

**SUMMARY OF THE U.S. EPA COLLOQUIUM  
ON A  
FRAMEWORK FOR HUMAN HEALTH RISK ASSESSMENT**

**Colloquium #2**

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## **NOTICE**

This report was prepared by Eastern Research Group, Inc. (ERG), an EPA contractor, as a general record of discussions during the U.S. EPA Colloquium on a Framework for Human Health Risk Assessment (Colloquium #2). As requested by EPA, this report captures the main points and highlights of discussions held during plenary sessions. The report is not a complete record of all details discussed nor does it embellish, interpret, or enlarge upon matters that were incomplete or unclear.

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## SECTION ONE BACKGROUND

### Developing a Framework for Human Health Risk Assessment

The U.S. Environmental Protection Agency (EPA) has recognized the need to develop a framework for human health risk assessment that puts a perspective on the approaches in practice throughout the Agency. Current human health risk assessment approaches are largely endpoint driven. In its 1994 report entitled *Science and Judgment in Risk Assessment*, the National Research Council (NRC) noted the importance of an approach that is less fragmented, more consistent in application of similar concepts, and more holistic than endpoint-specific guidelines. Both the NRC and EPA's Science Advisory Board have raised a number of issues for both cancer and noncancer risk assessments that should be reconsidered in light of recent scientific progress. EPA has recognized the need to develop a more integrated approach. In response, the Agency's Risk Assessment Forum (RAF) has begun the long-term process of developing a framework for human health risk assessment.

The framework will be a communication piece that will lay out the scientific basis, principles, and policy choices underlying past and current risk assessment approaches and will provide recommendations for integrating/harmonizing risk assessment methodologies for all human health endpoints.

As an initial step in this process, the RAF formed a technical panel in April 1996. An Issues Group (Gary Kimmel and Vanessa Vu, co-chairs; Jane Caldwell; Richard Hill; and Ed Ohanian) was formed, and this group developed a white paper, entitled *Human Health Risk Assessment: Current Approaches and Future Directions*, to provide an overall perspective on the issue (see Appendix A). The RAF peer-reviewed the white paper in February 1997. Its purpose is to serve as a basis for further discussion on current and potential future risk assessment approaches. The paper highlights a number of issues regarding the Agency's risk assessment approaches and their scientific basis, primarily with respect to dose-response and hazard assessment. The paper discusses the scientific basis for cancer and noncancer risk assessment, including differences and similarities. It also identifies knowledge/information gaps and areas where more work is needed.

As part of the continuing effort to develop a human health risk assessment framework, the RAF organized a colloquium series, consisting of two internal colloquia. The colloquia brought together EPA scientists for a dialogue on various scientific and policy issues pertaining to EPA's cancer and noncancer risk assessment approaches. The first colloquium, held on September 28 and 29, 1997, in Arlington, Virginia, focused on the role of mode of action information in re-examining and developing new risk assessment approaches. The second colloquium, held on June 2 and 3, 1998, in Bethesda, Maryland, explored the more quantitative aspects of mode of action, including dosimetry, dose-response relationships, and low-dose extrapolation methods.

The overall goal of the first two colloquia was to provide Agency scientists an opportunity to share perspectives on the role of mode of action in shaping future human health risk assessment approaches. The RAF invited a cross-section of senior Agency scientists (from headquarters, Research Triangle Park, Cincinnati, Las Vegas, and the regions) to participate in these discussions. As the Agency moves forward to develop this framework, additional colloquia are anticipated, as well as workshops to gather input and perspectives from scientists outside EPA.

## **The September 1997 Colloquium**

During the first colloquium, Agency scientists discussed the current standard default approach for cancer and noncancer risk assessment, and the advantages and limitations of departing from this approach in light of new information pertaining to chemical mode of action. The primary topics deliberated by the group included defining mode of action, evaluating what events are critical, formulating dose metrics, determining when enough information exists to support new risk assessment approaches, and strategizing on how mode of action information can be effectively and systematically used in low-dose extrapolations. Group discussions addressed general risk assessment issues and the overall use of mode of action in risk assessment. Case study discussions followed. The colloquium's final session included discussions on "critical harmonization issues" and quantitative dose-response issues to be covered at the second colloquium.

The "Summary of the U.S. EPA Colloquium on a Framework for Human Health Risk Assessment: Colloquium #1," dated November 24, 1997, provides a detailed account of the outcome of the first colloquium. A brief overview of the key results of the September 1997 colloquium was provided at the opening session of the second colloquium (see Section Two).

## **The June 1998 Colloquium**

Fifty EPA scientists and a small group of observers gathered for the second colloquium in June 1998 (see participant and observer lists in Appendix B). The 2-day colloquium focused on the role of mode of action information in developing descriptive quantitative models, applicable to a variety of needs for carrying out a risk assessment. Mode of action and harmonization issues were discussed in the context of four chemical-specific case studies: ethylene thiourea, ethylene oxide, trichloroethylene, and vinyl acetate.

Prior to the June colloquium each participant received one of the four case studies (Appendix C), including case-specific questions; a "charge" (Appendix D); and a list of general questions developed to guide colloquium discussions (Appendix E). During the colloquium, each participant was assigned to a breakout group to discuss assigned case studies. Appendix F includes a list of breakout group assignments, including the names of breakout group chairs and rapporteurs. As with the first colloquium, the RAF sought to ensure a mix of expertise and Agency representation in making group assignments.

After opening remarks were made, the first day of the colloquium was devoted to breakout group discussions on the case studies. During the second day, in plenary session, breakout group members presented their key findings. The closing plenary session involved an exchange of ideas on lessons learned from the colloquia series. Participants discussed next steps in developing a risk assessment framework in light of uncertainties and data gaps. The colloquium agenda is provided in Appendix G.

The following sections of this report highlight the outcome of the June 1998 colloquium. Section Two presents opening statements. Section Three captures the breakout group discussions on the case studies and Section Four presents highlights of the closing plenary session.

## **SECTION TWO OPENING PLENARY SESSION**

### **Welcoming Remarks**

William Wood, Risk Assessment Forum, EPA

Dr. Wood welcomed all participants, many of whom were at the first colloquium. He explained that this RAF project was directed at developing a framework on integrating approaches for cancer and noncancer risk assessment. Toward that end, the RAF workgroup's goal is to couple the outcome of the health effects colloquia series with Agency work on the final cancer guidelines in setting the course for how EPA will conduct future risk assessments. The outcome of the colloquium will also provide guidance for future research. Dr. Wood encouraged the input and active participation of Agency scientists throughout the second colloquium.

Dr. Wood acknowledged the hard work of the organizing committee whose members include Gary Kimmel (co-chair), Vanessa Vu (co-chair), Kim Hoang, Annie Jarabek, Jennifer Seed, Gina Pastino, and Wendy Yap. The colloquium participants then introduced themselves and their affiliations.

### **Goals of the Human Health Risk Assessment Framework**

Vanessa Vu, National Center for Environmental Assessment, EPA

Dr. Vu reviewed the overall goals of the framework project, accomplishments to date, additional short- and long-term plans, and the structure and charge of the second colloquium. She explained that the Agency intends to develop a framework to accomplish the following:

- # Develop a conceptual piece to communicate a risk assessment approach (for the Agency and public at large).
- # Layout past and current approaches.
- # Recommend approaches in integrating/harmonizing risk assessment approaches for all endpoints.

The major elements of the anticipated framework, she explained, include using mechanistic information to enable integrating risk approaches for different endpoints, considering a range of default approaches, and applying appropriate uncertainty factors.

Obtaining buy-in and input from Agency scientists, Dr. Vu emphasized, is very important, especially in the development stage of the framework. The RAF, therefore, has or plans to take the following steps:

1. *Development of a white paper.* The white paper, a "perspective" piece, was developed to identify key issues related to current risk assessment approaches and harmonization. The papers focuses on issues related to hazard and dose-response assessment and presents the scientific basis for assessing cancer and noncancer risks. It identifies uncertainties in the existing risk assessment process and areas requiring further guidance and research.
2. *Organization of the colloquia series.* The RAF organized the colloquia series to enable Agency scientists to discuss white paper issues and to provide recommendations on the approach of the framework. Agency scientists participating in the colloquia series were charged with discussing scientific and policy issues associated with developing a more consistent/holistic approach to risk assessment. During the first colloquium, discussions centered on the significance of qualitative implications of mode of action for various risk assessment endpoints. The second colloquium was designed to foster further qualitative discussions and initiate discussions on quantitative issues associated with the application of mode of action information (e.g., low dose extrapolation models).
3. *Draft the framework.* Based on the outcome of the colloquia series, the Agency anticipates preparing a draft framework document. It is anticipated that the framework document will undergo expert review, leading to future workshops and review by the Science Advisory Board.

Dr. Vu briefly summarized the outcome of the first colloquium. During Colloquium #1, participants developed a common appreciation for terminology and the role of mode of action in risk assessment. While Colloquium #1 participants recognized that strictly defining mode of action was difficult, mode of action was broadly defined as "knowledge of the series or sequence of biological events that influence the final toxic outcome." The group agreed that the traditional use of threshold/nonthreshold approaches may no longer be applicable in light of new scientific knowledge on mode of action. The group recommended greater use of mode of action information when extrapolating from high to low dose, across species, and across routes of exposure, as well as studying aggregate risk from chemicals that may have common mode of action. Colloquium #1 case studies enabled participants to begin to explore new approaches to low-dose extrapolation and evaluate commonalities across endpoints by reviewing toxicologic and mechanistic information for five chemicals. Participants agreed that issues related to commonalities across toxicities needed more emphasis. Continued development of the framework and future colloquia/workshops were encouraged to pursue the complex issues associated with harmonization of risk assessment approaches.

### **Introduction to Case Studies and Colloquium #2 Issues and Charge to Breakout Groups**

Dr. Vu explained that the purpose of the case study exercise at the second colloquium was to foster more in depth discussions on critical issues related to mode of action and its role in harmonizing



cancer/noncancer risk assessment. Dr. Vu emphasized that the intent of the case studies was not to perform chemical-specific risk assessments. Dose-response and mechanistic data were provided to help participants explore important factors related to developing descriptive quantitative models. Case-specific questions were provided to guide discussions and to promote deliberations on harmonization issues.

Lastly, Dr. Vu acknowledged the efforts of the issues group, organizing committee, RAF (Bill Wood, Jeanette Wilsey, and Carole Kimmel), and Eastern Research Group in helping to organize and coordinate the activities of the workshop. Dr. Vu also thanked participants and observers for taking part in the colloquia series.

### **Questions/Comments**

The group briefly discussed possible limitations of the case studies. Points raised by participants include the following:

- # Chemical-specific information presented in the case studies may not be 100 percent complete or correct. One participant questioned whether discussions should be limited to information provided in the case studies or if new information could be introduced.

The group recognized that it would be impossible to present a complete data set for one- or two-day discussions on a particular chemical. It was re-emphasized that participants were not performing full-blown risk assessments on case-study chemicals, but rather raising and evaluating case-specific issues related to more scientifically sound approaches to evaluating human health risks. While it was agreed that scientists should introduce pertinent data during the breakout sessions, it was also recognized that because of time constraints it is not possible, nor necessary, to consider every chemical-specific detail. The ultimate purpose of the case study exercise, the group was reminded, was to determine the best use of mode of action information and how to generate the most credible risk assessment.

- # One participant questioned how the group should approach the issue of multiple modes of action during case study deliberations, expressing concern that the group may try to "force fit" a single mode of action for multiple endpoints.

Multiple modes of action should be considered in terms of their relative contribution to pathogenesis. The intent of the case study exercise was to evaluate whether different endpoints should be treated differently when a common mode of action has been identified, not necessarily to identify a single mode of action.

### **SECTION THREE**

#### **BREAKOUT GROUP DISCUSSIONS ON CASE-SPECIFIC QUESTIONS**

The first day of the colloquium was dedicated to breakout group discussions on the following four case studies (See Appendix C).

- # Ethylene Thiourea (ETU)
- # Ethylene Oxide (EtO)
- # Trichloroethylene (TCE)
- # Vinyl Acetate (VA)

The case studies include a summary of key human and animal studies and describe primary acute and chronic effects. Depending on the chemical, the case study describes portal-of-entry effects; systemic toxicity; reproductive and developmental toxicity; neurotoxicity; mutagenicity; and carcinogenicity. The case studies also present pertinent dose-response, pharmacokinetic, and mode of action (MOA) information.

Each breakout group deliberated case-specific questions (included within each case study), but, in general, the following questions capture the key issues discussed by each group.

1. Given what is known about MOA, are there commonalities among endpoints that would be useful for quantitative analyses? For which endpoints should a common quantitative analysis be conducted? For which endpoints should a separate analysis be conducted?
2. What additional information would be useful for quantitative analysis?
3. In the absence of this information, are any of the available data sets useful for quantitative analysis?
4. Are dose and duration of exposure important considerations? If so, for which endpoints and how should they be handled?
5. In the absence of case-specific physiologically-based pharmacokinetic (PBPK) models, how should dose be adjusted for extrapolation to humans? Does choice of a specific endpoint influence this decision? Why or why not?

If a PBPK model is available, which dose metrics should be considered for the dose-response analysis?

6. What response/endpoint(s) would be useful for dose-response modeling in the observable range? Does MOA information influence this choice?

7. What quantitative method is recommended for low level exposures? Does this vary for different toxicities? Does MOA information influence the choice of models?
8. If a reference dose (RfD), reference concentration (RfC), or margin of exposure (MOE) were to be calculated, does MOA information influence the choice of uncertainty factors or influence uncertainties about data gaps?

The sections below summarize the main points discussed during breakout sessions, as captured by the group rapporteurs and presented in plenary session. Vicki Dellarco, Kerry Deerfield, Vanessa Vu, and Arnold Kuzmack presented the breakout group reports for ETU, EtO, TCE, and VA, respectively.

### **Ethylene Thiourea**

In reviewing the ETU case study, the group considered the adverse health effects associated with target organs/responses, common modes of actions across different responses, dose-related increases, exposure duration issues, critical windows of exposure, and the relevancy of animal data to humans. The group's responses to case-specific questions are provided below.

*Given what is known about MOA, are there commonalities among endpoints?*

The group identified the following ETU "targets:" thyroid, pituitary, liver, embryo/postnatal, and central nervous system (CNS). The group described the following three potential modes of action likely to be responsible for the effects in these target systems:

1. **Thyroid/pituitary:** In the rat, high concentrations of ETU result in decreased T3 and T4 and increased TSH levels. The severity of hyperplasia increases with dose and possibly with duration. These changes in T3/T4 and TSH levels are associated with thyroid hyperplasia and tumor development in the thyroid (adenomas and carcinoma). These events can eventually lead to pituitary tumors if substantial. Based on case study information, mutagenicity or a direct DNA reactive mechanism does not seem to be a major influence on tumor development. Perturbances of the pituitary-thyroid homeostasis is the essential event leading to tumor development (i.e., an anti-thyroid MOA).

Some developmental effects (related to brain development in late gestational/postnatal periods) are presumed to be thyroid-mediated.

2. **Liver:** A separate MOA appears responsible for liver effects. Effects appear to be metabolite-dependent (FMO) and species-specific.
3. **Non-thyroid developmental effects:** Seen primarily in the rat, CNS malformations result from necrosis of neuroblasts driven by ETU (parent compound). These effects are not considered to be thyroid-mediated. Effects are species-specific.



The group concluded that common modes of action for cancer and certain noncancer (e.g., CNS) endpoints are associated with the disruption of the thyroid/pituitary homeostasis. This knowledge enables one to use precursor events (e.g., changes in T3, T4, and TSH; increases in thyroid hyperplasia) instead of frank toxicologic effects in protecting for different outcomes. No conclusions could be reached on the reversibility of responses, however, because of the lack of data.

A question was raised following this discussion as to whether or not ETU exposures led to total endocrine disruption and whether the pituitary should be considered separately. Another participant questioned whether the group considered the relation of liver effects to thyroid/pituitary effects. It was noted that data were not available to suggest any such relation.

*What approaches should be considered for quantitative analysis?*

Upon consideration of available dose-response data, the group suggested different approaches for the quantitative analyses of the three identified MOAs. For thyroid/pituitary events and hyperplasia events, effects on thyroid hormones should be used as indicators of both cancer and noncancer endpoints. Given the understanding of MOA in the thyroid, the group suggested using a nonlinear approach for low-dose extrapolation. For liver effects, the group noted that, in the absence of quantitative information and a full understanding of MOA, the default linear approach should be used. The group commented, however, that this approach might be overly conservative—the group emphasized the need to point out data set uncertainties and the possibility that effects may be species-specific and not relevant to humans. For developmental effects, the group suggested using the default nonlinear approach, but data were available to also enable some benchmark modeling.

*What additional information would be useful for quantitative analysis? What are the research needs?*

In general, the breakout group agreed that more comparative metabolism information (within and across species) would be especially helpful in further evaluating MOA questions and the relevance of existing data to humans. Response-specific information needs to include the following:

*Thyroid:* Because thyroid hormones are a good biomarker and evidence exists that there is age-dependent susceptibility, it would be helpful to examine prenatal/early postnatal hormone levels. In addition, obtaining more dose-duration information would be helpful in studying the issue of reversibility. Comparative metabolism data (tissue distribution) between humans and rodents would be helpful to better understand species differences.

*Liver:* More information is needed specific to mouse metabolism. Comparative metabolism studies on FMO are needed.

*Nonthyroid Malformation:* More comparative metabolism data are needed to study differences in responses between humans and rats.

*Are dose and duration exposure important considerations?*

The breakout group considered patterns of exposure and critical windows of susceptibility. Responses in the thyroid/pituitary (severity of hyperplasia) appear to be dose limited and may be dependent on duration. Not enough information is available to assess dose/duration considerations for liver and developmental effects. Thyroid/pituitary and developmental effects were observed at similar ETU doses. Dose was species-dependent for liver effects, which is an example of why more species-specific metabolism data are needed.

