PEER REVIEW SUMMARY REPORT

External Peer Review Teleconference on the *Toxicological Review of Propionaldehyde (CAS No. 123-38-6)*

Prepared for:
John Stanek, Ph.D.
U.S. Environmental Protection Agency
National Center for Environmental Assessment
MD B-243-01
Research Triangle Park, NC 27711

Prepared by:
Versar, Inc.
Contract No. EP-C-07-025
Task Order 29

Peer Reviewers:
John Morris, Ph.D.
Andrew Salmon, Ph.D.
Richard Schlesinger, Ph.D., Fellow ATS
Jeffry Schroeter, Ph.D.

June 11, 2008

I. INTRODUCTION

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 in the risk assessment process, i.e., hazard identification and dose-response evaluation. In many instances, the IRIS database 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 available 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, within EPA's National Center for Environmental Assessment (NCEA), developed a Toxicological Review of Propionaldehyde. Propionaldehyde was nominated by EPA's Office of Air and Radiation (OAR) in 2000, 2001 and 2003 as a toxic air pollutant. The draft document slated for the external peer review contains a chronic inhalation RfC, but does not contain a chronic oral RfD or a quantitative cancer assessment.

Peer Reviewers:

John Morris, Ph.D. ***(Chair)***
University of Connecticut School of Pharmacy
Storrs, CT 06269

Andrew Salmon, D.Phil., C.Chem., M.R.S.C.[*]
Office of Environmental Health Hazard Assessment, State of California
Oakland, CA 94612

Richard Schlesinger, Ph.D., Fellow ATS
Dept. of Biology and Health Sciences, Pace University
New York, NY 10038

Jeffry Schroeter, Ph.D.
The Hamner Institutes for Health Sciences
Research Triangle Park, NC 27709

[*] Note: I am providing these comments on my own time and in my personal capacity. They do not represent the policy of the State of California or any of its agencies.

II. CHARGE TO THE REVIEWERS

The U.S. Environmental Protection Agency (EPA) is seeking an external peer review for a health assessment of propionaldehyde 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).

Peer review of this assessment is being sought to ensure that all available data relevant to the toxicological assessment of propionaldehyde have been appropriately and objectively evaluated. Below is a set of charge questions that address scientific issues in the assessment of propionaldehyde. Please provide detailed explanations for responses to the charge questions.

General Charge Questions:

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

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

3. Please discuss research that you think would be likely to increase the confidence in the database for propionaldehyde in future assessments.

4. Please comment on the identification and characterization of sources of uncertainty in sections 5 and 6 of the assessment document. Please comment on whether the key sources of uncertainty have been adequately discussed. Have the choices and assumptions made in the discussion of uncertainty been transparently and objectively described? Has the impact of the uncertainty on the assessment been transparently and objectively described?

Chemical-Specific Charge Questions:

(A) Oral reference dose (RfD) for propionaldehyde

1. No oral RfD has been derived in the current draft assessment based on the lack of studies available that examine the effects of propionaldehyde administered via the oral route. Are there available studies missing from the draft document that might be useful for deriving an oral RfD or that should be considered in this decision?

(B) Inhalation reference concentration (RfC) for propionaldehyde

1. The current draft IRIS assessment for propionaldehyde uses a combined reproductive/developmental exposure study by Union Carbide (1993) as the principal

study for the derivation of the RfC. Please comment on whether the selection of this study as the principal study has been scientifically justified. Has this study been transparently and objectively described in the document? Please identify and provide the rationale for any other studies that should be selected as the principal study. Is this study appropriate for use in this assessment?

2. Has the most appropriate critical effect (increase incidence of olfactory atrophy in male rats,) presented in Sections 4.3 and 4.5.2 of the Toxicological Review been selected? Is the rationale for this selection transparently and objectively described in the document? Please comment on whether the selection of this critical effect has been scientifically justified. Please comment on the choice of olfactory atrophy as the critical effect as opposed to other endpoints (e.g., vacuolization) and the rationale that this endpoint was chosen because it is on the continuum leading to overtly adverse effects such as cell death. Has the qualitative pathological relationship between effects observed been adequately and appropriately characterized? Please provide a detailed discussion. Please identify and provide the rationale for any other endpoints that should be used instead of increased incidence of olfactory atrophy in male rats to develop the RfC.

3. BMD methods were applied to incidence data on olfactory atrophy in male rats to derive the POD for the RfC. Please provide comments with regard to whether BMD modeling is the best approach for determining the POD. Has the BMD modeling been appropriately conducted and objectively and transparently described? Has the BMR selected for use in deriving the POD (i.e., 10% extra risk of olfactory atrophy) been scientifically justified? Please comment on EPA's decision to treat all cases of olfactory atrophy similarly, without consideration of the severity of the atrophy seen at different dose levels. Please provide a detailed discussion and any suggestions for consideration of severity in determining the POD including identifying and provide rationales for any alternative approaches for the determination of the POD and discussion of whether such approaches are preferred to EPA's approach considering the available data.

4. Please comment on the selection of the uncertainty factors applied to the POD for the derivation of the RfC. For instance, are they scientifically justified and transparently and objectively described in the document?

5. Please comment specifically on the database uncertainty factor of 3 applied in the RfC derivation. Please comment on the body of information regarding reproductive and developmental toxicity on propionaldehyde, the relevance of toxicity data on other aldehydes, and the relevance of toxicokinetic data regarding the likelihood of portal-of-entry effects as the critical effects in the determination of the database uncertainty factor. Please comment on whether the selection of the database uncertainty factor for the RfC has been scientifically justified. Has this selection been transparently and objectively described in the document?

(C) Carcinogenicity of propionaldehyde

1. Under the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (www.epa.gov/iris/backgr-d.htm), the Agency concluded that *data are inadequate for an assessment of the human carcinogenic potential* of propionaldehyde Please comment on the scientific justification for the cancer weight of evidence characterization. In addition, has the Agency properly characterized the potential for concern for carcinogenicity of propionaldehyde based on the available data on propionaldehyde and other aldehydes?

III. GENERAL IMPRESSIONS

John Morris

The propionaldehyde document provides a strong review of the available data relative to derivation of an RfC value for propionaldehyde. In my view the document correctly identifies the critical effect and does so in a logical, clearly expressed and transparent fashion. The overall conclusion that olfactory atrophy represents the critical effect is sound. Since the structurally related compound acetaldehyde also produces this lesion the text correctly highlights the current information available on this compound. A strength of the document is that it evaluates the toxicity information on propionaldehyde in the context of the complete database on structurally similar aldehydes. Weaknesses in the database are clearly delineated; the document appropriately identifies the principal study while clearly indicating its deficiencies. The methodology for estimating the benchmark dose and the application of the default RGDR-based species extrapolation are clearly described. The basis for uncertainty factors is similarly described with clarity and transparency. The use of summary tables on concentration-response relationships and sources of uncertainty add to the strength of the document.

