Page 4-3 lines 15-17. Suggest rewording to: "With five observed cancers of the urinary system (3 bladder and 2 kidney) the SMR was significantly elevated (300 compared to the DuPont population and 250 compared to the U.S…."

Page 4-3 line 23. Suggest "accrued" instead of "worked for"

Page 4-3 line 24. Should be "*was* identified" (subject is "a cohort")

Page 4-4 line 3. Were exposures determined or estimated?

Page 4-4 lines 8-10. The sentence as written doesn't actually state that males had increased exposure. Suggest "Males had statistically significant ($p<0.005$) greater exposure to chloroprene than females based on…"

Page 4-4 line 11. Subgroup has not been defined.

Page 4-4 line 13. "their *dates* of death"

Page 4-4 line 15. Suggest "sixteen reported cancer deaths occurred among…"

Page 4-5 Table 4-2, row "researcher". All cause cell needs slash between 21 and 176.

Page 4-5 line 1. Suggest "One limitation of the Li et al. (1989) study was insufficient comparison mortality data"

Page 4-5 line 2. "years *were* not"

Page 4-5 line 4. "*time* periods"

Page 4-5 line 6. Suggest "…during the time periods with no rates available,…"

Page 4-5 line 8. "there *were* no data…"

Page 4-5 line 17. "age at death was 12.7 years *younger*"

Page 4-6 line 7. Not clear whether "lasting and making" is one or two departments

Page 4-6 line 10. Locations or departments?

Page 4-6 lines 11-12. Suggest: "year. They therefore devised a relative exposure system. Workers in the high-exposure departments were assigned…"

Page 4-6 lines 19-20. Suggest: "Thirty-seven percent of cohort members (female/male distribution was not provided) contributing 26,063 person-years…"

Page 4-6 line 22. Suggest: "Mortality of the general population of Moscow was used for comparison."

Page 4-6 line 24. Suggest "available only"

Page 4-6 line 25. "the *rate* of expected deaths"

Page 4-6 lines 29-31. Need to specify that SMRs were elevated, not just statistically significant. What are "cancer-specific SMRs for liver cancer and leukemia" as opposed to "SMRs for liver cancer and leukemia"?

Page 4-7 line 4. "low number". Is this a statistically significant decrease? Or provide expected.

Page 4-7 line 8. Delete comma after leukemia.

Page 4-4 Table 4-4 header. All cases or just high-exposure cases?

Page 4-7 lines 10-11. Suggest: "…analysis by categories of duration of employment in high-exposure jobs (1-9…"

Page 4-7 line 12. Need new paragraph starting with "The cumulative.."

Page 4-7 line 15. "Kidney cancer was increased in all categories…" Are these categories of duration of employment as in lines 10-11 or tertiles or quartiles of cumulative exposure?

Page 4-8 line 13. "Similar to *the* Li et *al.* study…"

Page 4-8 line 14. Suggest: "…values if *mortality during* these years *was* not representative…"

Page 4-8 line 20. "Death certificates were coded by using *the* ICD-9…"

Page 4-9 line 9. Suggest: "Cancer incidence data *were available for* 1979-1999…"

Page 4-9 line 10. "…were identified, with six liver.."

Page 4-9 line 13. "lung cancer *in* both the total…"

Page 4-9 line 20. "noted *in analyses* using…"

Page 4-9 lines 21-22. "…five cases in *the* highest cumulative exposure *category* of…"

Page 4-10 line 7. "adjusted for *in either* mortality…"

Page 4-10 line 12. "time" of employment—era of employment or time of first employment?

Page 4-10 line 23. "…estimated *daily* exposure…" ?

Page 4-10 lines 29-30 states that 32 cancers occurred prior to 1977. How is that possible if the registry began in 1979? Does this mean 32 cancers occurred among those exposed prior to 1977?

Page 4-10 line 32 states all SIRs exceeded 100. Table 4-7 presents SIRs using base 1. Page 4-11 Table 4-7 header 3rd column. Cases Exposed before 1977?

Page 4-11 lines 2-3. "lung cancers *occurred* in workers with >20 years of exposure…, 3 in *those with* 11-20 years…and 1 in *those with* $\leq$10…"

Page 4-11 line 10. "the lung *cancer* excess…"

Page 4-11 line 11. "…smoking and alcohol consumption *were*…"

Page 4-11 line 18. Suggest: "…using external regional rates and internal comparisons…"

Page 4-11 line 20. "…both chloroprene and a potential…"

Page 4-12 throughout. As done in some places, but not consistently, label data with plant initials instead of providing a string of numbers and then stating "respectively". For example, line 9, change "1.54 and 0.094 ppm, respectively" to "1.54 (L) and 0.094 (M)". Similarly in lines 11, 24, 25.

Page 4-12 lines 4-6. Suggest: "Kentucky, and Ponchartrain (P), Louisiana. The third one was the Maydown (M) plant in Northern Ireland and the fourth facility was the Enichem Elastomer plant in Grenoble (G), France."

Page 4-12 line 8. Suggest "occurred at" instead of "existed in"

Page 4-12 line 14. "cohorts" (as in line 10)

Page 4-12 line 23 states 266, 48, 12, 10 for lung cancer deaths; table 4-8 has these numbers for all respiratory cancer deaths. Were all respiratory cancer deaths lung cancers?

Page 4-12 line 26. Suggest: "deaths *than expected* from liver cancer were…"

Page 4-12 line 29. Suggest: "~~when~~ compared to expectations based on the general population. When…"

Page 4-13 line 2. "trends across quartiles of exposure were examined"

Page 4-13 line 14. "included" instead of "contained"

Page 4-13 line 23. Delete "the" at end of line

Page 4-14 line 4. "…work status *was* so highly…"

Page 4-14 line 7. "They found inverse *associations*…"

Page 4-15 lines 7-8. "cohorts had *fewer* than 1000 workers, while the remaining cohorts had *fewer* than 6000."

Page 4-17, line 8. "…Louisville, Kentucky, plant."

Page 4-18 line 16. "found in workers *who*…"

Page 4-18 line 32. "…cohorts *on* different…

Page 4-19 line 7. "…much *lower* numbers…" or "many fewer numbers"

Page 4-20 line 1. "…19-23 employed…"

(I did not read section 4.2 as closely as the preceding section; there may be errors and ambiguities I did not catch.)

Page 5-15 line 3. Delete period preceding 1st word in line

Page 5-17 line 26. "multis*t*age-Weibull…"

Page 5-21 line 21. EPA 1994A or EPA 1994B?

Page 7-3 lines 19-20. Only articles by the same author (which these are not) should be labeled 2001a and 2001b.

Page 7-5 line 33. "…*life* table analysis…"

Page B-2 Figure B-1. Abbreviations should be explained in a caption (similarly for other figures). What is the metric for the doses (horizontal axis)?

Richard B. Schlesinger

Section 4.6. The first paragraph of this section should have a subsection 4.6.1. Human Studies and the Animal Studies should be renumbered as 4.6.2.

Section 4.7. This section could be better organized. The summary in section 4.7.1 should probably be moved to the end of the entire section on carcinogenicity. The human data are discussed separately in an Evidence for Causality section, yet this is not provided for the animal studies. A true synthesis would discuss Evidence for Causality across studies in all species. This could be integrated with the discussion in Section 4.7.3.3 on Mode of Action to provide a stronger rationale for effects of chloroprene