*In the absence of a PBPK-model, how should dose be adjusted for extrapolation to humans? Does choice of a specific endpoint influence this decision? What quantitative method is recommended for low level exposures? Does this vary for different toxicities? Does MOA information influence the choice of models?*

Although no single extrapolation method was recommended (e.g., lack of an interspecies adjustment versus using a scaling factor of body weight to the 3/4 power), the group strongly agreed that the approach should be the same for cancer and noncancer endpoints in the thyroid/pituitary.

*What endpoint(s) would be useful for dose-response modeling in the observable range? Does MOA information influence this choice?*

The group agreed that MOA is relevant to thyroid/pituitary responses. It plays less of a role in developing models for liver and developmental effects.

*If an RfD were to be calculated, does MOA information influence choice of uncertainty factors or influence uncertainties about data gaps?*

Yes. The group reiterated, however, that more comparative data between rats and humans are needed before fully answering this question. Qualitatively, the group agreed that uncertainty factors should be applied in the same way for cancer and noncancer endpoints. In comparing RfD and margin of exposure (MOE) approaches, the group agreed that, conceptually, the uncertainty factors applied are similar. In practice, however, they could be applied differently because the RfD approach is more compartmentalized and the MOE approach involves more scientific judgment/interpretation. This issue, therefore, warrants further study and careful consideration.

## Ethylene Oxide

The breakout group initiated their evaluation of EtO by preparing a matrix of observed effects. EtO induces a variety of effects including irritation, hematotoxicity, neurotoxicity, reproductive and developmental toxicity, and cancer. Group discussions focused primarily on the latter three. The group provided the following responses to case-specific questions:

*Given what is known about MOA, are there commonalities among toxicities that would be useful for quantitative analyses? Is there any reason to propose different mechanisms for the various endpoints?*

Based on available data, two plausible MOAs exist for EtO: the formation of protein adducts and the formation of DNA adducts. EtO distributes readily and is direct acting (no metabolite formation). Distribution is even throughout the body. Although it is highly reactive (e.g., hemoglobin binding, glutathione binding), free EtO distributes to target tissues. EtO binds to macromolecules (specific amino acids in protein) and forms specific DNA adducts (e.g, 7-hydroxyethylquanine). These two mechanisms are probably not mutually exclusive. The mechanisms related to neurotoxic outcomes are not completely understood; these effects are not fully explained by DNA adduct formation, and may relate primarily to the binding of EtO to protein.

The group categorized the endpoints and asked whether common MOAs exist.

*Cancer:* Tumors have been observed in multiple sites in animals (hematopoietic, brain, forestomach, lung, ovary, lymph). In humans, epidemiologic studies suggest a link between EtO and hematopoietic cancers. Because tumors appear in multiple locations, there is likely a common MOA for most of these cancers and that is related to DNA binding mechanisms. Forestomach cancers, however, appear to result from a local irritant effect, although this effect may be enhanced by the genotoxic action of EtO.

*Reproductive/Developmental Effects:* Observed effects include spontaneous abortion, zygotic death, lethality/viability, litter size, implant loss, and malformations. Dominant lethality appears to result from the formation of DNA adducts. While insufficient data exist for all of these endpoints, the group agreed that a common MOA probably exists for most reproductive/developmental endpoints.

Data suggest that MOA is similar in animals and humans for tumors, but unknown for developmental effects.

*What additional information would be useful for quantitative analysis of the various toxicities? (For example, is consideration of the entire spectrum of mutational changes, such as the induction of gene mutations, structural chromosome mutations, and numerical chromosome alterations important?)*

Several data needs were identified.

- # For mutagenic effects, existing information on point mutations needs to be considered. The case study concentrated on chromosome breaks (translocation) data.

- # Information on the shape of the curve at low doses. For example, is it linear or nonlinear? Are DNA adducts formed at low levels? This is a research need.
- # Additional information on the causality of different endpoints.
- # Cell proliferation information at all dose levels.
- # Information on background rates. What is the background load (endogenous EtO)?
- # Information on exposures to other agents that may have the same MOA or make one more susceptible to a MOA.

*What quantitative method is recommended for low level exposures? Does this vary for different responses? Does MOA information influence choice of models?*

The group proposed the same approach for both cancer and developmental/reproductive effects because the MOA suggests that both effects are related to the formation of DNA adducts. If one assumes linear behavior, then a linear quantitative method is appropriate for low dose extrapolation because of the mutagenic properties of EtO. The group, however, did discuss MOE and possible nonlinear approaches because the data suggest that protein binding and DNA adduct formation may not be linear. One participant noted that data on heritable effects versus dominant lethal effects suggest that a two-hit model and nonlinear dose response may exist. The overall impression of the group was that MOE eliminates the theoretical argument over linear versus nonlinear dose-response relationships and focuses on MOA. MOE would therefore be a viable approach to bring to the risk manager. In general, the MOA for all effects is probably related specifically to the electrophilic nature of EtO, and the ultimate action would be dependent on timing and duration of exposure, where and to what it binds, etc.

The question on linear versus nonlinear dose response triggered a fairly lengthy discussion among the plenary group. General and EtO-specific issues raised are highlighted below:

- # Because of the limited dose numbers in the NTP study, it is difficult to study linearity.
- # Adduct-formation is not the only factor to influence the shape of the dose-response curve. Although adduct formation may be considered a linear response, a certain level may need to be reached before a toxic outcome is observed. If adducts are easily repaired, a nonlinear response may in fact be observed. What is happening beyond adduct formation needs to be considered and is an argument for using the MOE approach.
- # "Toxicity" needs to be defined. Traditionally, toxicity was defined as an observable effect (e.g., a tumor or malformation). Now with activities at the cellular level being considered (e.g., biochemical changes or adduct formation), toxicologists need to agree on what the "toxic endpoint" is.

One participant noted a definition of toxicity by Doull (of Cassarett and Doull): toxicity is



not achieved until the first "irreversible step" is observed. Several others disagreed citing ethanol exposure as an example where reversible effects still result in "toxicity." Furthermore, RfDs have been developed based on nontoxic reversible effects. Doull's definition, therefore, may not be relevant to these discussions.

- # It is important to study the nature of the lesions before deciding on a linear versus nonlinear approach.

*Are dose and duration of exposure important considerations? If so, which responses and how should they be handled?*

Very little dose rate information is available for most endpoints, but the group agreed that it is an important consideration. For example, in a study of dominant lethality, dose and duration were found to be extremely important when considering the effects of EtO.

In summary, it was agreed that EtO presents a good case for quantitatively treating different endpoints similarly based on MOA. Although no specific approach was recommended, many felt that an integrated MOE approach for each of the effects would provide risk managers with useful information.

**Trichloroethylene**

TCE, the group agreed, was one of the more complex case studies because of the variety of systems affected and effects produced. It is further complicated because of the involvement of and uncertainties associated with the metabolites. The group reviewed TCE effects and its MOA in several target systems, but focused on effects in the liver, lung, and kidney.

Both the "minor" and "major" metabolic pathways for TCE were described (see case study figure in Appendix C). The group identified the role of metabolites in mediated TCE-induced toxicities and highlighted the relative species reactivity of the metabolites, as follows:

Effects	Metabolites	Species reactivity
liver	TCA, DCA	mouse>rat>humans
lung	Chloral	mouse>rat
kidney	DCVC	rat>mouse>human

TCA = trichloroacetic acid  
 DCA = dichloroacetic acid  
 DCVC = s-1,2-dichlorovinyl cysteine

The breakout group summarized the effects of TCE in the liver, lung, and kidney, highlighting cross-species and general dose duration differences. These discussions are summarized in Table 1.

**Table 1. Breakout Group Summary of TCE Effects**

<b>SPECIES</b>	<b>EXPOSURE</b>	<b>EFFECTS</b>
<b>Liver</b> human	acute/high  occupational	liver failure/necrosis  impaired liver function  some evidence of risk of cancer of the liver and the biliary duct
rat	acute/subchronic to high level  chronic/lower level	enlarged liver, hypertrophy, necrosis  enlarged liver
mouse	acute/subchronic to high level  chronic/relatively lower level	enlarged liver, hypertrophy, necrosis  hepatomegaly, hypertrophy, tumors
<b>Kidney</b> human	occupational	mild renal function changes suggestive evidence of kidney cancer
rat	acute exposure to high level  chronic to lower level	nephropathy  increased kidney weight mild karyomegaly tumors
mouse	acute/chronic to high level	nephrotoxicity no tumors
<b>Lung</b> human		no reported effects
rat	acute/chronic	no effects
mouse	acute  chronic	cytotoxicity to Clara cells  lung tumors

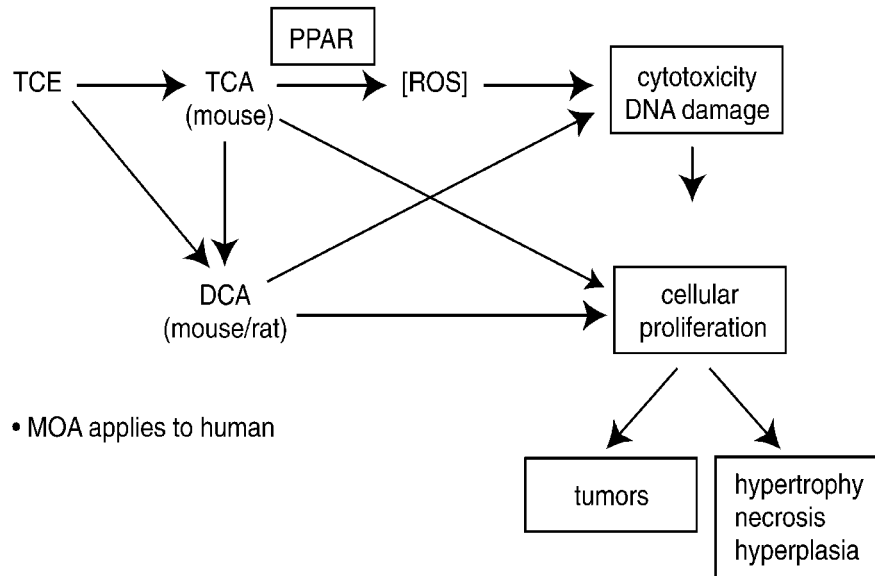
The group briefly discussed lympho/hematopoietic, reproductive/developmental, and CNS effects. TCE-related effects on the lympho/hematopoietic system include excess non-Hodgkin lymphoma in humans, lymphoma in exposed mice (via inhalation), and effects on the spleen in rats and mice. The group noted consistency across species. Inconclusive/conflicting evidence exists related to TCE-induced reproductive/developmental effects in humans. Eye and cardiac malformations have been observed in rats exposed *in utero*. Effects on sperm, implantations, and litter size have been observed in mouse reproductive studies. CNS effects are reported in humans exposed to high levels of TCE (acutely) and in occupational settings as well as in rats and mice exposed acutely, subchronically, and chronically.

Having highlighted key effects, the group then answered case-specific questions.

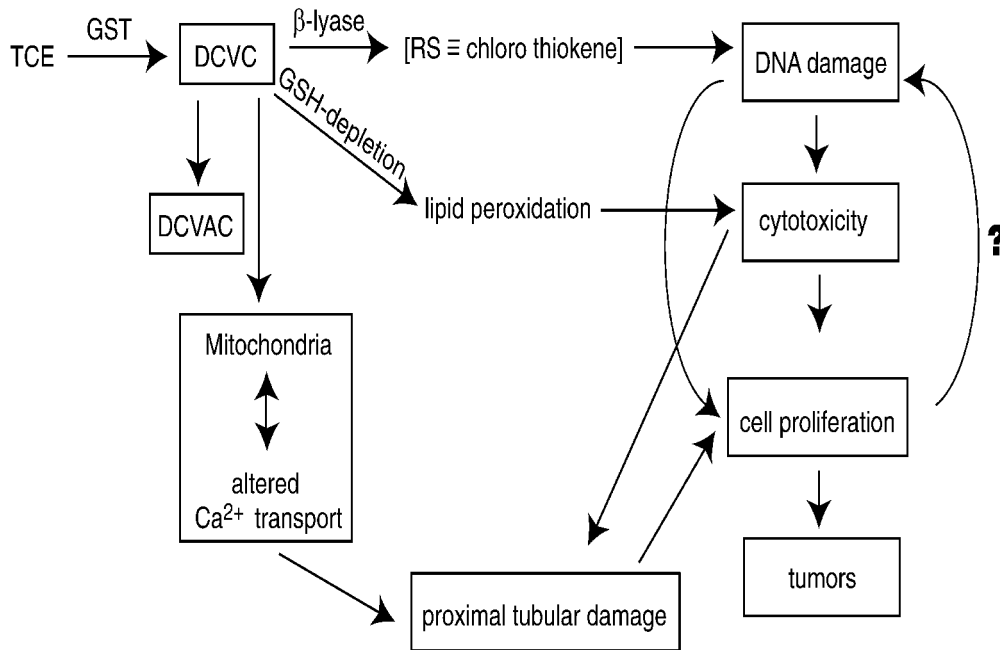
*What seems to be the series of events leading to each observed toxic response? Are there any reversible steps in the process? Can an irreversible step be identified in each process? Given that TCE-induced toxicities are mediated through metabolites, are there common biological responses across toxicities that would be useful for quantitative analyses?*

The group developed schematics depicting key events in the liver, kidney, and lung (see Figures 1, 2, and 3). Discussions centered around whether common modes of action are present for different toxic responses.

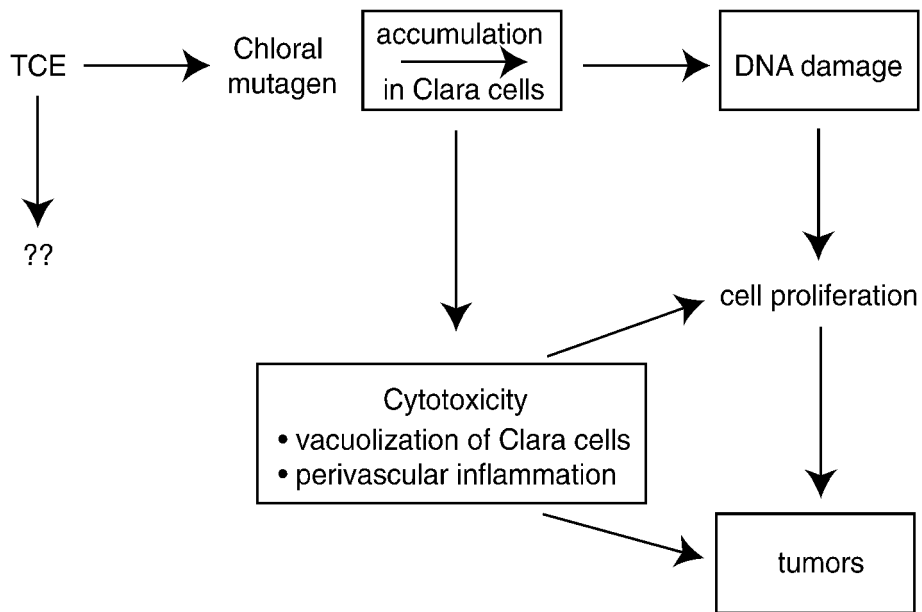
**Figure 1. TCE MOA in the Liver**



**Figure 2. TCE MOA in the Kidney (Rat)**



**Figure 3. TCE MOA in the Lung (mouse)**



The group also identified data gaps.

*Liver:* For the liver, the group emphasized that cellular proliferation appears to be the common event leading to both tumors and liver toxicity. The MOA is relevant to humans based on available data. Because quantitative information on cellular proliferation is lacking, it is not known whether reversible steps exist. The specific steps leading to tumors and liver toxicity are not clear. One group member noted that speculation exists as to whether DCA is a promoter or an initiator.

*Kidney:* While the metabolite DCVC is common to the two endpoints (i.e., tumors and proximal tubular damage), a common MOA is not observed for these endpoints.

*Lung:* TCE action in the lung of mice was described. Both cytotoxicity and DNA damage appear to be the result of the accumulation of chloral in the Clara cells. Because of many unknowns, no specific common biological events could be identified to account for either TCE-induced tumors or toxicity in the lung.

*Which of the above-selected responses is most relevant to humans regarding specificity (response concordance) and sensitivity (dose range of response)?*

Liver and kidney MOA and responses in test animals are relevant to humans. Lung responses, however, are not. Data are not sufficient to judge sensitivity of response. Epidemiologic data provide good qualitative information but do not enable quantification. Animal studies show more tumors in the liver versus the kidney following TCE exposure.

*What additional information would be useful for quantitative analysis?*

The group stressed that obtaining more dose-response information on cell proliferation was critical. No dose-response curve is available. Cell proliferation data are needed for initiated versus noninitiated cells. A labeling index study for age range is also needed.

*Are dose and duration of exposure important considerations? If so, for which toxicity and how should they be handled?*

Dose and duration appear to be important in the liver and the kidney. In animal studies, liver tumor response depends on dose, but not enough is known to specifically answer the dose/duration question. Not enough data are available to answer this question for the kidney. In addition, more information is needed on dose/duration issues in humans.

*What response(s) would be useful for dose-response modeling in the observable range for each toxicity? How does MOA information influence this choice? Given the availability of the PBPK models, what would be the appropriate dosimeters for the toxicity observed in the liver, lung, and kidney? Which quantitative models should be used for the observed data?*

Dose-response modeling could be considered for liver and kidney responses. Cell proliferation in the liver is the preferable response choice, but because of high background and species variability, coupled with the lack of quantitative data, it may be problematic. PBPK models could be used to estimate internal TCE dose. More information is needed, however, relating TCE to its metabolites so that an internal dose of metabolites can be obtained.