Andrew Salmon

The Review is generally well written, and reflects a thorough and careful review of the available data. The key difficulty with propionaldehyde is that the available data, although providing important indications of its chemical and toxicological properties in certain areas, fall short of the ideal in some key respects. This is particularly apparent with regard to the lack of a dedicated long-term inhalation toxicity study which would address the question of possible carcinogenicity. The Review is therefore to be applauded for its effort to use structural analogy with other reactive aldehydes such as formaldehyde, acetaldehyde and acrolein to identify critical endpoints, and likely consequences of the precursor lesions observed in the available shorter term experiments with formaldehyde. A critical study is identified which, although designed for a different primary purpose, nevertheless provides important data addressing the concerns about damage to the respiratory tract during short to medium-term exposures.

A second area of concern which appears to be largely unaddressed is the potential, demonstrated for other reactive aldehydes, to cause or exacerbate respiratory conditions with an immunological component, such as asthma. While the details of such effects are not always clear this is a recognized problem for reactive irritants in general, including formaldehyde, acetaldehyde and acrolein. Some aspects of this response evidently involve the immune system, and the effect is typically more severe for atopic individuals, and especially those with existing asthma. In the case of formaldehyde there have been extensive mechanistic studies of the effects in animals as well as humans. Anecdotally, it appears that reactions of this type in individuals who are, or have become, sensitive to formaldehyde are a more widespread and disabling problem than even the much discussed findings of carcinogenicity. It has to be acknowledged that the data for propionaldehyde do not include studies in animals or humans which address this

question. However, application of the structure-activity comparison approach successfully demonstrated in considering the potential carcinogenicity would have been useful here.

There are a few instances in which the reviewers content themselves with enumeration of the available data and fail to make a sufficiently critical analysis of its implications (as noted below in the detailed comments), but for the most part the analysis is well presented. However, I am left with the overall concern that while all the proper procedures have been followed and the relevant data evaluated, this Review falls somewhat short of providing decisive advice to protect public health from the consequences of exposures to propionaldehyde, especially by the inhalation route. The limitations of the data place constraints on what can reasonably be proposed, but there is a failure to address the problem of major uncertainties such as the unevaluated potential carcinogenicity of propionaldehyde, and the need to protect the health of the population of the United States from uncertain, but potentially significant, threats.

Richard Schlesinger

For the most part, the document is written in a clear manner. However, in Chapter 4, the arrangement of the specific sections is not optimal since there is repetition of the same studies in different sections due to the manner in which the chapter is currently formatted. It would be better to arrange the discussion either by exposure regime, i.e., acute, chronic, etc, or by specific endpoint across all temporal exposure regimes. There are a number of other sections, specifically Chapter 6 and some others noted below in Specific Observations, in which details of studies are constantly repeated. The latter sections should be summaries of the data and results do not have to be repeated each time a summary is presented. For example, for Section 6, the paragraph on page 40, lines 21-27, is the type of summarization discussion that should be in this section.

Jeffry Schroeter

The derivation of the inhalation reference concentration for propionaldehyde was based on olfactory atrophy observed in male CD rats from a 7-week inhalation study conducted by Union Carbide (1993). Limited data was available to derive a reference dose or cancer assessment for propionaldehyde. The inhalation study was designed to be a reproductive/developmental study and therefore did not include full nasal histology. However, there were clear concentration-response and concentration-severity relationships in olfactory atrophy observed in the two nasal sections analyzed, justifying the selection of the critical effect. In addition, some toxicity characteristics for propionaldehyde were induced by examining toxicity of other structurally similar reactive aldehydes (acetaldehyde, acrolein, and formaldehyde), most notably acetaldehyde.

The information provided in this review is clear and concise and accurately describes previous studies conducted with propionaldehyde that can be used to estimate effect levels for human health risks. Based on the paucity of data for this compound, the conclusions drawn from available studies are as sound as possible. The limitations in

using this data to derive human health risks are well documented in this review and the appropriate uncertainty levels are described. There is sufficient information to believe that propionaldehyde behaves similarly to acetaldehyde given the similar physical characteristics of these aldehydes, and so information on the toxicity of acetaldehyde (which is more plentiful) was used to assist in the development of health risks to propionaldehyde. The similarities between these two chemicals and the usefulness of acetaldehyde data in the propionaldehyde risk assessment are well described in this review. The use of standard default dosimetric information and techniques in RfC methodology (including use of BMD and UFs) were used to derive the inhalation RfC.

IV. RESPONSE TO CHARGE

(A) General Charge Questions

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

John Morris

The toxicological review is logical and generally clear and concise. (Specific comments in this regard are posted below.) The document clearly and objectively represents and synthesizes the available scientific evidence for the hazard evaluation. I think a strength of the document is that it doesn't consider propionaldehyde toxicity in a vacuum, but rather interprets the propionaldehyde toxicity data in the context of the information available on other aldehydes such as formaldehyde and acetaldehyde while recognizing that a full structure activity relationship analysis is not possible. In this regard inclusion of information on isobutyraldehyde toxicity would be highly valuable. The overall conclusions are quite sound with respect to the likely target organ and critical effect, especially when the data on propionaldehyde are compared to those on acetaldehyde. The conclusions are well reasoned and demonstrate a strong knowledge of the nasal toxicological issues that represent important considerations in a risk evaluation. The derivations are consistent with EPA policy; however, as noted below, the scientific basis for EPA policy relative to nasal dosimetric considerations is quite questionable.

I would make two small points relative to clarity and inclusion in the document of the toxicity data on aldehydes other than propionaldehyde. First, clarity would be enhanced if it is stated near the beginning of the document that these comparisons would be made. Doing so provides the reader with the rationale for the inclusion of text on formaldehyde and acetaldehyde. Second, data on many aldehydes is included that is not used in forming the overall conclusions (e.g. pentanal, hexanal). Data that is not explicitly utilized to formulate a recommendation is best not included in the document as it detracts from the overall focus.

Andrew Salmon

The Review is well written, and clearly lays out the available evidence for the various toxic effects of propionaldehyde. The description of effects, particularly mutagenicity and respiratory system toxicity, not only for propionaldehyde but also for other related aldehydes, and the use of this comparative structure-activity approach to address uncertainties and data gaps in regard to propionaldehyde is particularly useful in presenting a coherent overall picture.

Richard Schlesinger

As noted above, there is much repetition of details of studies used in support of the exposure levels. Thus, the document can clearly be made more concise. The database details should be presented only once and then summary statements made in the various subsequent sections can be made based upon these data without repeating the data.

Jeffry Schroeter

The toxicological review for propionaldehyde identified no subchronic or chronic inhalation studies for propionaldehyde. Several short-term inhalation studies were reviewed with the reproductive/developmental study conducted by Union Carbide (1993) selected as the principal study for deriving an RfC. I feel that this review accurately and succinctly synthesizes the available scientific evidence for noncancer effects in a clear manner. The available studies are accurately represented and the selection of the principal study is presented in an objective manner. There is inadequate information to determine an RfD or to assess the carcinogenic effects of propionaldehyde.