*Given what is known about the MOA for each toxicity, what quantitative approach would be recommended for characterizing risk associated with low level exposures (i.e., beyond the observable range) for each toxicity?*

The group focused on the liver response for this question. Opinions varied regarding the best quantitative approach to take in light of available data. Although no one approach was recommended, it was agreed that applying a biologically-based dose response (BBDR) model would be the ideal choice. The group considered two scenarios: (1) assume quantitative cell proliferation data are available, and (2) assume quantitative cell proliferation data are not available.

Assuming quantitative cell proliferation data were available, the group considered linear and MOE approaches. Half of the breakout group felt an MOE approach was preferable because it gives more consideration to science and nearly an equal number felt it is really a policy choice. One individual preferred a linear approach because it is more conservative and because the threshold for lifetime exposure is not known.

In the absence of cell proliferation data or a BBDR model (where tumor and liver toxicity would be considered as the responses), the group was again divided as to what approach is most appropriate. The following quantitative approaches were proposed, with the group divided equally on each of the three options.

1. *Status quo.* Several individuals supported using default approaches (i.e., linear for tumor and an RfD/RfC for noncancer effects). These individuals felt resorting to the existing models was more conservative in light of data gaps.
2. *Same approach for both responses.* Because of common MOA, others felt it was more appropriate to use the same approach for both cancer and noncancer outcomes. Both linear and MOE approaches were considered. The overall preference of the group was an MOE approach because of observed receptor-threshold effects. One member noted that, in the absence of data, no compelling reason exists to assume a linear curve at low doses; he emphasized, however, that all endpoints should be considered and the most sensitive should be used to select the RfD/benchmark dose.
3. *Policy choice.*

Presentation of these choices resulted in lively discussions both in breakout and plenary sessions. The group conveyed the following general points about choosing an appropriate quantitative approach.

- # How much information is enough to support a decision to choose a nondefault approach? Because of the uncertainties in most data sets, opinion will vary widely.
- # In the case of TCE, one participant questioned how one could conclude simply from the evidence of cell proliferation whether a threshold or nonthreshold response existed. He provided the dioxin example where several factors led to identifying a threshold. He could not accept the threshold concept for the complicated TCE story.
- # The group did not discuss other sensitive noncancer effects of TCE (e.g., neurotoxic effects). In focusing on the noncancer effects in the liver (cell proliferation), a potentially more sensitive outcome in another system (neurotoxic) may be overlooked.

*If an RfD or MOE were to be developed, which factors should be considered to account for uncertainties in risk assessment?*

The group agreed that the following uncertainty factors should be considered as common to both RfD and MOE approaches:

- intraspecies differences
- interspecies differences
- nature of response
- steepness of the dose-response curve at point of departure region
- lack of understanding

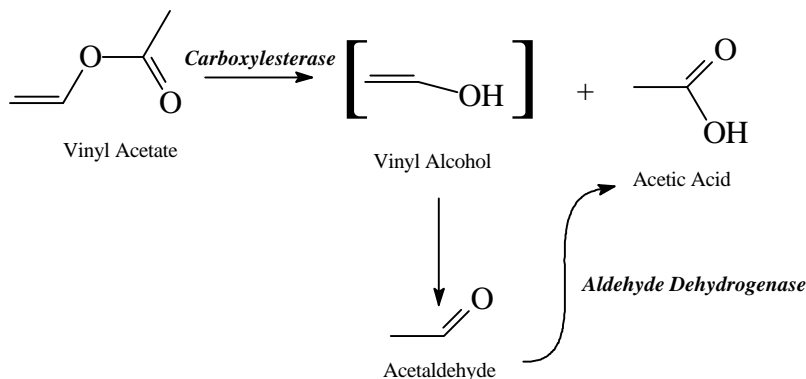
Further discussion on uncertainty factors was held in the final plenary session and is summarized in Section Four of this report.

## **Vinyl Acetate**

It was noted that the action of VA is unique from the chemicals evaluated in the other case studies in that it exhibits effects at the portal-of-entry (upper respiratory tract). There is a spatial specificity of lesion location, with most effects concentrated in the olfactory region of the rat. In mice, the location of the lesions is consistent with air-flow patterns and tissue-specific enzymes. Case-specific questions varied slightly, therefore, to foster discussions on this unique aspect of VA.

The group reviewed the established metabolic pathway for VA. Carboxylesterase catalyzes the initial hydrolysis of VA to vinyl alcohol and acetic acid (AA). Vinyl alcohol rearranges to acetaldehyde (AAld) which aldehyde dehydrogenase subsequently metabolizes to additional AA. These enzymes have been localized histochemically and are found in discrete cell types in the respiratory and olfactory mucosae. The metabolism scheme (as presented in the case study) is depicted in Figure 4:

**Figure 4. Metabolic Pathways for VA**



Two mechanisms of action were identified: (1) AA causes cytotoxicity which may progress to cell proliferation, (2) AAld, which is a known clastogen and sister chromatid exchange initiator, leads to multi-hit genetic damage. Tumors are seen only in male rats at the highest concentration tested, 600 parts per million (ppm), and only at the terminal sacrifice of a 2-year bioassay; no effects are observed at concentrations below 50 ppm. It was hypothesized that because mice can restrict respiration (reflex apnea), less of an effect is observed. This species difference was shown to be the case with formaldehyde, another upper respiratory tract (URT) irritant.

*Does the existing database support the URT lesions as the sentinel toxicity for inhalation exposures to VA?*

The group agreed the database clearly supports URT lesions as the sentinel toxicity. The proximal to distal pattern and the concentration response are both important to the argument.

*Can the cytotoxic changes caused by VA exposure be considered as sequentially linked to the observed tumor outcome? What are the key considerations to characterize the conditions of hazard (e.g., high dose versus low dose)? How do the genotoxic data factor in this characterization?*

- # Cytotoxic changes caused by VA are linked to tumors.
- # AAld are linked with different tumor types. Responses in both pathways appear to be at high doses only. The group noted that the spatial distribution of tumors was consistent.
- # A "good" PBPK model exists that relates metabolism, physical layout, and fluid mechanics in human and rodents. The PBPK model accounts for the observed species and gender differences.
- # Knowledge of cytotoxicity, cell proliferation and temporal aspects, and localization of enzymes is helpful.



- # Cytotoxicity may cause death but some cells will survive and those will have an increased probability for genotoxic effects, especially at high concentrations.

*What mechanistic data are most relevant to characterizing tumor outcome? Which would be useful for dose-response modeling in the observable range? What are the implications of the MOA information for extrapolation of risk to low dose?*

The data most relevant to tumor outcome include: cytotoxicity, cell proliferation, genotoxicity, site specificity (localization of effect), and metabolism. Dose-response modeling based on tumor outcome is not possible, however, because only two non-zero points (the second lowest with a response of 1) exist in the observable range. Because effects are seen only at the highest exposure concentration and only at the last sacrifice, the group overall felt this suggests that a nonlinear approach is appropriate for low-dose extrapolation. This was supported by clear relationships of genotoxicity, cytotoxicity, and cell proliferation only with high concentrations.

One breakout group member, however, disagreed that all effects are only at high concentrations. He noted that AA leads to cytotoxicity as a result of changes of pH, which may ultimately lead to cellular changes in the URT and to cancer. He agreed that the effect of AAlD is significant only at high doses. Evidence includes the fact that cross links are only significant at high doses and that there are no long-lived DNA adducts. He noted, however, that large-scale changes in DNA have been observed that may have required multiple events. He noted that these large-scale changes are important to humans and should be examined closely. Dose-response data are lacking for observed DNA damage. In addition, there is a lack of mechanistic understanding of the process. A low dose linear situation may, therefore, exist.

*Given the availability of the PBPK model, which dose metrics should be considered for the dose-response analysis? Does this choice of dose metric address consideration of the role of exposure duration?*

Limited time was spent discussing the PBPK model although its usefulness in addressing the toxicokinetic issue of species to species extrapolation was recognized. The dose metrics (about seven tabulated) need to be further explored for implications to quantitative dose-response assessment. At 50 ppm VA, the model predicts the same decrement in pH projected in animals and humans. The group concluded that, at lower doses, animal and human responses would be quantitatively the same, but that the case study did not present the model in sufficient detail to quantitatively explore the interspecies differences in dosimetry (e.g., airflow).

*What are the uncertainties in using these data to characterize human risk?*

The group identified several uncertainties and data gaps that, if filled, would enable further consideration of the mechanistic actions and commonalities across endpoints.

- # Reflex apnea in mice.
- # Description of lesions (coverage in case study was brief).

- # Effects of lowered pH in the respiratory tract on cancer.
- # Effects of acetic acid and other aldehydes.
- # Gender differences.
- # Differences in deposition patterns in the respiratory tract of humans versus rodents.
- # Dose-response data for DNA effects.
- # Human metabolism data (qualitatively metabolism between rodents and humans appear similar, but rates may be different).

*Should an RfC be developed separately? If an RfC or MOE were to be developed, which factors should be considered to account for uncertainties in the extrapolations applied?*

The group agreed that developing a separate RfC is justified. The potential role of lesions such as atrophy and hyperplasia would have to be considered in the context of later tumor outcome. Uncertainty factors would include one to account for animal to human extrapolation (based on further study of the PBPK model) and one for intrahuman variability.

*What mechanistic data would be useful for development of risk estimates of exposures via the oral route?*

The group did not evaluate the oral exposure route but agreed that more than site-specific (i.e., URT) effects need to be examined. More data are needed to learn whether using site of toxicity dose metrics is protective of other effects.

## SECTION FOUR FINAL PLENARY SESSION

### **Lessons Learned and Their Applications to the Development of a Human Health Risk Assessment Framework**

In efforts to integrate information deliberated throughout the two colloquia and to assist in the development of the framework, the group broadly discussed the questions listed below.

# Should a common quantitative analysis be conducted when there are commonalities among toxicities?

# In the absence of case-specific PBPK models, is there a common approach for dose adjustment for interspecies extrapolation for all responses? Does this differ for different routes of exposure?

In the presence of PBPK models, how does MOA information influence the dose surrogate in characterizing toxicity? Can it be different for different responses?

# In the absence of BBDR models, how does MOA information influence the default approach(es) to characterize in quantitative terms the potential risk of toxicities at low levels of exposure (i.e., beyond the range of observation)? Are there common default approaches?

# The 1996 "Proposed Guidelines for Carcinogen Risk Assessment" have recommended that five factors be considered when determining the margin of exposure. These included intraspecies variation, interspecies variation, nature of the response, steepness of the dose-response curve, and biopersistence.

The current quantitative approach for noncancer effects generally involves development of a single RfD/RfC for a "critical effect." Factors used include intraspecies variation, interspecies variation, subchronic to chronic extrapolation, LOAEL to NOAEL extrapolation, and completeness of the data base. An additional factor may be applied to account for scientific uncertainties in the study selected for derivation of the RfD/RfC.

If the goal is to harmonize across toxicities, can a consistent set of factors be identified? How does MOA information influence the choice of these factors?

Discussions focused on criteria and factors one should consider when evaluating integrated risk assessment approaches. In addition, factors relevant to MOE application and appropriate "uncertainty" factors were detailed. Prior to these discussions, the group clarified terminology related to dose response:

Linear: When assuming a linear dose response, the ED<sub>10</sub> (or point of departure) assumes that from the point of departure (POD) there is a linear extrapolation down to zero.

Nonlinear: For a nonlinear dose response, the ED<sub>10</sub> (or "benchmark dose") is divided by uncertainty factors to develop an RfD.

MOE: The MOE is the ED<sub>10</sub> divided by the human exposure estimate of interest. It can be applied to linear or nonlinear dose-response curves and for any endpoint.

The group agreed upon this definition of MOE but noted that the description of MOE in EPA's cancer guidelines is somewhat confusing and, therefore, needs to be clarified.

Some participants preferred the term "margin of protection;" however, it was pointed out that the term MOE was developed and used purposely so not to imply "safety" or "protection."

The group considered how adequate and useful MOE is to the risk management decision and discussed the possible basis on which an MOE should be set. The group agreed that regulators need these "numbers" for compliance purposes. Like RfDs, MOEs need to represent exposures "without appreciable risk." One participant noted that there are social, political, and legal issues as well as the science driving the decision. Another participant noted that it is ultimately a risk management decision—is the MOE acceptable given a certain set of conditions? It was noted that an MOE can be more powerful than an RfD because, in evaluating an acceptable MOE, the entire toxicity database is examined. It is the scientist/risk assessor's responsibility to bring the relevant information to the risk manager so that he/she can understand the significance of a given MOE.

Colloquium participants agreed on the following points or questions regarding the application of MOEs:

# IRIS needs to include additional risk characterization information. One participant commented that it could be included in Section 6.

# A criteria list is needed to guide risk assessors and managers in applying the MOE concept (a consistent series of questions). The list should include uncertainty issues for cancer and noncancer effects.

One participant noted that a consistent approach may be difficult (across programs and the different regions).

# Both the numerator (ED<sub>10</sub>) and denominator (human exposure of interest) values need to be clearly explained to the risk manager, including the confidence in each value.

# Adequacy of the MOE will be based largely on experience.

- # Factors considered when deriving an RfD and when deciding on an MOE are similar, but not identical. While both consider toxicity and dose-response, one important distinction is that application of an MOE also considers the magnitude and uncertainty in the exposure estimate. Furthermore, as mentioned previously, the entire toxicity database is considered when deciding on an MOE.
- # Mode of action needs to be carefully examined when deciding if MOE is the most scientifically viable approach for assessing risks.

The group listed the following key "uncertainty" factors for consideration when integrated approaches are applied. No "values" were assigned.

- # Intraspecies differences: Differences in toxicokinetics and toxicodynamics within species.
- # Interspecies differences: Differences in toxicokinetics and toxicodynamics across species.
- # Quantitative linkages between toxicokinetics and toxicodynamics.
- # Severity of endpoint/effects.
- # Structure activity relationship information.
- # Human exposure scenario information (e.g., frequency, pattern, etc.).
- # Confidence limits on ED<sub>10</sub> (experimental variability).
- # Shape and steepness of dose-response curve.
- # Integration of multiple factors.
- # Species specificity/sensitivity.
- # Quality of database.
- # Quality of individual studies.
- # Knowledge of MOA.
- # Reversibility/irreversibility of effects.
- # Biopersistence (e.g., is it sequestered in fat?) (toxicokinetics).
- # Bioavailability (toxicokinetics).

- # Particularly susceptible population (e.g., children, genetic susceptibility, pre-existing disease).
- # Route of exposure.
- # Route to route extrapolation.
- # Relationship between MOA and human exposure scenarios.
- # Confidence in PBPK models.
- # Biopersistence in the environment.
- # Biomarkers of effect/exposure.

### Overview/Next Steps

Both colloquia were instrumental in soliciting expert opinion on evolving issues related to MOA and integrated risk assessment approaches. Participants offered their impressions on the current state of scientific knowledge and on the next steps in developing a human health risk assessment framework. Having worked through the case studies, the group agreed that, in light of available knowledge, new more scientifically-based approaches can and should be applied. The group clearly recognized, however, that many uncertainties exist. The following ideas were communicated by participants and reiterated throughout the colloquium.

- # As was evidenced through case study discussions, a range of opinions still exist on the best approach (e.g., shape of the dose response curve, common MOAs, etc.).
- # Before integrated risk assessment approaches can fully evolve, more quantitative information is needed.
- # Risk assessors will inevitably be faced with limited data sets. The general scheme of toxicologic events may be known, but specific mechanisms may not be fully understood. What do we do if only limited MOA information is available? Do we fall back on current default approaches? Scientists will need to evaluate when "enough" data are available.
- # The process requires a good deal of data interpretation. Developing a system to aid in this process will be challenging. Others agreed, asking "Can we come up with an approach that is scientifically viable and useful from a regulatory perspective?"
- # As integrated approaches are explored further, a case study(ies) that would use an MOE approach needs to be developed. A set of key factors related to cancer and noncancer effects also should be formally developed.
- # The overall goal of the risk assessment framework is to consider how to practice and communicate the "best science" in predicting risks.

- # The best available science should be used to generate the most credible risk assessment, but presented in a way that is useful to the risk manager.
- # Scientists need to know when not to harmonize, even when similar MOAs exist.

In closing, members of the health effects framework planning committee provided a brief overview of next steps in the framework development. The input from agency experts during this colloquia series will be reviewed. Numerous questions and issues were raised that will need to be re-examined and/or further explored. The planning committee would like to see discussions from this colloquia series expanded. A collaborative workshop, including EPA and outside groups (e.g., SOT and SRA) is being contemplated.

Participants noted that additional forums would be helpful in offering additional insight. The group also expressed interest in future colloquia to discuss topics such as exposure and health outcome data and PBPK models.

**Human Health Risk Assessment:**  
*Current Approaches & Future Directions*

September 1997

**Risk Assessment Forum**  
**U.S. Environmental Protection Agency**

*Technical Panel*

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FINAL DRAFT- *Do Not Cite or Quote*

## 1. INTRODUCTION

Human health risk assessment entails the evaluation of available scientific information on the biological and toxicological properties of an agent to make an informed judgment about the potential toxicity in humans as a consequence of environmental exposure to the agent. The National Research Council (NRC), in its report entitled *Risk Assessment in the Federal Government: Managing the Process* (NRC, 1983) defined risk assessment as including some or all of the following components: hazard identification, dose-response assessment, exposure assessment, and risk characterization. This has been supported more recently in *Science and Judgment in Risk Assessment* (NRC, 1994). As recommended by the NRC, EPA has developed health risk assessment approaches, modified them over time and incorporated them into endpoint-specific guidelines for the evaluation of mutagenicity (USEPA, 1986), carcinogenicity (USEPA, 1986, 1996a), developmental toxicity (USEPA, 1986, 1991), reproductive toxicity (USEPA 1988a, 1988b, 1996b), and neurotoxicity (USEPA, 1995a). Guidelines on exposure (USEPA 1986, 1992a) and chemical mixtures (USEPA 1986) have also been developed.

The NRC, in *Science and Judgment in Risk Assessment* (NRC, 1994), noted the importance of an approach that is less fragmented, more consistent in application of similar concepts, and more holistic than endpoint-specific guidelines. The report also points out a number of issues in EPA's current risk assessment approaches that need to be reexamined in light of the current scientific knowledge. For example, the report questions the application of a non-threshold quantitative approach as a default in all cancer risk assessments. Conversely, the use of a threshold concept as a default for agents that cause neuro-, reproductive and developmental toxicity or that act on various systems through receptor-mediated events is also questioned. The need for explicit accounting of variability in sensitivity among individuals due either to inherent susceptibility or differential exposure was also a major point of discussion of the NRC report. EPA's Science Advisory Board, in its review of the *Draft Reproductive Toxicity Risk Assessment Guidelines*, raised similar concerns over the appropriateness of current default approaches, that include the assumption of a threshold (USEPA, 1995b). Finally, scientists are encouraging the use of mechanistic data in risk assessment (e.g. Butterworth et al., 1995, Purchase and Auton, 1995). Thus, there is a recognized need for the development of a framework for human health risk assessment which includes all of these perspectives.

In response, the Agency's Risk Assessment Forum is beginning the development of a human health risk assessment framework as a communication piece for risk assessors and risk managers, as well as members of the public who are interested in health risk assessment issues. The primary purpose of the framework document is to discuss the scientific bases and policy choices behind EPA's current risk assessment approaches and to lay out recommended future directions for health risk assessment in the Agency. The framework will emphasize the need for problem formulation at the beginning of the risk assessment process and for integration and harmonization of risk assessment methodologies and procedures of all health endpoints.

The present paper serves as the initial step in the development of a framework for a more integrated approach to human health risk assessment. This paper discusses a number of issues regarding the Agency's risk assessment approaches and their scientific bases to begin to examine their compatibility with current scientific developments. Several variations in health risk assessment approaches for carcinogenicity and for toxicological endpoints other than cancer and heritable mutations (hereafter "noncarcinogenic" or "noncancer" effects) are examined. These include several of the default assumptions and methodologic procedures used in the hazard and dose-response evaluations of cancer and noncancer effects, and in accounting for potential beneficial effects at low doses. This paper is intended as a perspectives piece and serves as a basis for further discussion of the scientific basis for current and future risk assessment approaches.

## **2. MODE OF ACTION / DOSE-RESPONSE CONSIDERATIONS: *CANCER VERSUS NONCANCER EFFECTS***

Assessment of risk from exposures to environmental agents has traditionally been performed differently, depending on whether the response is cancer or a noncancer health effect. This is because different modes of action were thought to be involved in the two cases. Cancer has been thought to largely be the consequence of chemically induced DNA mutations which unleash processes leading to tumor formation. Since a single chemical-DNA interaction may lead to a mutation and since cancer is thought to arise from single cells, it follows that any dose of an agent that produces mutations may be associated with some finite risk. This has led the Agency to employ a science policy that cancer risk should be estimated by a linear, nonthreshold dose-response method. On the other hand, noncancer effects have been thought to result from multiple chemical reactions within multiple cells of an anlage, tissue, organ or system. The Agency's science policy has been that threshold effects would pertain to noncancer risk assessment dose-response analyses.

### **2.1. Cancer Risk Assessment Approach**

#### **2.1.1. Overview of 1986 Cancer Risk Assessment Guidelines**

In the Agency's 1986 cancer guidelines, observation of tumors in animals and humans are the primary determinants of carcinogenic hazard to humans (USEPA, 1986). Other toxicologic and mechanistic information only play a modulating role. Cancer risk estimations use dose-response models to extrapolate tumor incidence observed in an epidemiologic or experimental study at high doses to the much lower doses typical of human environmental exposures. Since mode of action information is generally not available, the linearized multistage (LMS) procedure is employed as the default. An important feature of the LMS procedure is that it assumes increased risk is proportional to dose at low doses, even if it displays nonlinear behavior in the region of observation. A statistical confidence-limit procedure is incorporated in the LMS to generate what is known as an upper bound on excess lifetime cancer risk per unit of dose.

#### **2.1.2. Rationale for 1986 Cancer Risk Assessment Guidelines**

Since the inception of EPA's cancer policy in 1976 (USEPA, 1976), the Agency has taken risk averse positions on the identification of carcinogenic hazards and the estimation of risks. The Agency recognized a range of evidence bearing on carcinogenesis but relied primarily on human and especially chronic animal studies, in keeping with current scientific guidance at the time (NCAB, 1976). A single positive animal study was generally sufficient to identify potential carcinogens, and mutagenicity and other information played only supporting roles. A linear extrapolation of risk was assumed, based on experience with ionizing radiation, lung cancer from smoking and the induction of genetic mutations (Albert et al., 1977; Anderson et al., 1983; Albert, 1994). The Millers at the McArdle Institute developed the thesis that carcinogens were electrophiles (or were metabolized to them) which interacted with nucleophilic sites in cells, namely the DNA, to induce mutations and commence carcinogenesis (Miller & Miller, 1976). These positions were adopted broadly among Federal agencies (IRLG, 1979).

With time it was recognized that not all carcinogens seem to be mutagens. Some researchers suggested that mode of action could in some way be incorporated into the risk assessment process by dividing agents into genotoxic and epigenetic categories (Weisburger & Williams, 1981). Various groups, including EPA, considered the potential of using mode of action information, but given the paucity of chemical-specific information, thought that such actions were largely premature (USEPA, 1982a, 1982b; IARC, 1983; Upton et al., 1984).

By 1985, it was generally accepted that mode of action may play a part in cancer risk assessments, but there was still a significant emphasis on health-conservative default positions (OSTP, 1985; USEPA, 1986). In addition, arguments for linear dose-response relationships had centered upon the concept of additivity to background. This position asserts that if a chemical has a mode of action similar to any ongoing, background process (i.e., mutations), then the risk from the chemical will simply add to that of the background, resulting in no threshold of response and being consistent with low-dose linearity (Crump et al., 1976).

### **2.1.3. New Directions for Cancer Risk Assessment**

Within the last decade, it has become generally held by various groups that mode of action can influence significantly the conduct of risk assessments (IARC, 1991; Vainio et al., 1992; NRC, 1994; Strauss et al., 1994). Carcinogenesis is recognized to embody changes in key genes that regulate the cell replication cycle and can be influenced by mutagenic and non-mutagenic modes of action. Non-mutagenic events include mitogenic and cytotoxic events that result in an increase in cellular proliferation, immunotoxic events and modulation of key cellular control phenomena [e.g., hormonal, receptor-mediated processes (Purchase et al., 1995)]. These concepts have been incorporated into the EPA's 1996 Proposed Cancer Risk Assessment Guidelines (USEPA, 1996a).

Today, direct-acting mutagenic agents are assumed, as a science policy default, to influence the potential for cancer hazard and risk at any dose (e.g., linear, non-threshold), using the same rationale as the original 1976 EPA cancer policy. Linearity in the dose-response is also supported when anticipated human exposures are already in the part of the dose-response curve where effects are observed. However, when direct mutagenic events do not pertain and other mode of action considerations apply, the likelihood exists that cancer would be secondary to other events (e.g., stimulation of cell division). Under such conditions a potential for cancer would exist only at doses of an agent that are sufficient to produce the events. Such events can be anticipated to demonstrate significant nonlinearities in the slope of the dose-response curve. In some cases thresholds may apply. Accordingly, for secondary carcinogenic processes, a margin of exposure (MOE) analysis is proposed as the science policy default in the proposed revisions to the 1986 Cancer Risk Assessment Guidelines (USEPA, 1996a), similar to the approach that has been taken for non-cancer health effects (see below). Finally, in the absence of information on mode of action, the science policy position is to assume that a linear default will apply.

## **2.2. Noncancer Risk Assessment Approach**

### **2.2.1. Overview of Current Approach**

The Agency treats chemicals exerting noncancer health effects as if there is a dose below which there is no potential for risk and above which the potential for risk is undefined. Accordingly, it is assumed as a matter of science policy is that thresholds apply for the risks of health effect from exposure to such



pollutants.

Evaluating human risks for non-cancer effects has generally proceeded along two lines within the Agency. The first is derivation of the oral Reference Dose (RfD) or the inhalation Reference Concentration (RfC). The RfC is derived for continuous airborne exposures and includes adjustments based on respiratory physiology for animal to human extrapolation. The RfD/RfC is defined as an "estimate with uncertainty spanning perhaps an order of magnitude of a daily exposure to the human population, including sensitive subgroups, that is likely to be without an appreciable risk of deleterious effects during a lifetime" (Barnes & Dourson, 1988; USEPA, 1994a). The RfD/RfC is a dose operationally calculated from a human or animal study by dividing the no-observed-adverse-effect level (NOAEL) for a critical effect by various (usually 3-10X) Uncertainty Factors (UFs) and a Modifying Factor (MF) that reflect the various types of data used. UFs are applied on a case-by-case basis to compensate for application of a study that identifies a Lowest-Observed-Adverse-Effect-Level (LOAEL) instead of a NOAEL, subchronic instead of chronic study, within human variability, animal to human extrapolation, and an incomplete data base. The MF also varies by up to a factor of 10 and depends upon the uncertainties of the study and data base not explicitly treated above (Dourson and Stara, 1983; Barnes and Dourson, 1988; USEPA, 1994a; Ohanian, 1995). A more complete discussion of uncertainty factors is provided in section 5.0.

The second way of expressing noncancer risks is to calculate a Margin of Exposure (MOE), which is the ratio of the critical NOAEL to the expected human exposure level. The larger the ratio, the less likely an agent poses a risk to humans; the smaller the ratio, the greater the chance of some risk. Part of the evaluation of the adequacy of the MOE may include the UFs and MF that might have been applied for the case under investigation had an RfD/RfC been calculated.

### **2.2.2. Rationale for Current Approach**

Studies on many compounds show that before toxicity occurs, an agent must deplete physiologic reserves or overcome repair capacity. For instance, toxicity may occur within a cell when there has been sufficient lipid peroxidation or when levels of glutathione have been depleted and the chemical then has the ability to affect the cell. Likewise, toxicity is seen to occur when not just one cell is affected, but when

multiple cells in an embryonic anlage, tissue, organ or system have been perturbed. Thus as science policy, it is assumed that toxic effects occur only after homeostatic, compensating, repair, and adaptive mechanisms fail. Accordingly, if exposure is below that required to cause such failures, the noncancer effect should not be manifest.

### **2.2.3. New Directions for Noncancer Risk Assessment**

Over time it has been recognized that threshold considerations may not be applicable to all noncancer effects cases. Sometimes, effects are manifest at existing environmental exposure levels so that no apparent NOAEL exists, as is the case with exposure to lead (Markowitz et al., 1996). As studies on lead exposure in humans have been refined and conducted at lower and lower exposure levels, effects continue to be manifest. Thus, responses within the human population is already on the observed part of the dose-response curve, and obviously a threshold has not been defined for lead. The same seems to apply to certain receptor-mediated effects, like those associated with 2,3,7,8-TCDD and some hormones (e.g., estrogens).

Application of mode of action information, toxicokinetics and biologically based dose-response models may also play a role in the evolution of assumptions concerning dose-response relationships for noncancer effects. For instance, exposure to various mutagenic agents (e.g., ethylene oxide, ethylene nitrosourea) of pregnant mice carrying zygotes or two-celled embryos, leads to malformations and death later in embryonic and fetal stages (Generoso et al., 1987; Rutledge et al., 1992). Certainly these effects arise from single exposures at the 1- and 2-cell stages, but the mechanisms leading to them have not been determined. Maternal toxicity has been ruled out as an etiological agent, as have structural chromosome aberrations (Kato et al., 1989). Gene mutations are a potential cause of the effects, but they have not been directly investigated. Likewise, it is possible that the compounds are not working via mutagenesis but by changes in gene expression. Therefore, it is possible that thresholds would not apply in such cases.

In addition, it is not usually feasible to distinguish empirically between a threshold and a nonlinear dose response relationship. This has led the EPA Science Advisory Board, when deliberating the draft risk

assessment guidelines for reproduction (USEPA, 1996b) and neurotoxicity (USEPA, 1995a), to recommend a shift in the assumption about dose-response relationships from threshold to nonlinear. However, this recommendation does not fundamentally change the ways RfDs/RfCs are derived and interpreted.

### **2.3. SUMMARY**

The current scientific data base indicates that automatic application of traditional approaches of separating dose-response relationships for cancer and noncancer risk assessment, may no longer be justified. Given mode of action information available today, the Agency is proposing to depart from the assumption that all cancer effects show linear dose-response relationships (USEPA, 1996a). Likewise, it may not be reasonable to assume that all noncancer effects show threshold dose-response relationships. In addition, focus on mechanisms of carcinogenesis directs attention away from tumors per se toward earlier biological and toxicological responses that are critical in the carcinogenic process. Such responses are relevant to both noncancer effects and cancer and serve as a bridge to link their risk assessments.

### **3. POINT OF DEPARTURE FOR CANCER AND NONCANCER DOSE-RESPONSE EXTRAPOLATION: CENTRAL TENDENCY OR LOWER BOUND ESTIMATE**

The point of departure refers to that estimate of dose-response information in the observable range from which low-dose extrapolation occurs. Historically, EPA has used no observed adverse effect levels (NOAELs) as the point of departure for calculation of RfDs/RfCs or margins of exposure. Cancer risks were estimated using the linearized multistage procedure which incorporates all dose-response information for tumor incidence in projecting risks at any finite exposure level. In recent years, the Agency has been developing the benchmark dose (BMD) approach as an alternative for noncancer risk assessment (USEPA, 1995c). Using this method, uncertainty factors are applied to a BMD rather than a NOAEL. An approach similar to that of the BMD has recently been proposed for cancer risk assessment (USEPA, 1996). Comment is divided whether the lower bound on extrapolated dose should be used or the point estimate of extrapolated dose should be employed for the point of departure in cancer and noncancer dose-response assessments.

### **3.1. Proposed Departure Dose Point (Benchmark Dose) for Noncancer Assessment**

The historical approach to defining a NOAEL and calculating a RfD/RfC has a number of limitations. For example, this type of method does not specifically take into account both the slope of the dose-response curve and the baseline variability in the end point in question. The resulting NOAEL from a study using a small number of experimental animals may be significantly higher than the one identified from a study with a larger number of animals. Finally, the NOAEL is generally limited to one of the doses in a study and is contingent upon the dose spacing.

In response to these limitations, the Risk Assessment Forum has developed guidance on Agency use of an alternative approach, the BMD approach (USEPA, 1996c). The BMD is defined as a statistical lower confidence limit on the dose producing a predetermined level of change in adverse response compared with the background response. A BMD is derived by fitting a mathematical model to the dose-response data. In addition to the BMD approach, categorical regression analysis has been proposed to evaluate health effects sorted into categories of progressively greater severity (e.g., no adverse effect, mild-to-moderate effect, and severe effect) (Hertzberg, 1989; Dourson, 1994; Rees and Hattis, 1994).

With respect to the dose point of departure, participants at a workshop on the benchmark dose recommended the use of the lower confidence limit on the 10% incidence (or some other incidence level) of effect as the point of departure (Barnes et al., 1995). The lower confidence limit provides a means of including the variability of the data in the analysis, and addresses one of the limitations of the current RfD/RfC approach.

### **3.2. Proposed Departure Dose Point for Cancer Risk Extrapolation**

The proposed revisions to the cancer risk assessment guidelines (USEPA, 1996a), like the BMD

approach, divide dose-response assessment in two parts. The first is assessment of the data in the range of empirical observation. This is followed by low-dose extrapolations either by modeling, if there are sufficient information to support the use of case-specific model, or by a default procedure if there is not. The default procedure may utilize a linear or nonlinear approach, or both, based on information of the agent's likely mode of action. For those agents producing cancer that 1) lack mutagenic activity and 2) have sufficient evidence of a nonlinear dose response relationship, an analysis of margin of exposure (MOE) is conducted to provide perspective on how much risk reduction is associated with reduction in dose. The MOE is the ratio of the dose point of departure to the human exposure level. The point of departure can be obtained in several ways for cancer dose-response assessment. To be consistent with the process for the BMD for noncancer endpoints, the current proposal is to calculate either (1) the lower 95% confidence limit on dose for the observed or calculated 10% tumor incidence level, or (2) the lower 95% confidence limit on dose for the observed or calculated 10% incidence of some tumor precursor (e.g., hyperplasia, hormone levels) (USEPA 1996A).

At a workshop in the fall of 1994 (USEPA, 1994b) that evaluated an early draft of the cancer risk assessment guidelines, there was a strong recommendation that the Agency use dose associated with a particular tumor or tumor precursor response (e.g., 10%) instead of the lower confidence limit as is done for non-cancer health endpoints in the benchmark dose procedure as the point of departure. The importance of calculating the upper and lower 95% confidence limits on the 10% tumor incidence and conveying that information to risk managers as part of the risk characterization was recognized and recommended. It was thought that using the lower 95% confidence limit alone resulted in introducing a level of exactitude and public health conservatism that was unnecessary as a part of the analysis of observed data and given the uncertainties inherent in later extrapolation to lower doses outside the observed data range. However, in order to be consistent with the proposed noncancer BMD procedure, the Agency proposed in the 1996 cancer guidelines that the lower confidence limits on the 10% incidence dose be used. In the *Federal Register* notice of the proposed guidelines, the Agency specifically requested comments on how to proceed with defining the point of departure (USEPA, 1996a). At a more recent workshop on the BMD approach (USEPA, 1996d), in which there had been adequate time for reflection on the proposals for the cancer risk assessment guidelines, participants were divided as whether to use the lower confidence limit (BMD) or the point estimate (e.g., 10% response) as the departure point.