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

John Morris

I am not aware of any additional studies on propionaldehyde that might be included in the risk assessment. Although not without shortcomings, the recent studies of Oyama et al. (Susceptibility to inhalation toxicity to acetaldehyde in Aldh2 knockout mice, Frontiers in Biosci: 12: 1927-1934, 2007) that show enhanced nasal toxicity following acetaldehyde exposure in aldehyde dehydrogenase knock-out mice might provide useful contextual information. Similarly, the recent acetaldehyde inhalation study of Dorman et al (Inhal. Toxicol 20: 245-256, 2008) might be included. Since considerable data are available on the inhalation toxicity of isobutyraldehyde including an NTP cancer bioassay, it would be highly appropriate to include this information as well to provide a more comprehensive overview to the existing database on inhalation toxicity of aldehyde vapors.

Andrew Salmon

In the absence of a definitive bioassay, one of the key issues in assessing the weight of evidence for possible carcinogenicity of propionaldehyde is the evidence for mutagenicity. A well-researched and thoughtful analysis of the evidence of this topic was presented in the review. One of the key points, which downgrades the overall weight of evidence in support of a concern for carcinogenicity, is the lack of a clear indication of mutagenicity in the classic Ames test in *Salmonella* bacteria. This is in spite of the more evidently positive data for mutagenic endpoints (including clastogenicity) in mammalian test systems. However, a new report - Samson & Bobeck (2008) – not only demonstrates a clear positive mutagenicity result in the *Salmonella* test, but also indicates that bacteria of this genus have an extensive array of defenses against mutagens such as propionaldehyde, and that the mutagenicity of this compound is increased when these are inactivated. This not only supports the evidence in favor of mutagenicity, but diminishes the influence of the earlier negative results.

As noted with further discussion below, the recent publication by Teeguarden *et al.* (2008) on deposition of acetaldehyde in the upper respiratory tract of rodents and humans should be considered to determine whether it provides useful insights into the corresponding processes for propionaldehyde.

Richard Schlesinger

None that I am aware of.

Jeffry Schroeter

I know of no additional studies that should be considered in the risk assessment for cancer or noncancer effects of propionaldehyde.

3. Please discuss research that you think would be likely to increase the confidence in the database for propionaldehyde in future assessments.

John Morris

The identified areas of uncertainly provide sound direction for future research. For example, a well designed and performed chronic inhalation study for propionaldehyde which includes complete nasal sectioning would enhance the database, as would a multigenerational study. In addition, since there are no carcinogenicity data, a two year bioassay would provide useful information. The priority for such a study depends on the degree of importance one places on the carcinogenic response to acetaldehyde vs. isobutylaldehyde relative to the potential response to propionaldehyde. Finally, since the current dosimetric extrapolation procedures of the US EPA are so controversial, precise information on the inhalation dosimetry of propionaldehyde would strongly aid in the formation of a scientifically based quantitative inhalation risk assessment of this compound.

Andrew Salmon

A major deficiency of the existing database is the lack of a long-term inhalation carcinogenicity study. Supporting studies of deposition in the respiratory tract, cell proliferation and formation of DNA-protein crosslinks have provided extensive additional understanding of the effects of formaldehyde and, to a lesser extent, acetaldehyde. It would be helpful if at least some of these studies were extended to include propionaldehyde.

Richard Schlesinger

There is basically only one relatively short term study and one endpoint that is used in derivation of the exposure levels. Thus, it would increase confidence in the results if a longer term study with more endpoints was available.

Jeffry Schroeter

It was stated that the literature search was conducted before July, 2007. I found no other studies with propionaldehyde that are relevant to health effects. However, a main argument in the RfC derivation is that propionaldehyde is toxicologically similar in many aspects to acetaldehyde. There were recently two publications on acetaldehyde inhalation: Teeguarden et al. A PBPK Model for Evaluating the Impact of Aldehyde Dehydrogenase Polymorphisms on Comparative Rat and Human Nasal Tissue Acetaldehyde Dosimetry. Inhalation Toxicology 20:375-390, 2008, and Dorman et al. Derivation of an Inhalation Reference Concentration Based Upon Olfactory Neuronal Loss in Male Rats Following Subchronic Acetaldehyde Inhalation. Inhalation Toxicology 20:245-256, 2000. These publications should be considered in future risk assessments of propionaldehyde. As far as the current review is concerned, I think these publications

only strengthen the argument for using olfactory atrophy as the critical effect, since this was also the endpoint selected for acetaldehyde.

4. Please comment on the identification and characterization of sources of uncertainty in sections 5 and 6 of the assessment document. Please comment on whether the key sources of uncertainty have been adequately discussed. Have the choices and assumptions made in the discussion of uncertainty been transparently and objectively described? Has the impact of the uncertainty on the assessment been transparently and objectively described?

John Morris

The document appropriately identifies and characterizes sources of uncertainty. Table 5-1 is quite useful in this regard. The impact of the uncertainties on the assessment has been transparently and objectively described.

Andrew Salmon

The presentation of key uncertainties in the data on histological changes in the upper respiratory tract, and the potential for carcinogenicity, are presented thoroughly and systematically in section 5. As noted previously in the general comments, the potential for reactive respiratory irritants such as aldehydes to produce or exacerbate functional impacts such as asthma is not addressed at all. This is a significant omission since earlier in the narrative the potential for special impacts on infants and children is summarily dismissed by saying that there are no studies having a bearing on this question, which is not considered further. The potential for asthma or related respiratory effects is especially significant for children, who suffer disproportionately from this condition. There is a discussion of the uncertainties in the mutagenicity data, the analogy with the upper respiratory tract lesions with aldehydes known to be carcinogenic, and the lack of data addressing the possible carcinogenicity of propionaldehyde. If anything, the narrative understates the degree of uncertainty (thus diminishing the apparent concern), although correctly identifying the key issues.

The level of uncertainty on several key points of the assessment is substantial, which is for the most part correctly recognized in the description.

Richard Schlesinger

It is certainly stated in the document that there is a paucity of data for propionaldehyde and Table 5-1 provides an excellent summary of the thinking that went behind justification for each of the "areas of consideration." Furthermore, the impact of the uncertainty has been clearly and objectively described. However, there are some issues regarding justification for decision on some of these issues. In the table noted above, the human relevancy of the animal data was based upon the fact that an irritative type mode of action involving the reactivity of aldehydes should be similar or identical across species. While this is certainly true, the relative effects at different sites in the respiratory tract may differ in different species. Animals with more extensive nasal passages than found in humans may have greater effect in the upper respiratory tract, while there may

be greater penetration into the lower respiratory tract in humans. There is also assumed to be no systemic uptake due to the high tissue reactivity of these materials upon contact. However, ozone is a highly reactive chemical and there is evidence for penetration into the lower respiratory tract and for some systemic uptake of subsequent reaction products with airway fluid or tissue components. In fact, the study noted on page 39, lines 24-25, indicates that there is systemic uptake of reaction products of aldehydes.