### 3.3. Summary

The Agency is interested in developing consistent principles both for analysis of observed data and extrapolation below the observed range of exposures. However, a number of issues have been raised with the revision of the cancer risk assessment guidelines and the development of the BMD approach for noncancer risk assessment. There is still debate over the use of lower confidence limit on the dose or the point estimate as the proposed departure point for low-dose extrapolation. Is there a reason to apply different approaches to cancer or other health effects? Cancer testing in animals regularly uses 50 or more animals per dose group, a number greater than in most testing of noncancer endpoints. Would it be preferable to use a point of departure that is based on the power of the study, yet may differ for different endpoints? There are numerous options to consider.

## 4. INTERSPECIES ADJUSTMENTS FOR DOSE

There are a number of uncertainties in the extrapolation of dose-response data from animals to humans. EPA's risk assessment guidelines and procedures provide specific guidance for the application of default approaches and procedures to compare dose between species and to account for potential species differences in the carcinogenic and noncarcinogenic responses to environmental agents. One of the critical steps in risk assessment is the selection of the measure of exposure for definition of the exposure-dose-response relationship. EPA's exposure guidelines (USEPA 1992a) describes several types of exposure measures for such definition. *Administered dose* is the amount of chemical ingested, inhaled, or applied to the skin. *Internal dose* is the amount of a chemical that has been absorbed across the applicable barriers (i.e., the gut wall, the skin, or the lung lining) and is available for biological interactions. *Delivered dose* is the amount transported to an individual organ, tissue, or fluid of interest. *Biologically effective dose* is the amount of the chemical that actually reaches cells, sites, or membranes where adverse effects occur. Ideally, the biologically effective dose is used as the basis for defining the dose-response relationship and for assessing risk.

EPA has recommended the use of physiologically-based pharmacokinetics (PBPK) models as the procedure of choice to account for metabolism and pharmacokinetics processes and, thereby, improve

confidence in dose estimation (USEPA, 1986, 1994). This approach for dose extrapolation between species, however, is not possible for most compounds since the use of PBPK models requires extensive comparative metabolism and pharmacokinetics data for use in the modeling process, as well as a good understanding of the agent's mode(s) of action. These data are generally not available for most compounds. As a result, EPA has developed default procedures to compare dose between species in the absence of sufficient pharmacokinetics information. The default assumption is that the administered dose and biologically effective dose are directly proportional.

#### **4.1. Default Procedure for Dose Extrapolation for Noncarcinogens**

The RfD/RfC methodologies represent quantitative approaches to estimate levels of exposure with little appreciable risk of adverse effects for noncancer endpoints. A major difference between the two approaches is that the RfC methodology includes dosimetric adjustments to account for the relationship between exposure concentrations with that of deposited or delivered doses, whereas the RfD does not.

##### **4.1.1. Oral Exposure**

In the derivation of a RfD, it is assumed that the dose administered orally is proportional to the delivered dose as well as the biologically effective dose, and is equivalent across species on a body weight basis (BW<sup>1</sup>). The underlying scientific bases for this assumption are not provided in the guidance describing the methodology. However, such procedures are common among other agencies as well as internationally.

##### **4.1.2 Inhalation Exposure**

In the RfC methodology, the disposition of inhaled toxicants is determined by several factors. EPA has established standard methods for derivation of the human equivalent concentration (HEC) estimates from animal exposure data. Disposition is defined for inhalation exposure as encompassing the processes of deposition, absorption, distribution, metabolism, and elimination. Major factors include the respiratory

tract anatomy and physiology, as well as the physicochemical characteristics of the inhaled toxicant. In addition, the relative contribution of these factors is also influenced by exposure conditions such as concentration and duration. Finally, default adjustment factors are used which are based on default dosimetry models for relatively insoluble and non-hygroscopic particles and three categories of gases (USEPA, 1994).

The default deposition model for particles provides estimates of regional deposition of the major respiratory tract regions [i.e., extrathoracic (ET), tracheobronchial (TB), and pulmonary (PU) regions]. The model, however, does not take into account the clearance and distribution of the deposited dose which would allow for a more accurate estimation of the retained dose and would be a better measure of chronic dose for the derivation of a RfC. For particles, a multiplicative factor (  $RDDD_r$  or regional deposited dose ratio), is used to adjust an observed inhalation particulate exposure concentration of an animal to that of a human that would be associated with the same dose delivered to a specific regional (r) tissue. Depending on whether the observed toxicity is in the respiratory tract or at distal (extrarespiratory) sites,  $RDDR_r$  is used in conjunction with default normalizing factors for the physiological parameter of interest. Because insoluble particles deposit and clear along the surface of the respiratory tract, dose per unit surface area is the recommended normalizing factor for respiratory effects due to particulate deposition. Body weight is often used to normalize dose to distal target tissues.



For gases, the dosimetric adjustments are dependent on the type of gas as well as the effect to be assessed, i.e., respiratory effects or extrarespiratory toxicity. The two categories of gases with the greatest potential for respiratory effects are those that are highly water soluble and/or rapidly irreversibly reactive in the respiratory tract (Category 1), and those that are water soluble and rapidly reversibly reactive, or moderately to slowly irreversibly metabolized in respiratory tract tissue (Category 2). Because they are not as reactive in the respiratory tract tissue as Category 1 gases, gases in Category 2 also have the potential for significant accumulation in the blood and, therefore, have a higher potential for both respiratory and distal toxicity. Gases in Category 3 are relatively water insoluble and unreactive and their uptake is predominantly in the pulmonary region. The site of toxicity of these gases is generally at sites remote to the respiratory tract.

For gases, a ratio of regional dose of a gas in the laboratory animal species to that of humans for region (r) of interest for the toxic effect ( $RGDR_r$ ) is used to dosimetrically adjust the experimental NOAEL to an HEC. The default equations to calculate the  $RGDR_r$  for the different gas categories are dependent on the types of effects - respiratory effects versus effects at remote sites. For respiratory effects, the default  $RGDR_r$  is based on species differences of ventilatory parameters and regional respiratory surface areas (i.e., ET, TB, PU) of concern. For extrarespiratory effects, the default approach assumes that the toxic effects observed are related to the arterial blood concentration of the inhaled agent, and that the animal alveolar blood concentrations are periodic with respect to time for the majority of the experiment duration. Thus, the  $NOAEL_{[HEC]}$  is dependent on the ratio of the blood to gas (air) partition coefficient of the gas for the animal species to the human value. For the situation in which blood to gas (air) partition coefficients are unknown the default value of 1 is recommended.

#### **4.1.3. Dermal Exposure**

No official Agency guidance has been developed for evaluating health risks from dermal exposure to chemicals. However, EPA's Office of Research and Development (ORD) has developed interim methods and procedures for estimating dermally absorbed dose resulting from direct contact with environmental contaminants in soil, water, and contact with vapors (USEPA, 1992c). The guidance document provides a range of default values to be used in situations where exposure information and chemical-specific data

(e.g. permeability coefficient) are not available.

Due to the paucity of dose-response data from dermal exposure to chemicals, the default practice for characterizing noncancer risks from dermal contact with contaminants in soil and water is to utilize chemical-specific oral RfD, with some adjustment for dermal bioavailability when feasible.

## **4.2. Default Procedure for Dose Extrapolation for Carcinogens**

### **4.2.1. Oral Exposure**

To derive a human equivalent oral dose from animal data, the default procedure as recommended in the 1986 Cancer Risk Assessment Guidelines was to scale the lifetime average daily dose by  $2/3$  power of body weight as a measure of differences in body surface area. Dose extrapolation on the basis of body surface area was thought to be appropriate because certain pharmacological effects commonly scale according to surface area (USEPA, 1986). Recently, the Agency has adopted the recommendation made by an interagency workgroup that interspecies scaling be based on  $3/4$  power by body weight (USEPA, 1996a). The underlying assumption is that lifetime cancer risks are equal in animals and humans when average daily administered dose are proportional to each species' body weight. This default procedure is based on empirical observation that rates of physiological processes consistently tend to maintain proportionality with  $3/4$  power by body weight (USEPA 1992b).

#### **4.2.2. Inhalation Exposure**

The default procedure to derive a human equivalent concentration of inhaled particles, gases, and vapors is that for estimating inhaled dose in the derivation of RfC (see discussion above).

#### **4.2.3. Dermal Exposure**

As discussed in section 4.1.3, interim guidance is available for the estimation of dermally absorbed dose resulting from direct contact with environmental contaminants in soil, water, and contact with vapors (USEPA, 1992c). Potential cancer risk from dermal exposure to systemic carcinogens for which dose-response information by the oral route is available can be estimated with some adjustment for dermal bioavailability. This default procedure is only applicable for chemicals that are expected to be readily absorbed via animal and human skin.

#### **4.3. Summary**

As illustrated from the discussion above, different default assumptions and methodologies are being utilized to account for interspecies differences for dose in the assessment of cancer and noncancer risks. There are also differences in the methods applied to different routes of exposures. The underlying scientific bases for these default assumptions need to be re-examined in light of the need to better harmonize and integrate the assessment for potential human cancer and non-cancer health effects. A number of questions have been raised: (1) Should EPA's science policy for dosimetric adjustments be the same for cancer and noncancer assessments from lifetime oral exposure, as it has now been recommended for inhalation exposure? (2) What would they be? (3) What are the interagency and international implications of adopting similar default procedures? In addition, more guidance is needed for the evaluation of potential cancer and noncancer risks from dermal exposures. Current EPA risk assessment guidelines primarily focus on oral and inhalation pathways.

## **5.0 APPROPRIATENESS OF UNCERTAINTY FACTORS**

Efforts have been made to account for major sources of variation in responses when estimating levels of human exposure that may not be attended with significant risk for noncancer and, more recently, for certain cancer risk assessments. Uncertainty factors (UFs) have been used to account for response differences of various types. They have often been used, along with a modifying factor (MF) which is dependent on the completeness of the data, for calculation of an RfD/RfC or evaluation of the significance of a margin of exposure (MOE) (NOAEL/estimated human exposure). Questions have arisen concerning the magnitude of individual uncertainty factors and the appropriateness of compounding a number of such factors together for evaluation of potential risk.

### **5.1. Noncancer**

Traditionally, UFs of up to 10X have been used to adjust for differences in variability of response following oral exposures for differences: (a) within species, (b) between species, (c) when using less than chronic data, (d) when using a lowest observed adverse effect level (LOAEL) instead of a NOAEL, and (e) incompleteness of the data base (Barnes & Dourson, 1988; USEPA, 1994).

The initial choice of 10X for these UFs was somewhat arbitrary (Lehman and Fitzhugh, 1954) . Empirical analyses presented in Table 1 (see page 20 ) indicate that these values are usually conservative estimates of the underlying variability (Dourson & Stara, 1983; Calabrese, 1985; Lewis et al., 1990). For instance,

- a. Nair et al. (1995) investigated NOAELS for a large number of subchronic and chronic studies in rats, mice and dogs that were investigated by FAO/WHO and a smaller number of studies conducted by Monsanto. Interspecies comparisons could be made for 7 to 73 studies. Of these cases, 80-100% of interspecies comparisons are covered by a 10X factor, and the median is usually less than a factor of 3X, although there is one exception.

- b. Human variability can be quite marked for certain inherited conditions, but about 80 to 95% of cases people are covered by a 10-fold factor (Calabrese, 1985). This is also born out when comparisons are made for various pharmacokinetic factors as well as for the elimination half life or the therapeutic dose of pharmaceuticals (Naumann, 1995).
- c. Variability in extrapolating from subchronic to chronic studies ranges from 9 to over 40 study comparisons (Weil & McCollister, 1963; McNamara, 1976; Abdel-Rahman, 1995; Nair et al., 1995; Nessel et al., 1995). Median differences are 4 fold or less; the 90th percentile is usually about 5 fold; and essentially 100% of cases are within a factor of 10 fold.
- d. In comparisons of the LOAEL vs. a NOAEL in a study, investigators have noted median differences of less than 4 fold and 90th percentile fold differences of about 5, with almost all cases being covered by a factor of 10 fold (Weil & McCollister, 1963; Abdel-Rahman, 1995; Kadry et al., 1995).

These data indicate that uncertainty factors of 10 are generally inclusive of the variation that exists for the various factors, often with the median significantly less than 10X. Even the 90th percentile for a number of the factors may only be about a factor of 5X.

**Table 1. Observed Variability of Responses**

Factor		Fold level at named %		Proportion of cases below 10-fold level
		50th	90th	
<b>Interspecies</b>	Nair et al., 1995 rat/mouse (N=31)	3.0		80%
	(N= 7)	5.3		85%
	rat/dog (N=73)	2.0		92%
	(N= 7)	1.8		100%
	mouse/dog (N=30)	2.9		83%
<b>Intraspecies</b>	Calabrese, 1985			80-95%
	Hattis et al., 1987 p'kinetic factors			100%
	Naumann, 1995 elimination $t_{1/2}$ therapeutic dose			100% 88%
<b>Subchronic to chronic</b>	Weil & McCollister, 1963 (N=33)	<2.0	<5.0	97%
	McNamara, 1976 (N=41)		<5.0	100%
	Abdel-Rahman, 1995 (N= 3)		$\leq$ 5.0	100%
	Nessel et al., 1995 oral (N=22)	2.0	3.5	96%
	inhalation (N= 9)	4.0	7.6	100%
	Nair et al., 1995 (N=22)	3.3		68%
<b>LOAEL to NOAEL</b>	Weil & McCollister, 1963 (N=33)	<3.0	<5.0	100%
	Kadry et al., 1995 (N= 9)	2.0	5.0	100%
	Abdel-Rahman, 1995 (N=24)	<3.5		96%

Given the inclusive nature of individual 10X UFs, compounding of multiple factors all with this magnitude could result in a significant overestimation of the inherent total variability. For instance, the combination of five factors of 10X to calculate an RfD is 100,000. If the individual UFs were actually 3X each instead of 10X, the overall estimate of variability would be 27, a value nearly 4000 times smaller than the default value. Partially in recognition of this problem, the Agency limits the maximum product of the UFs and MF for RfD/RfC calculation to 3000. If factors in a given case are in excess of 3000, then an RfD is not calculated. An empirical analysis of the influence of compounding UFs on 231 RfDs found that none of the calculated values was greater than the 30th percentile of the distribution of potential human threshold doses and over half were below the 5% level (Baird et al., 1996).

In addition, for calculation of some RfDs EPA has deviated from using the default 10X factors: (a) when human variability is less than the default, (b) when the database is partially complete, (c) for essential nutrients when default factors would result in exposures below maintenance levels, (d) when the LOAEL is a minimal effect, and (e) when animal studies warrant reduction, as when they share a common target toxicity with humans (Cicmanec & Poirier, 1995).

## **5.2. Cancer**

In the 1996 proposed cancer risk assessment guidelines, an MOE approach is used when there is sufficient information to conclude the agent is not mutagenic and mode of action findings support a non-linear dose-response relationship. In evaluating MOEs, default factors of not less than 10X are suggested to account for differences in sensitivity (a) within species and (b) between species. If humans are less sensitive than animals, the default value is 0.1. Basically all hazard and dose response information are to be considered in evaluating the adequacy of the MOE. Other factors should be evaluated include things like (c) slope of and uncertainties about the dose response curve at the point of departure, (d) nature of the endpoint used for dose response assessment, and (e) persistence of the agent in the body. Only qualitative guidance is given as to how to use this information.

### **5.3 Summary**

Traditional use of 10X uncertainty factors seems to account for the variability in responses of a number of factors and may overestimate it in most cases. Exceptions do exist, however. Compounding multiple UFs may only propagate either over or underestimates in calculating RfDs/RfCs and in evaluating MOEs.

Several issues deserve consideration such as the following. Should default UFs remain the same as in the past or be changed? Should assessments include the use of central tendency values for UFs or continue with default 10X positions? How should the employment of multiple UFs be presented and characterized in risk assessments?

## **6. NONCANCER RISK ASSESSMENT**

### **6.1. Critical Health Endpoints Versus Entire Spectrum of Adverse Effects**

As discussed in the introduction section, the Agency has published several guidelines for assessing specific non-cancer, non-mutagenic endpoints, such as developmental toxicity (USEPA, 1986, 1991); reproductive toxicity (USEPA 1988a, 1988b, 1996b), and the proposed neurotoxicity (USEPA, 1995a). These guidelines set forth principles and procedures to guide EPA scientists in the interpretation of studies that follow EPA's testing guidelines and other toxicologic and epidemiologic information to make inferences about the potential hazard to specific health endpoints and identification of data and knowledge gaps. In practice, EPA risk assessments do not routinely make a full evaluation and characterization of various potential health effects. Rather, most EPA non-cancer assessments focus on the "critical effect" of an agent (i.e., the adverse effect or its known precursor which occurs at the lowest dose) to derive an RfD or RfC for oral and inhalation exposures, respectively. The RfD/RfC approach assumes that if exposure can be limited so that such a critical effect does not occur, then no other effects of concern will occur. Consequently, this approach fulfills the regulatory needs in various EPA programs for defining an exposure level(s) below which there is negligible risk of adverse non-cancer and non-mutagenic effects from exposure to a given agent.



EPA also conducts endpoint specific assessments for identification of potential hazards for priority setting or hazard ranking, for making decisions whether to invest resources in collecting data for a full assessment, or for determination of whether there is scientific basis for listing an agent on the Agency's regulatory lists of hazardous substances of concern. These hazard assessments can be of screening or comprehensive level depending mainly on the regulatory need. Accordingly, the scope and depth of a given EPA assessment for noncarcinogenic effects vary depending on its intended purpose, the available data and resources, and other factors including the nature of risk management needs. Critical to the process is communication between risk assessors and risk managers to insure that scientific information is best analyzed and used.