Jeffry Schroeter

As with most chemical-induced nasal histology studies, there will be uncertainty as to what biological endpoint to select and whether nasal effects are the most appropriate endpoint. These are accurately summarized in Table 5-1 of the review. Regarding the latter point, given the high nasal uptake in rodents, the known high solubility and reactivity of propionaldehyde, and the comparative toxicity with other aldehydes (especially acetaldehyde), I think the selection is justified and has been clearly stated in the review. One other area of uncertainty would be the relevance of nasal lesions in rodents in determining health effects in humans, given the different geometry and function of the respective nasal passages in each species. Specifically, the olfactory epithelium and inhalation airflow patterns are vastly different in rats and humans, and rats typically absorb a higher percentage of inhaled soluble gases in their nose. Lower nasal uptake may lead to a higher lung dose in humans, where the gas could penetrate deeper into the lung. Another source of uncertainty is the use of only 2 nasal sections, which may have resulted in missed lesions, specifically throughout the olfactory epithelium. Also, the Union Carbide (1993) study states that microscopic examination was conducted on “the anterior 2 sections of the nasal cavity”, yet I could not find more descriptive information as to the exact locations of these sections. It would be helpful to know where these sections were taken and how much of the sections were comprised of olfactory epithelium. This uncertainty is addressed in Table 5-1. The other sources of uncertainty have been objectively described.

Chemical-Specific Charge Questions:

(A) Oral reference dose (RfD) for propionaldehyde

1. No oral RfD has been derived in the current draft assessment based on the lack of studies available that examine the effects of propionaldehyde administered via the oral route. Are there available studies missing from the draft document that might be useful for deriving an oral RfD or that should be considered in this decision?

John Morris

I am not aware of any studies that would be useful for deriving an oral RfD for propionaldehyde. It might be noted that formaldehyde and acetaldehyde appear to be less hazardous by the oral than inhalation route (e.g. see Morris et al., Regul. Toxicol. Pharmacol. 24: 251-263, 1996).

Andrew Salmon

In practical terms this is fine, since the U.S. EPA is unlikely to be involved in regulatory situations for oral propionaldehyde. Some insight into oral intake levels which could be regarded as tolerable might be gained by examining the levels of propionaldehyde which occur in some common foods.

Richard Schlesinger

There are no relevant studies that I am aware of that would inform a positive decision regarding any RfD development.

Jeffry Schroeter

I could find no human or animal studies on the oral effects of propionaldehyde.

(B) Inhalation reference concentration (RfC) for propionaldehyde

1. The current draft IRIS assessment for propionaldehyde uses a combined reproductive/developmental exposure study by Union Carbide (1993) as the principal study for the derivation of the RfC. Please comment on whether the selection of this study as the principal study has been scientifically justified. Has this study been transparently and objectively described in the document? Please identify and provide the rationale for any other studies that should be selected as the principal study. Is this study appropriate for use in this assessment?

John Morris

The selection of the Union Carbide study as the principal study has been scientifically justified. That this is not an ideal study is clearly stated. As appropriately noted , this study does provide sufficient (but not ideal) data for an RfC formulation particularly considering the nature of the toxic response relative to the current state of the art on nasal toxicology. In short, the basis for selection of this study has been transparently and objectively described. (Below I provide a long list of suggestions that might improve clarity of the descriptions of the toxicological information. These represent minor issues; nonetheless, they might serve to enhance clarity).

The section on comparative toxicity of aldehydes represents a strong component of the overall risk assessment. The recent study of Dorman et al 2008 on subchronic acetaldehyde inhalation toxicity might be cited. Given the structural similarity between acetaldehyde and propionaldehyde and the similarity of nasal lesions induced by both compound I feel it is particularly appropriate to include information on our knowledge of acetaldehyde toxicity. Similarly information on inhalation toxicity of butyradehyde and isobutryaldehyde including the NTP studies should be included as well.

Andrew Salmon

It appears that this study was used as a result of necessity rather than choice, there being no plausible alternatives available. It is far from ideal for the purpose, but nevertheless appears to be sufficient to provide a basis for the RfC. The description in the document is appropriate.

Richard Schlesinger

This was basically the only study that could be used for derivation of the RfC due to paucity of the database. It is clearly described in the document.

Jeffry Schroeter

I feel that the use of the reproductive/developmental exposure study by Union Carbide (1993) as the principal study for an RfC derivation for propionaldehyde is justified. Propionaldehyde is a soluble, reactive aldehyde that has been shown to have high uptake

efficiencies in the nasal passages of dogs exposed by inhalation. This is similar to other reactive aldehydes such as acetaldehyde, acrolein, and formaldehyde, which demonstrate high uptake in the nasal passages. Due to the high nasal deposition, one would expect portal-of-entry effects, so an inhalation study where nasal histology has been examined would be the most appropriate study upon which to base an inhalation risk assessment. There are several concerns in using the study by Union Carbide (1993), and these have been adequately addressed in this document. Despite these shortcomings, I feel that this study is appropriate for use in this assessment.

2. Has the most appropriate critical effect (increase incidence of olfactory atrophy in male rats,) presented in Sections 4.3 and 4.5.2 of the Toxicological Review been selected? Is the rationale for this selection transparently and objectively described in the document? Please comment on whether the selection of this critical effect has been scientifically justified. Please comment on the choice of olfactory atrophy as the critical effect as opposed to other endpoints (e.g., vacuolization) and the rationale that this endpoint was chosen because it is on the continuum leading to overtly adverse effects such as cell death. Has the qualitative pathological relationship between effects observed been adequately and appropriately characterized? Please provide a detailed discussion. Please identify and provide the rationale for any other endpoints that should be used instead of increased incidence of olfactory atrophy in male rats to develop the RfC.

John Morris

I concur with the conclusion that olfactory atrophy represents the critical response. Olfactory atrophy represents a common nasal lesion being induced by a variety of compounds including weak organic acids (acetic, acrylic acid), aldehydes (acetaldehyde) and esters (ethyl acetate, dibasic esters, etc.). The description of the lesions is adequate to relate propionaldehyde-induced lesions to the lesions induced by these other nasal toxicants. Any additional information on the localization of propionaldehyde-induced lesions would be helpful. This decision to use olfactory atrophy as the critical lesion is sound and scientifically justified. The rationale for the selection of the critical response is transparently and objectively described. Table 4-1 is particularly helpful in evaluation the concentration-response relationships for the olfactory lesions. In my view, olfactory atrophy represents a toxic lesion. The data clearly indicate a concentration response continuum for these lesions. Use of 150 ppm as the LOAEL, based on olfactory atrophy and vacuolization is appropriate in my view. The qualitative pathological lesions and their concentration-response relationships have been adequately and appropriately characterized.

Andrew Salmon

The general choice of critical effect as histopathological changes in the upper respiratory tract is evidently reasonable in view of the somewhat limited overall database, and is consistent with the critical effects for other reactive aldehydes such as formaldehyde, acetaldehyde and acrolein. However, the justification for placing a specific cut-off level for these effects at the level of atrophy as opposed to vacuolization is questionable. The analysis is entirely correct in characterizing the various types of effect seen as a continuum with vacuolization at the lower end and more severe effects, including various degrees of metaplasia and even carcinogenesis, at the upper end. However, it seems inappropriate to argue that the vacuolization is for some reason an “adaptive” or “compensatory” response which is not relevant, as opposed to the slightly more severe level where atrophy is observed. This choice is particularly problematic in that the more severe levels of response have typically been observed either at higher doses or, importantly, in longer term studies. The available data for propionaldehyde include only

a relatively short-term study in comparison to the other aldehydes for which lifetime studies are available. The narrative as presented does not fully address the question of progression to these more severe endpoints, which are presented as particularly critical in determining either the adverse nature of the initial response or, at the further end of the severity spectrum, the potential for progression to frank carcinogenicity. In reality, it seems more reasonable to see all these response as a continuum, which could probably be spanned by exposures to any of the reactive aldehydes considered if sufficient levels of exposure and duration of study were available for consideration. This obviously presents a problem for an analysis which starts with the premise of defining a single "adverse" level as the basis of a POD and thus an RfC. There are some alternatives which address this dilemma. The U.S. EPA has for instance attempted to use "categorical regression" to deal with different levels of effect severity, although frankly, this has not been a universally accepted or even readily applicable concept to date (we look forward to further developments in this area!). The State of California has recently used a procedure where a scoring approach for different degrees of response (including relatively small increments) is used to develop a pseudo-continuous variable for incorporation into a benchmark dose analysis (see for example a current draft assessment for acetaldehyde shown of the OEHHA web site: http://www.oehha.ca.gov/air/hot_spots/pdf/AppendixDRELSummaries042408.pdf). This might be a useful concept to apply in the current situation, rather than applying an artificial cutoff to what is obviously a continuum of responses which increase progressively with both level and duration of exposure.

Richard Schlesinger

Due to the paucity of the database, this was basically the only effect that could be selected for derivation of the RfC. Had other studies been available, perhaps this would not be the one having the greatest biological significance, but in the absence of other appropriate data, this was a justifiable endpoint.

Jeffry Schroeter

I agree that olfactory atrophy incidence in male rats is the appropriate critical effect. There is a clear concentration-response relationship with very low incidence (2/15) at the lowest exposure concentration (150 ppm), and 100% incidence at the highest concentration (1500 ppm). In addition, lesion severity also increased with increased exposure concentration, being minimal at 150 ppm, minimal to moderate at 750 ppm, and mild to marked at 1500 ppm. This results in a well-defined dose response curve for establishing a POD and extrapolating to human health effects. The choice of this selection has been transparently and objectively described and justified in this review. As indicated in the review, olfactory atrophy is an effect on the continuum leading to cell injury and death, whereas vacuolization may not be observed with decreased cellular function. This was observed in the highest exposure group in males where olfactory lesion incidence was 100% and vacuolization had decreased to 2/15. I believe that the selection of olfactory atrophy was appropriate. In addition, it is similar to effects seen in rats exposed by inhalation to acetaldehyde.

3. BMD methods were applied to incidence data on olfactory atrophy in male rats to derive the POD for the RfC. Please provide comments with regard to whether BMD modeling is the best approach for determining the POD. Has the BMD modeling been appropriately conducted and objectively and transparently described? Has the BMR selected for use in deriving the POD (i.e., 10% extra risk of olfactory atrophy) been scientifically justified? Please comment on EPA's decision to treat all cases of olfactory atrophy similarly, without consideration of the severity of the atrophy seen at different dose levels. Please provide a detailed discussion and any suggestions for consideration of severity in determining the POD including identifying and provide rationales for any alternative approaches for the determination of the POD and discussion of whether such approaches are preferred to EPA's approach considering the available data.

John Morris

I am not an expert on appropriate utilization of BMD versus straightforward NOAEL for determination of the point of departure. It appears that the BMD calculation has been performed appropriately. Thus, the 10% extra risk level is calculated correctly. This approach is often used by EPA, thus this appears to be consistent with EPA policy. I would note that the derived BMCL10 appears to be consistent with the animal data and, from this perspective, makes sense. I can't comment on the strengths of including or excluding severity of response data in the BMD analysis because I am unaware of how such data would be objectively and quantitatively incorporated into the calculations.

Although not a charge question, it is appropriate to comment on the RGDR based species extrapolation of the rat data. The calculations were performed properly, however the current state of the art clearly indicates that this procedure itself is not appropriate. The derivation of the RGDR formulae was based on inappropriate assumptions (e.g. uniformity of air phase mass transfer coefficient throughout the nose) and the predictions are counter to data (e.g. Hinderliter et al, Toxicol Sci 85: 460-467, 2005) as well as being counter to more theoretically supportable modeling efforts (see any of the work of J. Kimbell, J. Schroeter, C. Frederick, M. Bogdanffy, J. Morris or others).

Andrew Salmon

The benchmark dose (BMD) methodology is clearly the preferred method for determining a POD from which to extrapolate to the RfC. There is a large body of literature now supporting this approach and demonstrating its superiority to the earlier NOAEL/LOAEL approach whenever data permit. It is also worth noting parenthetically that in the few cases where the BMD approach cannot be used, the result obtained by a NOAEL/LOAEL analysis is a low-confidence result, and may even be deceptive in implying that a conclusion can be drawn when perhaps it should not. See the previous comment for a discussion of the possible treatments of severity. It is not considered appropriate to limit this consideration merely to those endpoints categorized as "atrophy" as opposed to other more, or less, severe histological diagnoses. Our experience has been that the dose producing a 10% response rate (or even the lower confidence bound on this

dose level) is not an appropriate BMR to use as a substitute for a NOAEL. Both conceptually and practically, based on our analysis of a significant number of data sets for a range of both acute and chronic toxicants, this looks more like a LOAEL than a NOAEL for straightforward quantal responses in animal toxicity studies. In general it is more appropriate to choose a BMR of 5%, and the 95% lower confidence limit on the dose producing this response rate, as the POD for this type of data. If this is chosen then the uncertainty factors chosen (see below) can be the same as those traditionally used with a NOAEL result in a similar study. However, it should be acknowledged that this simple criterion may not be as readily applicable to more complex analyses such as those using continuous or pseudo-continuous data, as advocated above in addressing results where there is a continuum of results of varying severity.

Richard Schlesinger

The BMD approach seems justified for derivation of the RfC for this chemical. It has been clearly described in the document. Furthermore, the biologically significant response of atrophy is consistent with observed vacuolization at comparable exposure levels. The critical effect endpoint is clearly a component of a continuum that could eventually lead to more severe responses, such as cell death and tissue necrosis or other tissue responses. However, when considering severity and incidence, there may be some justification for reduction to the 5% level from the proposed 10%.

Jeffry Schroeter

The BMD approach is suitable for determining the POD for this study. A standard alternative would be to use the NOAEL/LOAEL approach. The LOAEL for olfactory atrophy (the critical effect) occurred at the lowest exposure concentration (150 ppm), so there was no NOAEL. Therefore, I assume that the POD using this approach would be the LOAEL, which is close to the POD using the BMD approach. I believe that an additional UF of 3 would be applied for absence of a NOAEL, and now we are near the $BMCL_{10}$ of 53.7 using the BMD approach. So it appears both approaches are consistent. A BMR of 10% is typically used in absence of any other relevant value, and this number is close to the incidence level at the LOAEL (2/15 = 13%), so I feel this level is justified as a biologically minimal significant response level. I don't have any suggestions on how to use severity in determining the POD. The lesion incidence demonstrates a clear concentration-response relationship and is appropriate to use with a BMD approach. I feel that the value in the severity scores (increasing with exposure concentration) serves as *justification* for the use of olfactory atrophy as the critical effect. For example, if severity did not increase with increased exposure concentration and lesion incidence then this would lead to additional questioning of this effect as being critical.