Risk assessments that focus only on the critical health endpoint, in effect, minimize characterization of other adverse effects the chemical may cause and the doses where they are found. As such, the full spectrum of potential effects are not characterized. In trying to identify potential health effects in humans from studies of an agent in experimental animals, the assessor seldom knows which effects are predictive of those which may occur in humans. Therefore, there is merit in presenting the myriad of effects in experimental animals at differing dose levels. As a result, risk managers may have a better appreciation of the potential effects in humans and can better evaluate risk reduction options. In addition, performing non-cancer effects in this way would have several advantages: 1) a better appreciation of possible hazards at various exposures is developed with little more investment of time and effort, 2) because it is not known whether sensitivity to different effects is the same for humans as that of the test animals, a more full consideration of effects that may be closely spaced in appearance with increasing exposure could be realized; and 3) non-cancer effects that may underlie potential carcinogenic endpoints could be discerned and examined. A presentation of a spectrum of effects is currently being accomplished in the ATSDR toxicological profiles which feature graphic means to summarize observed effects.

## **6.2. Exposure-Duration Relationships**

Historically, the risk assessment of noncancer effects has placed emphasis on the potential health effects from continuous lifetime exposures. However, there is an increasing recognition that other exposure scenarios such as intermittent occupational and consumer exposures, as well as accidental exposures are also of regulatory concern. As a result, various EPA regulatory program offices have developed or are developing exposure guidelines or advisories for acute, short-, or intermediate-term exposures. For example, the Office of Water has developed health advisories for 1-day and 10-day consumption levels, which consider exposures to both adults and children. The Office of Pollution Prevention and Toxics is leading an Agency effort, in collaboration with other federal and state agencies, to develop acute exposure guideline levels (AEGL) for the general public from emergency or accidental exposures to hazardous chemicals. The risk evaluation method for AEGL is based on the methodology developed by the National Academy of Sciences (NAS, 1993). The Office of Pesticides Program has recently completed its effort in the development of risk assessment methods for less-than-lifetime exposures to pesticides.

However, all of the available approaches, described above to estimate short-duration exposure limits, assume a constant relationship between level of an exposure and its duration with respect to the expected response. Specifically, the exposure basis used in risk assessment calculations is a "daily exposure", regardless of the actual timing, duration, or frequency of exposure. Even in the derivation of a reference dose or reference concentration for developmental toxicity ( $RfD_{DT}$ ,  $RfC_{DT}$ ), the risk assessment is based on the overall daily exposure.

Consequently, while approaches for incorporating less-than-lifetime exposures in the risk assessment process have been developed, our understanding of the influence of the timing, duration, and frequency of exposure on chemical toxicity is limited at best. There is a need for the development of an Agency risk assessment guidelines for the evaluation of "less-than-lifetime exposures". These guidelines should set forth the general principles and approaches, and the underlying assumptions of available methodologies for various exposure scenarios other than continuous lifetime exposures and stress the use of toxicokinetic data where possible. These guidelines should also be useful in identifying major gaps in our scientific knowledge.

### **6.3 Dose-Response Assessment for Contaminants with Beneficial Effects at Low Doses**

Essential elements are those elements that must be present in small quantities in the human diet to maintain normal physiological and biochemical functions. The 10th edition of the NRC's Recommended Dietary Allowances (NRC, 1989) identifies nine essential elements. For four of these (iodine, iron, selenium, and zinc), the database was considered acceptable to set a Recommended Dietary Allowance (RDA), and for the other five (chromium, copper, fluoride, manganese, and molybdenum), a range of estimated safe and adequate daily dietary intakes (ESADDIs) was generated. The NRC also addressed several other trace elements (e.g., arsenic, boron, nickel and silicon), for which there is some evidence of essentiality but where physiological/biochemical requirements and functions in humans have not been proven.

For each essential element, there are two ranges of exposure or intake associated with adverse health effects: intakes that are too low and result in nutritional deficiency, and intakes that are too high and cause toxicity. The general dose-response for adverse effects for these elements thus has been visualized as U-shaped, composed of overlapping curves for deficiency and toxicity (ILSI, 1994). Ideally, the "trough" of the U-shaped curve would define the region of acceptable (safe and adequate) intakes. In practice, the available data are seldom adequate to clearly describe the shape of the curve, and values such as the RDA are established with a margin of safety based on the best scientific evidence available.

On the toxicity side of the U-shaped relationship, EPA establishes oral RfDs. Because human data on the toxicity of these elements are limited, RfDs often must be based to a considerable extent on experimental data from animal studies, and in most cases, there is a large uncertainty factor associated with such RfDs. In fact, in one case, zinc, the RDA and RfD were found to be almost identical, and for other cases the values were within an order of magnitude or less. This apparent convergence of values associated with beneficial effects on one hand and minimal risk of toxicity on the other suggests the need for a closer look at the Agency's risk assessment methodology for contaminants with beneficial effects at low doses (Calabrese, 1995). The following examples illustrate this point of view (ILSI, 1994).

1. The RDA for zinc (15 mg/day for males, 12 mg/day for females) and the RfD (0.3 mg/kg/day, or 21 mg/day for a reference 70-kg adult) represent somewhat convergent doses. Furthermore, the

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RfD for this element is below the RDA for infants, children, adolescents, and (possibly) pregnant or lactating women, an overlap that is acknowledged in IRIS.

2. Selenium has an RDA of 70 µg/day for males and 55 ug/day for females, compared with an RfD of 5 ug/kg/day (350 µg/day). Both the RDA and RfD for selenium are based on studies in China. The actual estimated dietary selenium intakes of Americans vary, ranging from 60 to 234 µg/Se/day. For some apparently healthy individuals, however, selenium intakes appear to be greater than the RfD, with no apparent adverse effects.

Based on the above discussion, it is quite timely that the Agency evaluates its existing risk assessment methodologies to apply "common sense" while attempting to maximizing beneficial effects at low doses and minimizing toxic effects at high doses.

## 7. REFERENCES

- Abdel-Rahman, M.S. & Kadry, A.M. 1995. Studies on the use of uncertainty factors in deriving RfDs. *Hum. Ecol. Risk Assess.* 1: 614-624.
- Albert, R.E. 1994. Carcinogen risk assessment in the U.S. Environmental Protection Agency. *Crit. Rev. Toxicol.* 24: 75-85.
- Albert, R.E., Train, R.E. & Anderson, E. 1977. Rationale developed by the Environmental Protection Agency for the assessment of carcinogenic risks. *J. Natl. Cancer Inst.* 58: 1537-1541.
- Anderson, E.L. and the Carcinogen Assessment Group of the U.S. Environmental Protection Agency. 1983. Quantitative approaches in use to assess cancer risk. *Risk Anal.* 3: 277-295.
- Baird, S.J.S., Cohen, J.T., Graham, J.D., Shlyakhter, A.I. & Evans, J.S. (1996) Non cancer risk assessment: a probabilistic alternative to current practice. *Hum. Ecol. Risk Assess.* 2: 79-102.
- Barnes, D.G. & Dourson, M. 1988. Reference dose (RfD): Description and use in health risk assessments. *Regul. Toxicol. Pharmacol.* 8: 471-486.
- Barnes, D.G., Daston, G.P., Evans, J.S., Jarabek, A.M., Kavlock, R.J., Kimmel, C.A., Park, C. & Spitzer, H.L. (1995) Benchmark dose workshop: Criteria for use of a benchmark dose to estimate a reference dose. *Regul. Toxicol. Pharmacol.* 21: 296-306.
- Calabrese, E.J. 1985. Uncertainty factors and interindividual variation. *Regul. Toxicol. Pharmacol.* 5: 190-196.
- Calabrese, E. J. 1995. Expanding the RfD concept to incorporate and optimize beneficial effects while preventing toxic responses from non-essential toxicants. *BELLE Newsletter* 4: 1-10.
- Cicmanec, J.L. & Poirier, K.A. 1995. Selected applications of reduced uncertainty factors in noncancer risk assessment. *Hum. Ecol. Risk Assess.* 1: 637-640.
- Crump, K.S., Hoel, D.G., Langley, C.H. & Peto, R. 1976. Fundamental carcinogenic processes and their implications for low dose risk assessment. *Cancer Res.* 36: 2973-2979.

Dourson, M.L. and Stara, J.F. 1983. Regulatory history and experimental support of uncertainty (safety) factors. *Regul. Toxicol. Ind. Health.* 3: 224-238.

Generoso, W.M., Rutledge, J.C., Cain, K.T., Hughes, L.A. & Braden, P.W. 1987. Exposure to female mice to ethylene oxide within hours after mating leads to fetal malformation and death. *Mutat. Res.* 176: 269-274.

International Agency for Research on Cancer (IARC) 1983. Approaches to classifying chemical carcinogens according to mechanism of action. IARC internal tech. rept. no. 83/001. Lyon: International Agency for Research on Cancer.

International Agency for Research on Cancer (IARC) 1991. A consensus report of and IARC monographs working group on the use of mechanisms of carcinogenesis in risk identification. IARC internal tech. rept. no. 91-001. Lyon: International Agency for Research on Cancer.

International Life Sciences Institute (ILSI) 1994. Risk assessment of essential elements, ILSI Press, Washington, D.C.

Interagency Regulatory Liaison Group (IRLG) 1979. Scientific bases for identification of potential carcinogens and estimation of risks. *Fed. Regist.* 44: 39858-39879. Also published in *J. Natl. Cancer Inst.* 63: 241-268.

Kadry, A.M., Skowronski, G.A., Khodair, A.I., & Abdel-Rahman, M.S. 1995 Determining “safe” levels of exposure: The validity of the use of 10X safety factors. *Hum. Ecol. Risk Assess.* 1: 565-575.

Kato, M., Cacheiro, N.L.A., Cornett, C.V., Cain, K.T., Rutledge, J.C. & Generoso, W.M. 1989. Fetal anomalies produced subsequent to treatment of zygotes with ethylene oxide or ethyl methanesulfonate are not likely due to the usual genetic causes. *Mutat. Res.* 210: 337-344.

Lehman, A.J. & Fitzhugh, O.G. 1954 100-fold margin of safety. *Assoc. Fd. Drug Offic. U.S. Quart. Bull.* 18: 33-35.

Lewis, S.C., Lynch, J.R. & Nikiforov, A.I. (1990) A new approach to deriving community exposure guidelines from “no-observed-adverse-effect levels.” *Regul. Toxicol. Pharmacol.* 11: 314-330.

Markowitz, M.E., Bijur, P.E., Ruff, H.A., Balbi, K., and Rosen, J.F. 1996. Moderate lead poisoning: Trends in blood lead levels in unchelated children. *Environ. Health Perspect.* 104: 968-972.

FINAL DRAFT- *Do Not Cite or Quote*

McNamara, B.P. 1976 Concepts in health evaluation of commercial and industrial chemicals. In Mehlman, M.A., Shapiro, R.E. & Blumenthal, H., eds. *New Concepts in Safety Evaluation*. Washington, DC: Hemisphere. pp. 61-115.

Miller, E.C. & Miller, J.A. 1976. The metabolism of chemical carcinogens to reactive electrophiles and their possible mechanisms of action in carcinogenesis. In Searle, C.E., ed. *Chemical carcinogens*. ACS monogr. 173. Washington, DC: American Chemical Society. pp. 737-762.

Nair, R.S., Sherman, J.H., Stevens, M.W. & Johannsen, F.R. 1995 Selecting a more realistic uncertainty factor: Reducing compounding effects of multiple uncertainties. *Hum. Ecol. Risk Assess.* 1: 576-589.

National Academy of Sciences (NAS). 1993. Guidelines for developing community emergency exposure levels for hazardous substances. Committee on Toxicology. Washington DC: National Academy Press.

National Cancer Advisory Board (NCAB). 1976. General Criteria for assessing the evidence for carcinogenicity of chemical substances: Report of the Subcommittee on Environmental Carcinogenesis, National Cancer Advisory Board. *J. Natl. Cancer Inst.* 58:461-465.

Naumann, B.D. & Weideman, P.A. 1995 Scientific basis for uncertainty factors used to establish occupational exposure limits for pharmaceutical active ingredients. *Hum. Ecol. Risk Assess.* 1: 590-613.

Nessel, C.S., Lewis, S.C., Stauber, K.L., & Adgate, J.L. 1995 Subchronic to chronic exposure extrapolation: Toxicologic evidence for a reduced uncertainty factor. *Hum. Ecol. Risk Assess.* 1: 516-526.

National Research Council (NRC). 1983. Risk Assessment in the Federal Government: Managing the Process. Washington D.C.: National Academy Press.

National Research Council (NRC). 1989. Recommended dietary allowances, 10th ed. National Academy Press, Washington, D.C.

National Research Council (NRC). 1994. Science and judgment in risk assessment. Washington, DC: National Academy Press.

Ohanian, E.V. 1995 Use of the reference dose in risk characterization of drinking water contaminants. *Hum. Ecol. Risk Assess. J.* 1: 625-631.

FINAL DRAFT- *Do Not Cite or Quote*

Office of Science and Technology Policy (OSTP). 1985. Chemical carcinogens: A review of the science and its associated principles. Fed. Regist. 50: 10371-10442. Also published Environ. Health Perspect. 67: 201-282. 1986.

Purchase, I.H.F. & Auton, 1995. Thresholds in chemical carcinogenesis. Regulatory Toxicol. Pharmacol. 22: 199-205.

Rutledge, J.C., Generoso, W.M., Shourbaji, A., Cain, K.T., Gans, M. & Oliva, J. 1992 Developmental anomalies derived from exposure of zygotes and first-cleavage embryos to mutagens. Mutat. Res. 296: 167-177.

Strauss, B., Hanawalt, P., Swenberg, J. 1994 Risk assessment in environmental carcinogenesis. An American Association for Cancer Research special conference in cancer research cosponsored by the Environmental Mutagen Society. Cancer Res. 54: 5493-5496.

Upton, A.C., Clayson, D.B., Jansen, J.D., Rosenkranz, H. & Williams, G. 1984 Report of ICPEMC task group on the differentiation between genotoxic and non-genotoxic carcinogens. Task group 5. Mutat. Res. 133: 1-49.

U.S. Environmental Protection Agency (USEPA). 1976. Interim procedures and guidelines for health risk and economic impact assessments of suspected carcinogens. Fed. Regist. 41: 21402-21405.

U.S. Environmental Protection Agency (USEPA). 1982a. Report on workshop on estimating ambient water quality criteria for epigenetic carcinogens. Cincinnati, OH: Environmental Criteria Assessments Office. (February 17).

U.S. Environmental Protection Agency (USEPA). 1982b. Report on workshop for the review of the use of uncertainty factors in developing ambient water quality criteria for epigenetic carcinogens. Cincinnati, OH: Environmental Criteria Assessments Office. (March 12).

U.S. Environmental Protection Agency (USEPA). 1986. The risk assessment guidelines of 1986. EPA/600/8-87/045.

U.S. Environmental Protection Agency (USEPA). 1988a.. Proposed guidelines for assessing female reproductive risk. Notice. Fed register 53:24834-24847.

U.S. Environmental Protection Agency (USEPA). 1988b.. Proposed guidelines for assessing male reproductive risk and request for comments.. Notice. Fed register 53:24850-24869.

U.S. Environmental Protection Agency (USEPA). 1991. Guidelines for Developmental Toxicity Risk Assessment. Fed Register 58(234):63798-63826.

U.S. Environmental Protection Agency (USEPA). 1992a. Guidelines for exposure assessment. Fed. Register 57(104):22888-22938.



FINAL DRAFT- *Do Not Cite or Quote*

U.S. Environmental Protection Agency (USEPA). 1992b. Draft report: A cross-species scaling factor for carcinogen risk assessment based on equivalence of  $\text{mg/kg}^{3/4}/\text{day}$ . Fed. Register 57(109):24152-24173.

U.S. Environmental Protection Agency (USEPA). 1992c. Dermal Exposure Assessment: Principles and Application. EPA/600/8-91/011B. Office of Health and Environmental Assessment, Washington D.C.

U.S. Environmental Protection Agency (USEPA). 1994a. Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. EPA/600/8-90/066F.

U.S. Environmental protection Agency (USEPA). 1994b. Report on the workshop on cancer risk assessment guidelines issues. Risk Assessment Forum. USEPA: Washington, DC

U.S. Environmental Protection Agency (USEPA). 1995a. Proposed guidelines for neurotoxicity risk assessment. Fed. Register 60(192):52032-52056.

U.S. Environmental Protection Agency (USEPA ).1995b. Report of the SAB on the draft Reproductive Guidelines.

U.S. Environmental protection Agency (USEPA). 1995c. The Use of the Benchmark Dose Approach in Health Risk Assessment. EPA Document EPA/630/R-94/007.

U.S. Environmental protection Agency (USEPA). 1996a. Proposed guidelines for carcinogen risk assessment. Fed. Register 61(79):17960-18011.

U.S. Environemntal protection Agency (USEPA). 1996b. Reproductive Toxicity Risk Assessment Guidelines. Fed. Register 61(212):56274-56322.

U.S. Environmental Protection Agency (USEPA).1996c. Draft benchmark dose technical guidance document. EPA/600/P-96/002A.

U.S. Environmental Protection Agency (USEPA) .1996d. Benchmark dose peer consultation workshop. Holiday Inn Bethesda, Bethesda, MD, September 10-11, 1996. Washington, DC: U.S. Environmental Protection Agency.

Vainio, H., Magee, P.N., McGregor, D.B. & McMichael, A.J. 1992 Mechanisms of carcinogenesis in risk identification. IARC scientif. publ. no. 116. Lyon: International Agency for Research on Cancer.

Weil, C.S. & McCollister, D.D. 1963 Relationship between short- and long-term feeding studies in designing an effective toxicity test. Agric. Fd. Chem. 11: 486-491.

Weisburger, J.H. & Williams, G.M. 1981 Carcinogen testing: Current problems and new approaches. Science. 214: 401-407.