4. Please comment on the selection of the uncertainty factors applied to the POD for the derivation of the RfC. For instance, are they scientifically justified and transparently and objectively described in the document?

John Morris

The application of uncertainty factors appears to follow EPA policy. They represent the factors that are often employed and are consistent with precedent. Selection of uncertainty factors is clearly and transparently described. The use of a factor of 3 for interspecies extrapolation is appropriate because the RGDR approach was used for pharmacokinetic differences. The factor of 10 for inter-individual differences is appropriate because of the known human polymorphisms in aldehyde dehydrogenase and the likely role of this enzyme in detoxifying propionaldehyde. Presumably this factor of 10 is sufficient to account for the potential sensitivity of persons with chronic lung disease such as asthma or COPD. This point might be explicitly stated in the text. A factor of 10 is appropriate for chronic to subchronic adjustment. Certainly it is known that the severity of acetaldehyde lesions progress as exposure duration increases from subchronic to chronic. I can't comment on whether or not the NOAEL and/or LOAEL differ between these dosing regimens however. The recent study of Dorman et al (2008, cited above) might also be useful in assessing progression of acetaldehyde-induced lesions. Analogously information on the inhalation toxicity of isobutylaldehyde might also inform decisions regarding the progression of propionaldehyde-induced lesions. A UF=3 for database deficiencies appears warranted as well. Table 5-1 on uncertainties is highly useful.

Andrew Salmon

If the proper POD (i.e. the lower 95% confidence limit on the dose producing a 5% rather than 10% response) for a quantal analysis had been selected the UFs selected and their justification would be acceptable, apart from the questions of how the pharmacokinetic analysis should be handled (see below) and the evaluation of database uncertainty. However, if a more sophisticated approach to determining a POD, such as a pseudo-continuous score-based variable, were used, this section would have to be developed differently. The usual approach for continuous variables assumes that any statistically distinguishable deviation constitutes an unacceptable impact (especially if the continuous change constitutes a demonstrably adverse and undesirable change at higher levels of response, as here).

Richard Schlesinger

The rationale for each of the UFs applied to the POD is stated. However, the basis for the default value of 3 for interspecies extrapolation is not clear. The value of 10 for intraspecies uncertainty is clearly justified, as is the value of 10 for adjustment from subchronic to chronic.

Jeffry Schroeter

A cumulative uncertainty factor of 1000 was applied to the POD: 3 for interspecies extrapolation (pharmacodynamic effects), 10 for intrahuman variability, 10 for subchronic to chronic duration, and 3 for database deficiency. The UFs for interspecies extrapolation, intrahuman variability, and subchronic to chronic duration appear to be standard values from the RfC methodology, and are described as such in the review. These are appropriate to apply since there is no information on human variability of propionaldehyde uptake or metabolism and there are no other inhalation studies of longer duration. The reasons for applying these UFs are objectively described in the review. The UF for database deficiency is described below.

5. Please comment specifically on the database uncertainty factor of 3 applied in the RfC derivation. Please comment on the body of information regarding reproductive and developmental toxicity on propionaldehyde, the relevance of toxicity data on other aldehydes, and the relevance of toxicokinetic data regarding the likelihood of portal-of-entry effects as the critical effects in the determination of the database uncertainty factor. Please comment on whether the selection of the database uncertainty factor for the RfC has been scientifically justified. Has this selection been transparently and objectively described in the document?

John Morris

As noted above the uncertainty factor of 3 for database deficiencies is appropriate in my view. Certainly a limitation of the database is the absence of chronic toxicity data, however a UF=10 was included for subchronic to chronic extrapolation. This uncertainty factor should be adequate for this database gap. My interpretation of the document it that the database uncertainty factor of 3 was used due to the lack of a multi-generational study. I believe a UF=3 is adequate for this purpose, and it is my recollection that use of the UF=3 for this deficiency is consistent with other EPA documents that I have reviewed.

Andrew Salmon

The selection of the database uncertainty factor is appropriate for the data cited, which is restricted to consideration of possible deficiencies in the animal study database. However, no allowance for or recognition of the lack of information on postnatal developmental effects, such as possible exacerbation of asthma, which is a particular problem for children, was made in this calculation. This particular issue is one which has received scant attention in the analysis as a whole, and should be addressed here at least if not elsewhere.

The pharmacokinetic considerations in interspecies extrapolation were addressed using the RGDR methodology, which has a long history of use in U.S. EPA RfC derivations. However, the theoretical justification for this approach has always been somewhat tenuous, and although it has been found useful in the past there remains a suspicion that its acceptance is on a "*faute de mieux*" basis. In particular it entirely ignores local metabolism, and also takes a very limited view of the factors affecting deposition. In recent draft guidance the State of California recommended that these uncertainties be recognized by reducing the UF_A only to 6, rather than 3, when this method is used, in order to recognize that it falls well short of providing a full pharmacokinetic model of deposition, distribution and metabolism of inhaled toxicants. In fact, there have been several efforts recently to describe at least the deposition process more exactly, including a recent model (Teeguarden *et al*., 2008) for acetaldehyde, which is recognized as being the aldehyde most similar to propionaldehyde in its general properties. The analysts should consider whether this could be used to provide greater insights into the pharmacokinetics of propionaldehyde.

Richard Schlesinger

The justification of the UF of 3 for database deficiency is not adequate. The database is clearly lacking a long term study and, in fact, the justification provided in the document does not support the value of 3 but would support a value of 10.

Jeffry Schroeter

The UF of 3 for database deficiency was well described in the review. It was stated that even though the principal study by Union Carbide (1993) was a reproductive/developmental study, it actually provided limited information in this regard since pathology in pups was not evaluated. This is not my area of expertise, but this was also a major criticism in the external review of the Union Carbide study. These points were objectively addressed in this review. It was stated that the main reason for application of this UF was the lack of a multi-generation reproductive toxicity study. However, it was stated earlier in the review that the nasal pathology from the Union Carbide study was lacking since they only evaluated nasal pathology at 2 nasal sections, as opposed to the standard 3-6 sections. Would this not also be a valid reason for applying this UF for database deficiency?

(C) Carcinogenicity of propionaldehyde

1. Under the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (www.epa.gov/iris/backgr-d.htm), the Agency concluded that *data are inadequate for an assessment of the human carcinogenic potential* of propionaldehyde Please comment on the scientific justification for the cancer weight of evidence characterization. In addition, has the Agency properly characterized the potential for concern for carcinogenicity of propionaldehyde based on the available data on propionaldehyde and other aldehydes?

John Morris

The document properly concludes that there are insufficient data to characterize the carcinogenic potential of propionaldehyde. The text states that data on formaldehyde and acetaldehyde carcinogenicity might be useful in assessing potential carcinogenicity of propionaldehyde. This might be true were the state of the art sufficiently advanced. It is also highly appropriate to include statements about the inhalation carcinogenicity (or lack thereof) of isobutyraldehyde. Failure to include this information may raise concerns relative to the objectivity of the document. Given the differences in overall nasal dosimetry, and potential for induction of DNA-protein crosslinks it is not certain that the formaldehyde database would be of much use for such determinations. In essence formaldehyde may represent a special case because its deposition is so focal and because it's ability to form crosslinks is so much greater (orders of magnitude?) than the other aldehydes.