# Framework for Human Health Risk Assessment Colloquia Series

## Colloquium #2

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# Framework for Human Health Risk Assessment Colloquia Series

## Colloquium #2

**Holiday Inn Bethesda**  
**Bethesda, MD**  
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# FRAMEWORK FOR HUMAN HEALTH RISK ASSESSMENT

## Colloquium #2

### *Case Study: Vinyl Acetate*

#### Executive Summary

Vinyl acetate is a synthetic organic ester with a wide range of uses. The 1990 Clean Air Act Amendments include vinyl acetate as one of the 189 hazardous air pollutants listed under Title III because it has been shown to be a respiratory tract toxicant in experimental species.

Vinyl acetate induces nasal tumors in rats, but not mice, following long-term inhalation exposure. Tumors have also been shown in the portal of entry (buccal cavity, esophagus, and forestomach) after oral administration at high concentrations (10,000 ppm), but the focus of this case study is inhalation exposure. All but one of the nasal tumors were observed at the terminal sacrifice indicating a late-life dependency of tumor formation. Non-neoplastic lesions of the nasal cavity were observed in both rats and mice. These data show that the upper respiratory tract (URT), and in particular the olfactory mucosa, is the primary target of vinyl acetate toxicity in both rats and mice. This portal-of-entry toxicity is not surprising given that vinyl acetate is metabolized to acetic acid and acetaldehyde by nasal carboxylesterase. Rats are chosen as the most sensitive experimental species because, while olfactory degeneration was shown in both species at comparable exposure concentrations, tumors were observed only in rats.

Olfactory degeneration is proposed as a sentinel lesion in rodents based on dosimetry considerations and on the pathogenesis continuum believed to be involved in tumor development. Degeneration of the olfactory epithelium is rapidly followed by an induced proliferative response, which is likely the driving force or rate limiting step behind the observed tumor formation. Dose-response modeling of olfactory degeneration is thereby proposed as a relevant basis for human health risk assessment of both noncancer toxicity and carcinogenesis. Olfactory degeneration is mechanistically linked to the *in situ* formation of acetic acid in olfactory sustentacular cells and the consequent loss of control over intracellular pH. Acetaldehyde, a known clastogen (chromosome breaking agent) and inducer of sister chromatid exchanges, is also formed during the hydrolysis of vinyl acetate. Acetaldehyde's involvement in the development of nasal tumors is unclear, and is more



likely to occur only at high exposures where saturation of its detoxification would occur. Acetaldehyde is rapidly oxidized almost exclusively to acetic acid by NAD<sup>+</sup>-dependent aldehyde dehydrogenase. Airflow dynamics and the distribution of enzymes which metabolize vinyl acetate are shown to be key determinants of uptake and distribution in the tissues of the URT of the rat.

Mechanistic studies have been conducted to test the hypothesis that both the genotoxicity and degenerative cytotoxic effects were related to carboxylesterase-mediated hydrolysis of vinyl acetate to acetaldehyde and acetic acid. These studies support the hypothesis that the cytotoxic and carcinogenic effects of vinyl acetate are related to the carboxylesterase-mediated formation of acetic acid, a strong cytotoxicant, and acetaldehyde, a clastogen. *In vitro* studies on both vinyl acetate and acetaldehyde suggest that neither induces point mutations. Research on the stability of acetaldehyde-induced DNA-protein crosslinks show the crosslink is unstable at physiological temperature and pH ( $t_{1/2} \cong 6.5$  hr) raising the possibility that the carcinogenic effect of vinyl acetate is less dependent on acetaldehyde-induced DPXL and more dependent on acetic acid-induced cytotoxicity. Furthermore, research on the effects of pH on clastogenic activity *in vitro* show that low pH alone can induce clastogenic responses similar to those induced by vinyl acetate. Therefore, conditions under which intracellular pH is maintained in a physiological range such that cytotoxicity-induced cell proliferation is prevented will likely minimize any potential contribution of metabolite, acetaldehyde, to the formation of DNA protein crosslinks and potential consequent clastogenesis.

Cell proliferation studies showed a rebound response of olfactory epithelium to extended vinyl acetate exposure (1 day vs. 5 days, vs. 20 days). These results suggest that restorative cell proliferation within the basal cell compartment, to replace lost sustentacular cells, becomes the driving event for neoplastic growth. Thus, in olfactory epithelium, the carcinogenic response to vinyl acetate exposure appears to be driven largely by a cytotoxic proliferative mechanism.

Determinants of uptake and nasal tissue dose were evaluated extensively including quantitative estimation of kinetic constants governing carboxylesterase and aldehyde dehydrogenase activities and histochemical localization of their cellular distributions. Separate methods of analysis of carboxylesterase activity suggested that the enzyme is

localized in nasal tissue in functionally distinct compartments. Experiments utilizing a unique *in vitro* whole tissue gas uptake system demonstrated that vinyl acetate is almost completely metabolized in the most superficial compartment of olfactory epithelium (sustentacular cells). Therefore, sustentacular cells are likely the primary target of vinyl acetate-induced olfactory toxicity. Also of importance is the observation from the histochemical analyses that in olfactory epithelium, the basal cells, which are stem cells for proliferative regeneration and presumably are progenitor cells for neoplastic growth, are devoid of carboxylesterase activity.

A physiologically-based model of the URT has been developed that describes vinyl acetate vapor deposition and metabolism, and acetic acid-induced changes in intracellular pH in the rat. Dosimeters generated from the modeling showed a pattern that is consistent with the overall mechanistic hypothesis. The intracellular pH of olfactory epithelium was predicted, through simulations, to drop at external exposure concentrations above 50 ppm. This is consistent with observations from the 2-year inhalation bioassay that olfactory degeneration occurs at concentrations above 50 ppm. Therefore, dosimeters related to intracellular pH (total amount of acetic acid formed, and final proton concentration in olfactory tissue) appear to be tenable measures of tissue dose on mechanistic grounds.

## Case Specific Questions for Vinyl Acetate

- I. Does the existing database support the URT lesions as the sentinel toxicity for inhalation exposures to vinyl acetate?
- II. Can the cytotoxic changes caused by vinyl acetate exposure be considered as sequentially linked to the observed tumor outcome? What are the key considerations to characterize the conditions of hazard (e.g., high dose versus low dose)? How do the genotoxic data factor in this characterization?
- III. What mechanistic data are most relevant to characterizing tumor outcome? Which would be useful for dose-response modeling in the observable range? What are the implications of the mode of action information for extrapolation of risk to low dose?
- IV. Given the availability of the PB-PK model, which dose metrics should be considered for the dose-response analysis? Does this choice of dose metric address consideration of the role of exposure duration?
- V. What are the uncertainties in using these data to characterize human risk?
- VI. Should an RfC be developed separately? If an RfC or MOE were to be developed, which factors should be considered to account for uncertainties in the extrapolations applied?
- VII. What mechanistic data would be useful for development of risk estimates of exposures via the oral route?

## **I Introduction**

Vinyl acetate monomer is a synthetic organic ester with a wide range of uses including application in polyvinyl acetate emulsion of latex paints and as a copolymer with ethylene in adhesives, paper, and paper board coatings. Vinyl acetate is also used in the manufacture of polyvinyl alcohol. Because vinyl acetate has been shown to be a respiratory tract toxicant in experimental species and is emitted from production facilities, concern exists over its potential adverse human health effects. The 1990 Clean Air Act Amendments include vinyl acetate as one of the 189 hazardous air pollutants listed under Title III (42 U.S.C.A. §7412(b)).

Vinyl acetate is a highly flammable, colorless liquid with an acrid, ether-like sweetish odor. It is soluble in most organic solvents and moderately soluble in water. The physical properties are summarized in Table 1. A typical commercial sample of technical vinyl acetate has a purity of  $\geq$  99.8% (w/w) and may contain trace quantities of water, acetic acid, and acetaldehyde. Hydroquinone is typically added at 1.5 - 20 ppm to inhibit polymerization (ECETOC, 1991).

## **II Summary of Key Studies**

### **A. Effects in Humans**

In an occupational study by Deese and Joyner (1969), no adverse effects associated with long-term occupational exposure were detected in a review of medical records and multiphasic examinations of workers in three vinyl acetate production units of a chemical plant. The mean concentration of vinyl acetate in the air of the units was 8.6 ppm based on a range of 0 to 49.3 ppm. No significant eye or throat irritation was noted below 10 ppm. There is inadequate evidence in humans for the carcinogenicity of vinyl acetate based on epidemiology studies (IARC, 1995).

**Table 1.**  
**Physical and Chemical Properties of Vinyl Acetate**

<b>Name:</b>	Vinyl acetate
IUPAC name:	Vinyl acetate
Synonyms:	Acetic acid, vinyl ester Acetic acid, ethenyl ester 1-Acetoxyethene 1-Acetoxyethylene Ethanoic acid, ethenyl ester Ethenyl acetate Ethenyl ethanoate Vinyl acetate monomer Vinyl A monomer Vinyl ethanoate
Chemical Abstracts Index name:	Acetic acid, ethenyl ester
CAS Registry No.	108-05-4
Formula:	C <sub>4</sub> H <sub>6</sub> O <sub>2</sub>
Molecular weight:	86.09
Density:	0.932 at 20°C
Vapor pressure:	108 mm Hg at 25°C
K <sub>ow</sub>	0.73
Conversion factors (20°C, 760 mm Hg):	1 ppm = 3.57 mg/m <sup>3</sup> 1 mg/m <sup>3</sup> = 0.28 ppm

## **B. Laboratory Bioassay Data**

### ***Non-Cancer Respiratory Tract Effects***

Exposure of mice or rats for up to four weeks to concentrations greater than 150 ppm or 500 ppm, respectively, produced clinical signs consistent with irritation of the respiratory tract (Owen, 1979a, b). Concentrations as high as 2,000 ppm caused a decreased rate of weight gain eye and nose irritation, and increased numbers of lung macrophages in rats (Cage, 1970).

Rats and mice were exposed for up to three months to vinyl acetate vapors ranging from 50 ppm to 1,000 ppm. At 1,000 ppm decreased body weight gain and increased lung weights were accompanied by histopathological changes in the lower respiratory tracts of rats and in the entire respiratory tract of mice (Owen, 1980a,b). Inflammatory and metaplastic changes were apparent in the respiratory epithelium of mice. The no-observable adverse effect level (NOAEL) in these studies was 200 ppm in rats and 50 ppm in mice.

The effects of chronic exposure (0,50,200, and 600 ppm) to vinyl acetate in rats and mice (60/sex/group) were evaluated (Bogdanffy *et al.*, 1994). The study also included three satellite groups of 10 of each species and sex for interim evaluations and recovery studies (data not shown in tables). There were no exposure-related tumors observed in any satellite group animal. Exposure-related effects in both species were confined to the respiratory tract. There was no evidence of systemic toxicity or systemic oncogenicity.

Non-neoplastic lesions in rats. A summary of significant non-neoplastic lesions of the respiratory tract is presented in Table 2 and Figure 1. In the respiratory epithelium of the nasal cavity, no non-neoplastic treatment-related changes were observed. The most prominent and consistent compound-related nasal lesion consisted of thinning of the olfactory epithelium accompanied by basal cell hyperplasia. In severe cases, low cuboidal cells lined the thickened submucosa in the olfactory region. In less severe cases, proliferating basal cells were covered by epithelium that resembled respiratory epithelium. In most animals of the 600 ppm groups, these changes were associated with submucosal edema and with loss of nerve bundles and Bowman's glands or with hyperplasia of glandular structures. Infiltrations of inflammatory cells in the epithelium and submucosa as well as leukocytic exudate were seen regularly in 600 ppm-exposed animals.

In 200 ppm groups, the location of the lesions described above were often restricted to, or most pronounced in, the anterior part of the dorsal meatus. This region is normally covered by olfactory epithelium. In the 600 ppm concentration group the lesions extended to the posterior part of the olfactory epithelium. Focal squamous metaplasia of olfactory epithelium (without keratinization) was observed in many 600 ppm-exposed rats and was located mainly on the top of the dorsal lamellae of the ethmoturbinates.

Regeneration of the olfactory epithelium was evident in many rats of the 200 ppm groups and in a few rats of the 600 ppm group. The regenerated epithelium was seen as a layer of stratified undifferentiated epithelium containing small foamy structures resembling nerve bundles and groups of epithelial cells containing yellow-brown pigment resembling acinar cells of the Bowman's glands. The regenerating epithelium was most prominent in the anterior part of the dorsal meatus.

Table 2

## Summary of Statistically Significant Non-neoplastic Changes in Lungs and Nose Rats: Main Study

Concentration (ppm):	Incidence of Lesions Other than Tumors <sup>a</sup>							
	Males				Females			
	Control	50	200	600	Control	50	200	600
<b>Lungs:</b>	(58)	(59)	(60)	(60)	(60)	(60)	(60)	(59)
Bronchial exfoliation								
very slight	0	0	0	8**	0	0	0	0
slight	0	0	0	26***	0	0	0	4
moderate	0	0	0	2	0	0	0	0
Intraluminal fibrous projections								
very slight	0	0	0	16***	0	0	0	3
slight	0	0	0	14***	0	0	0	28***
moderate	0	0	0	1	0	0	0	8**
severe	0	0	0	0	0	0	0	1
Pigment macrophage								
very slight	1	0	0	0	0	0	0	1
slight	1	3	3	33***	6	4	1	10
moderate	0	0	1	2	0	0	0	4
Peribronchiolar/perivascular lymphoid aggregates								
very slight	5	1	0*	0*	0	1	2	0
slight	15	18	21	14	11	14	14	23*
moderate	1	4	1	2	2	1	2	5
<b>Nose:</b>	(59)	(60)	(59)	(59)	(60)	(60)	(60)	(59)
Olfactory epithelial atrophy								
very slight	0	1	4	0	0	1	4	0
slight	0	2	47***	7*	0	0	23***	18***
moderate	0	0	2	33***	0	0	0	30***
severe	0	0	0	10**	0	0	0	3
Olfactory epithelial squamous metaplasia								
very slight								
slight	0	0	0	2	0	0	5	4
moderate	0	0	0	12**	0	0	0	26***
severe	0	0	0	9**	0	0	0	7**
	0	0	0	1	0	0	0	0
Olfactory epithelial regeneration								
very slight	0	0	3	0	0	0	3	2
slight	0	0	30***	1	0	0	16***	7**
moderate	0	0	2	0	0	0	3	0
Olfactory epithelial inflammatory cell infiltrate								
very slight	0	0	0	1	0	0	0	0
slight	0	0	0	7*	0	0	0	5*
moderate	0	0	0	1	0	0	0	1
Epithelial nest-like infolds								
very slight	0	0	0	0	0	0	1	0
slight	0	0	1	0	0	0	0	0
moderate	0	0	15***	5	0	0	5	5*
severe	0	0	1	5	0	0	0	2

Table 2 (continued)  
Summary of Statistically Significant Non-neoplastic Changes in Lungs and Nose Rats: Main Study

Concentration (ppm):	Incidence of Lesions Other than Tumors <sup>a</sup>							
	Control	Males			Females			
		50	200	600	Control	50	200	600
Olfactory epithelial leukocytic exudate								
very slight	0	0	0	0	0	0	1	0
slight	0	0	0	11***	0	0	0	5*
moderate	0	0	0	2	0	0	1	3
severe	0	0	0	1	0	0	0	0
Basal cell hyperplasia								
very slight	2	5	3	1	0	0	7*	0
slight	0	0	40***	21***	0	0	24***	35***
moderate	0	0	11***	22***	0	0	3	16***
severe	0	0	0	2	0	0	0	0
Turbinate leukocytic exudate								
very slight	0	2	0	0	1	1	3	1
slight	4	8	5	5	4	3	3	7
moderate	3	6	3	8	0	1	1	7**
severe	0	0	0	1	0	0	0	0
Submucosal inflammatory cell infiltrate								
slight	2	0	1	2	0	0	0	0
moderate	1	3	1	6	0	0	0	5*
severe	0	0	0	1	0	0	0	0
very severe	0	0	1	0	0	0	0	0

<sup>a</sup> Figures in parenthesis represent the number of animals from which this tissue was examined microscopically. Significance of differences in a pairwise (Fisher's) test between each treatment and control incidence is indicated by \*p<0.05, \*\*p<0.01, \*\*\*p<0.001.

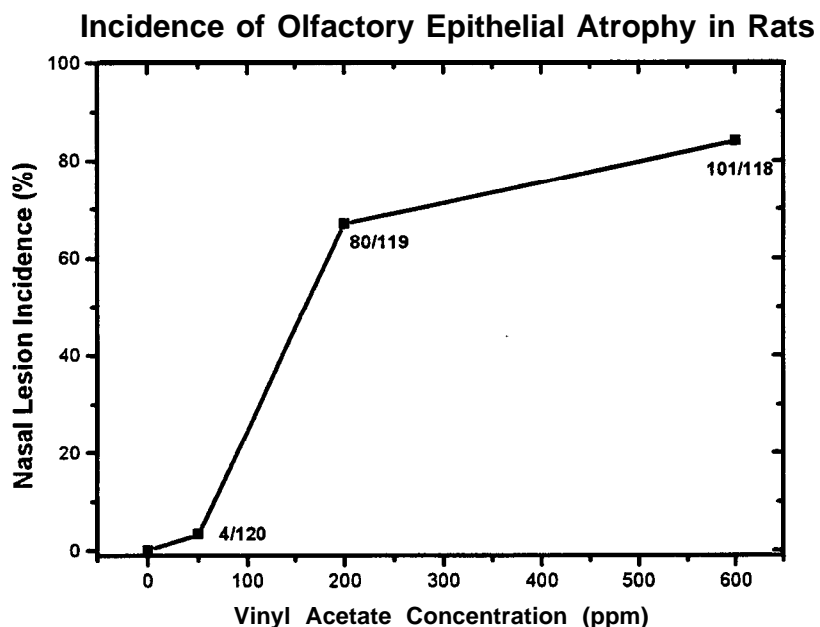
In the anterior part of the nose, signs of rhinitis, such as leukocytic exudate, hyperplasia of the epithelium covering the nasal and maxillary turbiates, and epithelial and submucosal infiltrates of inflammatory cells were observed to about the same incidence and severity in test and control animals.