Andrew Salmon

Given the lack of direct evidence either for or against the carcinogenicity of propionaldehyde by inhalation, it would be difficult to reach a conclusion different from the Agency's on this point. The review actually makes a very creditable effort to use structure/activity comparisons with other aldehydes for both carcinogenicity and mutagenicity in order to place the potential concern for carcinogenicity in context. If anything this evaluation tends to underestimate the concern in my opinion. This is based first on the review of the mutagenicity data, which place too much evidence on the Ames test (*Salmonella*) results which as reported are mostly negative. In practice this test is difficult to use with volatile materials, and several of the apparently negative results were plate incorporation assays which may not have achieved sufficient contact time between the test material and the bacteria. The known rodent carcinogen acetaldehyde is not identified as a mutagen in the *Salmonella* assays reviewed, and even formaldehyde (the most reactive of the group) is inconsistently mutagenic in this class of assays. The negative findings for propionaldehyde in *Salmonella* are therefore essentially uninformative either as supporting or detracting from the concern for carcinogenicity. In fact, the recent report (Sampson and Bobik, 2008) in which mutagenicity in *S. typhimurium TA1534* was clearly demonstrated, very significantly increases the level of concern, in view of the clear positive findings in several other types of genotoxicity assay described in the Agency's review. The finding of a concentration-related (although

weak) increase in DPXs, the indicator lesion in the extensive studies of formaldehyde carcinogenicity, is noteworthy.

The decision to place a lower level of concern on the histological findings of atrophy and squamous metaplasia for propionaldehyde than that associated with the parallel findings for the known carcinogens acetaldehyde and formaldehyde seems unwise. The more advanced and possibly pre-neoplastic changes such as atypia, disorganization and hyperplasia which are reported for the latter compounds are characteristic of the longer term exposures and observation periods in the studies available for those compounds, whereas only short-term data are available for propionaldehyde. The most compelling aspect of the comparison between upper respiratory tract lesions in these three compounds is that all three show progression with exposure concentration and duration on a similar continuum of lesion severity.

Richard Schlesinger

As noted in the document, there are no appropriate cancer bioassay studies specifically for proprionaldehyde. The document appropriately indicates that it is not possible to extrapolate the carcinogenic potential for this material from data related to other related aldehydes. However, some indication of comparative effects from other aldehydes based up SAR may be in order.

Jeffry Schroeter

Propionaldehyde was shown to induce a weak, concentration-related increase in DPX in cultured human lymphoma cells (Costa et al., 1997), but there is no available information from in vivo animal bioassays. There have been several studies examining acetaldehyde-induced DPX formation in rats, but these studies have shown mixed results (Lam et al., 1996; Stanek and Morris, 1999; Dorman et al., 2008). Based on the lack of data with propionaldehyde and the comparative toxicity with acetaldehyde, I think it is justified in this review that there is inadequate data for an assessment of the human carcinogenic potential of propionaldehyde. The potential for concern for carcinogenicity from propionaldehyde has been properly characterized in this review.

V. SPECIFIC OBSERVATIONS

John Morris

Although minor in nature there are many area of the toxicological review that might benefit from additional clarity.

The description of the inhalation dosimetry studies of Egle (p5, bottom) might include more precise information on the inspiratory flow regimes and flow rates at which dosimetric parameters were obtained. Egle's manuscript does provide errors bars, some indication of the statistical reliability of these data might be provided.

The descriptions of the metabolic data for acetaldehyde (p6 mid) might be more complete. The Zhang et al reference is not in the reference section. My recollection is that metabolism rates were measured indirectly via disappearance of acetaldehyde from the headspace air of the incubation vials. If true, this might be mentioned. Direct data on acetaldehyde metabolism rates are provided in manuscripts by Casanova-Schmitz or Morris. The relevance of the information on downstream acetyl-CoA metabolic pathways (p7, top) is unclear to this reviewer. As noted above studies on acetaldehyde toxicity in Aldh knockout mice have been performed. This information might be sighted after p7, line 12.

The section on inhalation studies (p8, lines 14-20) merely states that "all principal" organs were examined. A listing of the organs would benefit clarity.

At some point in the text it would be beneficial to state that the sensory irritation response is characterized by a pause at the onset of expiration. I mention this so that uninformed readers do confuse this response with a pause at the end of expiration which is reflective of pulmonary irritation.

I was unable to precisely discern the total exposure times of the females in the inhalation reproductive toxicity study of Union Carbide. True, the text states there was a two week pre-mating period, etc., but an explicit statement of the number of days or weeks of exposure would be very helpful (p 8-12) especially since this is the critical study relative to derivation of the inhalation RfC. A more complete description of the precise locations of the nasal sections (p10 top) would be extraordinarily useful. Perhaps this cannot be discerned from the Union Carbide study, if so, it should be explicitly stated. At a minimum, it should be stated (if known) whether or not the lesions were localized to the most anterior olfactory epithelium lining the dorsal medial meatus (as opposed to the ethmoid turbinates themselves). In addition, wherever possible, statements of injury (or absence of injury) should include information on localization. For example on p10, line 23, (relating to female rats) the statement that "No evidence of squamous metaplasia was found" is difficult to fully interpret without knowledge of whether this refers to respiratory, transitional and/or olfactory mucosa. The statement that squamous metaplasia was "primarily localized to the olfactory epithelium" (p12 line 4) might warrant further explanation. Was squamous metaplasia also localized to the respiratory

epithelium? Did the olfactory metaplasia represent respiratory metaplasia that had become more squamous in nature? I am not a nasal histopathologist but I suspect that squamous metaplasia of the olfactory epithelium might represent an unusual response. In discussing the potential basis for the gender difference in toxicity two possible explanations are provided in differing portions of the text, either that the difference might reflect either the shorter exposure duration for females or the multiple days for repair of lesions in females compared to male rats. Either a single explanation should be provided throughout the text or both explanations should always be provided.

The text provides a great amount of data regarding the genotoxicity (or lack of same) of propionaldehyde. Would there be any way to provide some information on the biological relevance of the in vitro concentrations that were used in these studies? At least a comment on the potential relevance or lack thereof would be of value. Comparisons are made among formaldehyde, acetaldehyde and propionaldehyde subsequently in the document, thus the information on these aldehydes is of interest. The relevance of information on pentanal, hexenal, and higher aldehydes is not clear (e.g. Table 4-5 p 17) and might be removed for the sake of simplicity and clarity. The text might benefit from an explicit statement that the complete database on mutagenicity of all aldehydes was not summarized. What is provided is a review of a selected database. In this regard the studies that are most convenient to cite (e.g. those in which both propionaldehyde and other aldehydes were examined) may not be fully reflective of the database as a whole.

Perhaps more emphasis and interpretation on DNA-protein crosslink potential might be of value since this measure is of such importance in assessment of formaldehyde toxicity. The paragraph on p 18 (lines 3-12) is fairly succinct. The relevance of in vitro findings might be a subject of discussion. Certainly there does not appear to be a concordance between in vitro and in vivo DNA-protein crosslink formation with acetaldehyde (e.g. Stanek and Morris, Tox Sci 49: 225-231, 1999). At face value it would appear that it requires a concentration five times higher for propionaldehyde compared to acetaldehyde (p18, lines 5 and 10) to cause crosslinks in vitro. Thus the basis for the statement that acetaldehyde and propionaldehyde are of equivalent potency (p19, lines 24) is not clear. Finally it should be noted that many of the reported DNA effects occurred only at cytotoxic concentrations. This might be emphasized because it places these effects into context.

The statement on p22 (lines 8-10) about diminished lymphocyte proliferation leading to favorable conditions for tumor growth is highly speculative and should not be included.

The text also provides much information on the cardiovascular effects of propionaldehyde (pp19-21). Although this information is not utilized for the RfC formulation, it is appropriate to include for the sake of completeness. The use of such extraordinarily high concentrations in these studies limits their usefulness, however.

Andrew Salmon

Page 8, line 14 *et seq.* It is stated that the Gage (1970) study offers a NOEL for short-term exposure to propionaldehyde. Although the general statement is made that "all principal organs and tissues" were examined histologically, it is not immediately obvious that this actually included the specialized examination that would have identified the initial stages of the nasal vacuolization/atrophy/metaplasia sequence identified elsewhere as the critical effect. In view of the age and very general nature of the report this needs clarification before the finding of a NOEL can be accepted without further qualification.

Page 12, line 21 *et seq.* (Section 4.4.1.1). See comments above about the limitations of the Ames test for aldehydes, and volatile materials in general: these limitations should be noted here. Also the new positive result, Sampson and Bobik (2008) should be included.

Page 19, line s 9-10. This statement should be modified in the light of the positive result found by Sampson and Bobik (2008).

Page 26, line 1 *et seq.* The discussion of the QSAR model developed by Benigni *et al.* (2003) makes their conclusion that propionaldehyde is inactive as a genotoxin appear to be an entirely circular argument, since they input the negative *Salmonella* results then available as a fixed point in the model. Perhaps the description is not quite clear on how this model is supposed to work, but in any case the input premise is out of date. It is unclear whether this analysis really adds anything to the discussion.

Page 30, line 26. Lack of studies on child susceptibility. See comment earlier on induction or exacerbation of asthma by reactive irritants. There are no studies of which I am aware looking at propionaldehyde from this perspective. However, it seems that some useful insight could be gained by reviewing the data on asthma and similar conditions, a key health problem for children, using the comparison approach with other aldehydes which has been used with evident success elsewhere in the report.

Page 32, line 32. A certain level of intracellular autophagy, visualized by the presence of vacuoles, is certainly a normal cellular function. However an increase of this activity above the normal level, visualized as the appearance of more vacuoles than normal per cell volume (vacuolization) is a compound-induced effect which is not "normal", but indicates the early stages of intracellular damage control in response to a higher than usual rate of impairment or structural degradation of cellular components. It should therefore be regarded as an early precursor lesion in a continuum with the more severe expressions, such as atrophy, necrosis, and metaplasia.

Page 34, lines 13-14. See earlier discussion of appropriate BMR, and the preferable consideration of a response measure which is reflective of degree of severity rather than just a quantal measure of one particular response level.

Page 36, lines 19-21. As noted there is no basis for a compound-specific quantitative cancer risk assessment. However the extensive structure/activity comparisons with other

aldehydes have in a number of cases (including consideration of mutagenicity, nasal toxicity and general reactivity) identified propionaldehyde as having similar properties to acetaldehyde. It might therefore be helpful to risk managers trying to put the concern for carcinogenicity in context to suggest that if the concerns for carcinogenicity were to be confirmed the slope factor estimated for acetaldehyde could be used as an indicator of the level of risk expected for propionaldehyde.

Page 40, line 10 *et seq.* The conclusion was noted earlier that the responses seen in the female are less severe possibly because a recovery period was included after exposure and before examination for the females (due to the design of the study as a reproductive toxicity study), but this was not the case for the males. This is a significant point in interpreting the significance of the difference in response between the sexes in this study, and should be included here also.

Page 40, line 29. Update the conclusion to reflect the new data described earlier showing mutagenicity of propionaldehyde in *Salmonella.*

Page 41, line 25. Although the evidence for carcinogenicity is inadequate, the overall assessment here should note the existence of significant concern for such an effect. The following section could usefully suggest a quantitative comparison with acetaldehyde for which a cancer slope factor is available.

Richard Schlesinger

p. 5, lines 25-26. Are these percentages of totally amount inhaled?

p. 9, lines 25-28. The sentence is unclear. The way it is written it seems that the change in body weight is similar at both 1500 and 2500 ppm.

p. 26. Section 4.5. The purpose of this section is unclear since it repeats information already discussed. This is especially evident in Section 4.5.2. The section should be rewritten as a synthesis of the information from the previously discussed studies rather than a rehash of the results of the studies themselves.

p. 27, line 3. Add “histopathology” after “irritation.”

p. 29, lines 1-2. What “higher centers” are being referred to here? In addition, what would be the rationale for low and high doses affecting different aspects of the autonomic nervous system?

p. 29, lines 12-14. What is meant by “indirect” sympathomimetic activities? The discussion earlier on lines 1-2 suggests a direct effect on the sympathetic nervous system.

p. 29, line 22. There is no need for the subsection title of 4.6.1. Since there are no additional subsections in Section 4.6

p. 29, lines 27-38 to p. 30, lines 1-22. This is a repetition of prior information. It should be rewritten as a summary related to the title of the section.

p. 30, lines 20-22. It needs to be made clear that the presence of squamous metaplasia alone is considered to be a nonneoplastic lesion only in the context of the discussion related to propionaldehyde. The way it is currently written it seems like a generic statement and as such may not be true.

p. 30, line 25. Change subtitle to 4.7.1 to "Age –Dependent Related Susceptibility."

p. 30, line 26. Change sentence to read, "No studies are available on possible age-dependent, i.e. children, elderly, susceptibility to propionaldehyde."

p. 30, line 28. Change subtitle 4.7.2. to "Gender Related Susceptibility."

p. 30, line 4.7.3. Change subtitle 4.7.3 to "Genetic Related Susceptibility."

p. 32, line 17. Is it the most biologically relevant or the only biologically relevant endpoint studied based upon the available database.

p. 32, lines 32-33. This statement seems to contradict the statement on lines 15-16.

Jeffry Schroeter

1. Section 5.2.3, paragraph 2: Using the BMD method, a BMC(10) of 149.8 ppm was calculated using the Weibull model, followed by a BMCL(10) of 53.7 ppm. These data were most likely obtained by running the BMD software on the EPA website, but is it possible to provide more information on how the BMCL(10) value was obtained? The reason is that it is about 3 times lower than the BMC(10) so some justification would be warranted.

2. Section 5.2.3, page 35, lines 27-28: I would recommend re-wording these sentences as follows to emphasize that the repro/dev study by Union Carbide was limited: "Although the principal study used for the RfC derivation was a reproductive/developmental study (Union Carbide, 1993), this study provided limited …"

3. Section 5.2.3, page 36, line 8: The two references Stanek and Morris, 1999 and Morris and Blanchard, 1992 are not listed in the References.