The lack of non-neoplastic effects reported for the respiratory epithelium of rats is remarkable considering the appearance of two inverted papillomas in this region and two squamous cell carcinomas in the anterior (non-olfactory) region in male rats of the 600 ppm exposure group. It is unlikely that the inverted papillomas arose spontaneously. In a retrospective study of material in the NTP archives, Brown (1990) reported a spontaneous papilloma incidence (not otherwise specified) of 0/1596 male rats and 0/1643 female rats. The appearance of non-neoplastic effects in the respiratory epithelium of rats may have been masked by the pathology



induced by tumor formation. Nasal respiratory epithelium of mice was reported to be affected by vinyl acetate (see below). It is also possible that non-neoplastic effects were evident at an earlier time during the study and were subsequently repaired. Evidence for this is as follows.

Higher exposure concentrations have been shown to affect respiratory epithelium. Exposure of rats for up to 4 weeks show a very low incidence of respiratory epithelial damage and repair (see Cell Proliferation Effects, below). *In vitro* cytotoxicity studies discussed below show that vinyl acetate has the potential to produce cytotoxicity in rat respiratory epithelium, but at relatively high concentrations (Kuykendall *et al.*, 1993). Therefore, it appears that the respiratory epithelium is susceptible to the cytotoxic effects of vinyl acetate, but is substantially more resistant than the olfactory epithelium. These data also suggest that the respiratory epithelium is capable of rapid repair and adaptation.



**Figure 1.** Dose-response for olfactory degeneration (atrophy). The incidences are for males and females combined, regardless of severity qualification. All lesions observed at 50 ppm were considered either “very slight” or “slight”.

No compound-related non-neoplastic changes were seen in larynx or trachea. Treatment-related changes in the lower respiratory tract were restricted to male and female rats of the highest exposure concentration and generally involved the bronchi and bronchioli. Bronchial exfoliation of the lining epithelium was observed in many 600 ppm group males and in a few females, without showing apparent associated acute bronchitis. In addition, intraluminal fibrosis was observed. This lesion was characterized by fibrous plaques and buds covered by normal bronchial epithelium that projected into the lumen of the airways. An increased incidence of macrophages laden with brown pigment granules located in the main bronchi, in bronchioli, in alveolar spaces, and in the interstitium was observed in the 600 ppm groups.

In general, treatment-related nasal and lower respiratory tract lesions similar to those seen in the main study were present in the interim and recovery groups and occurred to about the same incidence and severity as in the main study. In the lower respiratory tract, however, bronchial exfoliation was not observed in any of the interim or recovery group rats.

Vinyl acetate is metabolized to acetic acid and acetaldehyde. Acetaldehyde is also a nasal toxicant (Woutersen *et al.*, 1986). Morphologically, the non-neoplastic lesions induced by vinyl acetate bear only slight resemblance to those induced by acetaldehyde. The main similarities include a high incidence of olfactory epithelial atrophy and basal cell hyperplasia of the olfactory epithelium (Woutersen *et al.*, 1984; 1986). Olfactory epithelial atrophy induced by acetaldehyde appears as early as 28 days after exposure of rats to 400 ppm acetaldehyde (Appelman *et al.*, 1982). Squamous metaplasia was found with high incidence among rats exposed to >750 ppm acetaldehyde (lower levels were not tested) or >200 ppm vinyl acetate. In the case of vinyl acetate on the other hand, squamous metaplasia was not associated with keratinization. A particularly interesting difference observed between the non-neoplastic effects of vinyl acetate and acetaldehyde, under the conditions of their respective bioassays, is the lack of pronounced effect on nasal respiratory epithelium in rats exposed to vinyl acetate. Squamous metaplasia, with or without keratinization, and simple epitheliomatous hyperplasia was observed in respiratory tissue of rats exposed to 1500 ppm acetaldehyde.

The non-neoplastic lesions induced by vinyl acetate bear greater resemblance to lesions induced in rodents by inhaled organic acids and esters. Examples include propylene glycol monomethyl ether acetate, ethyl acrylate, methyl acrylate, n-butyl acrylate, formic acid, and acrylic acid (Miller *et al.*, 1981; Miller *et al.*, 1984; Miller *et al.*, 1985b; Reininghaus *et al.*, 1991; National Toxicology Program, 1992). For all of these compounds the critical lesion of the nasal passages is degeneration of the olfactory epithelium, primarily of the epithelium lining the dorsal meatus. Respiratory epithelium is generally less sensitive.

The strongest data set linking the mechanism of vinyl acetate-induced non-neoplastic nasal lesions to that of other inhaled esters is the work on dibasic esters. Keenan *et al.* (1990) showed that 13 week exposures of rats to dibasic esters mixtures produces degeneration of only the olfactory epithelium while Lee *et al.* (1992) showed that high concentrations of dibasic esters (5900 mg/m<sup>3</sup> aerosols plus unspecified amounts of vapor) damage both respiratory and olfactory epithelium. The pathogenic responses were similar to that of vinyl acetate in that the lesions progressed from reduced olfactory epithelial thickness and degeneration to a reparative state of hyperplasia and/or metaplasia with prominent basal cell mitotic activity. In the case of

dibasic esters, the carboxylesterase-rich sustentacular cell was shown at the ultrastructural level to be the primary target of cytotoxicity (Trela *et al.*, 1992).

Non-neoplastic lesions in mice. A summary of the statistically significant non-neoplastic lesions in mice is presented in Table 3. In general, the morphology of the non-neoplastic lesions observed in the nasal cavity of mice was similar to that of rats, however, several specific differences were noted. In mice, some atrophic areas of the olfactory epithelium were accompanied by foci of respiratory epithelium (respiratory metaplasia). This type of respiratory epithelial metaplasia occurred locally both at the dorsal meatus in the mid-region and at the dorsal parts of the nasal cavity in the ethmoturbinate region. The ciliated cells often appeared to be continuous with the ciliated lining epithelium of the ducts of the underlying Bowman's glands. In rats, areas of regeneration of the olfactory epithelium were often accompanied by a keratinizing squamous epithelium and epithelial nest-like infolds.

Another notable difference between rats and mice was the appearance of non-neoplastic lesions in the respiratory epithelium of mice. Focal non-keratinizing squamous metaplasia of respiratory epithelium of the maxilloturbinates and lateral wall of the nasal cavity at the naso/maxilloturbinate region, and occasionally of olfactory epithelium at the dorsal meatus, was observed in the 600 ppm mice. Also, eosinophilic hypertrophic sustentacular cells along with local loss of sensory cells was observed in all groups, including controls, but occurred more frequently in mice of the 200 ppm and 600 ppm groups.

**Table 3**  
**Summary of Statistically Significant Non-neoplastic Changes in Lungs and Nose of Mice: Main Study**

Concentration (ppm):	Incidence of Lesions Other than Tumors*							
	Control	Males			Females			
		50	200	600	Control	50	200	600
<b>Lungs:</b>	(51)	(51)	(56)	(53)	(56)	(55)	(55)	(51)
Accumulation of alveolar macrophages								
very slight	5	1	4	3	5	2	6	1
slight	10	2*	4	7	3	8	4	10
moderate	0	4	8**	4	2	1	1	12**
severe	1	1	4	0	1	3	1	1
Intra-alveolar eosinophilic material								
very slight	0	0	3	1	0	0	2	1
slight	3	1	1	19***	0	0	0	7**
moderate	0	0	0	10**	0	0	1	15***
severe	0	0	0	2	0	0	0	1
Accumulation of brown pigmented macrophages								
very slight	2	2	1	11*	3	5	1	2
slight	0	0	5	12***	1	1	4	21***
moderate	0	0	1	1	0	0	0	2
Intraluminal fibroepithelial projections								
very slight	0	1	2	3	1	0	0	6
slight	0	0	0	17***	0	2	1	19***
moderate	0	0	0	3	0	0	0	7**
Bronchial gland dilatation	14	16	26	17	8	17	20*	15
Bronchial/bronchiolar epithelial flattening and/or exfoliation								
very slight	0	0	0	4	0	0	0	4*
slight	1	0	0	25***	0	0	0	28***
moderate	0	0	0	7*	0	0	0	4*
severe	0	0	0	0	0	0	0	1
Bronchial/bronchiolar epithelial disorganization								
very slight	0	0	0	0	0	0	0	5*
slight	0	0	0	11**	0	1	0	18***
moderate	0	0	0	4	0	0	0	0
<b>Nose:</b>	(52)	(48)	(53)	(50)	(56)	(57)	(55)	(51)
Inflammatory exudate	0	0	2	15***	0	0	1	5**
Mucosal inflammatory infiltrate	1	0	0	12**	1	2	0	5
Submucosal gland hyperplasia								
slight	3	3	28***	25***	2	5	42***	35***
moderate	0	0	8**	15***	0	0	7**	13***
Olfactory epithelial atrophy (mainly dorsal meatus)								
very slight	0	0	2	0	0	0	0	0
slight	0	0	5	0	2	4	8	0
moderate	0	0	28***	2	0	0	26***	0
severe	0	0	4	3	0	0	4	1

**Table 3 (continued)**  
**Summary of Statistically Significant Non-neoplastic Changes in Lungs and Nose of Mice: Main Study**

Concentration (ppm):	Incidence of Lesions Other than Tumors*							
	Males				Females			
	Control	50	200	600	Control	50	200	600
Olfactory epithelial atrophy (widespread)								
slight								
moderate	0	0	1	0	0	0	0	0
severe	1	0	8*	5	0	0	12***	5*
	0	0	4	39***	0	0	2	45***
Squamous metaplasia at the naso/maxilloturbinate region								
very slight	0	0	0	0	0	0	0	1
slight	1	1	2	13**	4	2	0	13*
moderate	0	1	0	11***	0	0	0	6**
severe	0	0	0	0	0	0	0	1
Replacement of olfactory by respiratory epithelium								
slight	0	0	5	11***	0	0	15***	10***
moderate	0	0	1	0	0	1	5*	10***
severe	0	0	0	0	1	0	0	0
<b>Trachea/bronchi:</b>	<b>(49)</b>	<b>(46)</b>	<b>(51)</b>	<b>(48)</b>	<b>(55)</b>	<b>(56)</b>	<b>(52)</b>	<b>(48)</b>
Epithelial hyperplasia	0	0	2	19***	1	1	0	11***

\* Figure in parenthesis represent the number of animals from which this tissue was examined microscopically. Significance of differences in a pairwise (Fisher's) test between each treatment and control incidence is represented by \*p<0.05, \*\*p<0.01, \*\*\*p<0.001.

In mice (53 and 83 week interim sacrifices), the pattern of lesions was similar to that of the main study although the incidence and severity of the lesions was not as great at the terminal sacrifice.

Tracheal epithelial hyperplasia was significantly increased in incidence in 600 ppm mice of the main study. A few mice of the 600 ppm group showed tracheal epithelial flattening and/or exfoliation, metaplasia, or intraluminal fibroepithelial projections, similar to the treatment-related changes observed in the intrapulmonary conducting airways.

As with the rats, the treatment-related changes of the conducting airways of mice occurred only in the 600 ppm group. There was flattening and/or exfoliation of the bronchial and bronchiolar lining epithelium, without obvious evidence of an associated inflammatory response. Moreover, intraluminal fibroepithelial projections, seen as finger-like projections,

plaques, and buds, protruding into the lumen of the bronchi and bronchioli were observed. The projections were lined by flattened epithelium and they incorporated a stromal component. Epithelial disorganization of the bronchial and bronchiolar epithelium was defined as the presence of foci or areas of dedifferentiated lining epithelium, seen as a pleomorphic picture of swollen basophilic epithelial cells showing pronounced nucleoli, together with relatively flattened or cuboidal epithelial foci, suggesting regeneration, and occasionally multilayered or hyperplastic foci. These changes were grouped since they generally occurred together in the same area and appeared to be stages of a process of continuous degeneration and regeneration. Focal metaplasia of the bronchi/bronchioli was occasionally observed. A single male of the 600 ppm group showed a small area in the alveolar tissue with cornifying squamous metaplasia.

In the alveoli there were compound-related accumulations of alveolar (foamy) macrophages in 600 ppm females. Accumulation of brown pigmented macrophages was observed in the 600 ppm group in both sexes and perhaps also in males of the 200 ppm group. Moreover, there was intra-alveolar accumulation of eosinophilic material. Occasionally this material was taken up by macrophages, resulting in an eosinophilic appearance of the macrophages. Although no statistically significant increases in non-neoplastic lesions were noted in the larynx of mice, one female in the 600 ppm group showed a focus of squamous epithelial hyperplasia with dysplastic changes. Several other females showed epithelial hyperplasia.

In general, treatment-related changes occurred to about the same degree and severity in mice of the interim sacrifice groups as occurred in those of the main study. Among mice of the recovery groups the results were also similar to the main study except that the severity of the lesions appeared to be slightly lower. Squamous metaplasia in the naso/maxilloturbinate region and compound-related inflammatory exudate in the nasal cavity were not observed in the recovery groups of mice.

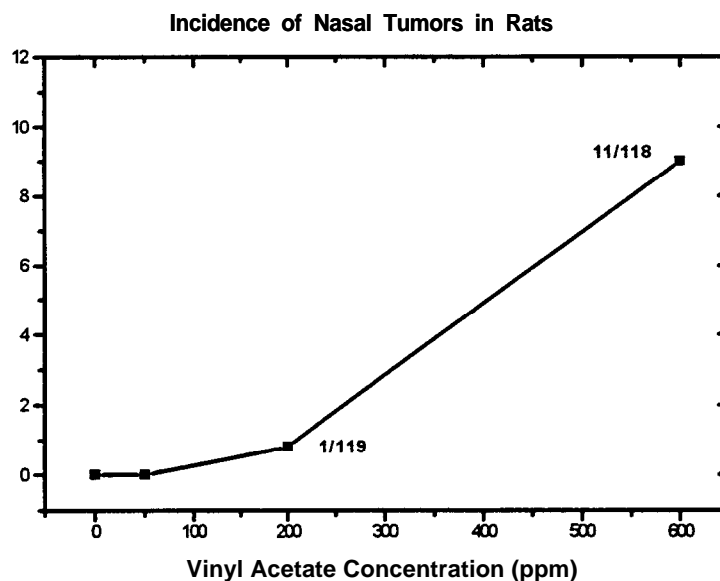
#### ***Other Non-neoplastic endpoints***

Irvine (1980) exposed rats (24 mated females/concentration) to 0, 52, 198, or 1004 ppm to VA on gestation days 6 through 15 for 6 hr/day. Toxicity in the dams exposed to the highest concentration only was noted as a decrease in body weight gain of 10-12% from day 10 of gestation to the end. Fetal growth retardation (decreased mean litter weight, mean fetal weight, and crown/rump length; increase in retardation of sternebral and occipital ossification) also occurred only at the highest concentration.

An oral (drinking water at 0, 200, 1000, and 5000 ppm) 2-generation study in rats (Shaw, 1987) indicated a marginal effect (not statistically significant) on reproductive performance in males dosed at 5000 ppm. No effects in offspring parameters were noted at any concentration.

### *Neoplastic Effects*

Neoplastic lesions in Rats. A summary of the significant neoplastic lesions of the respiratory tract is presented in Table 4 and Figure 2 (Bogdanffy *et al.*, 1994). A total of twelve tumors of the nasal cavity were observed. Four of them were classified as benign inverted, endophytic papillomas and were found in the 600 ppm males only; one was classified as a benign exophytic papilloma and was found in one male in the 200 ppm concentration group. The papillomas were characterized by pseudoacinar structures with cuboidal to columnar epithelium and, in some cases, multilayered epithelium with atypical cells and flattening in some areas.



**Figure 2.** Dose-response for tumor incidence. All tumors included The one tumor observed at 200 ppm was a benign exophytic papilloma.



The tumors were seen in various regions of the nose (Table 5). One was seen in the respiratory area on the lateral wall in zone C, one in the respiratory area of the lateral meatus in zone A, and three in the ethmoturbinate olfactory region in zone E. The other tumors all were observed in the 600 ppm group and were classified as malignant carcinoma *in situ* (one case) or squamous cell carcinomas with varying degrees of keratinization (six cases). The carcinoma *in situ* was found in a male rat in the olfactory area in zones C and D. One small squamous cell carcinoma was found in another male rat in zone B in the ventral floor normally covered by cuboidal cells. In one male rat a squamous cell carcinoma was noticed in the maxilloturbinate area in zone B.

One small squamous cell carcinoma occurred in the ethmoturbinate olfactory area (zone E) in a female rat. Three large squamous cell carcinomas were observed in female 600 ppm rats. These tumors obstructed one side of the nasal cavity and were characterized by invasive growth in nasal bones, soft tissues, and the maxillary sinus. The origin of these tumors could not be established. A subsequent review of these three tumors indicated that one of these might have arisen from respiratory regions, but its size and invasiveness prevented a definitive assignment (KT Morgan personal communication).

In the larynx a squamous cell carcinoma was found in one female rat of the 600 ppm group. No tracheal or treatment-related lung tumors were found in the terminal sacrifice animals nor were any neoplasms observed in the 53 week, 83 week, or recovery groups.

Neoplastic lesions in mice. No treatment-related tumors were observed in the nose, larynx, trachea, or other tissue besides lung of mice of the main study group nor in any airway tissue or other tissue of mice in the satellite groups. A single moderately invasive squamous cell carcinoma was found in a major bronchus of the lung of a male of the 600 ppm group while a single adenocarcinoma occurred in the lung of a male of the control group. These were not statistically significant.

**Table 4**  
**Summary of Statistically Significant Neoplastic Changes in Lungs and Nose of Rats:**  
**Main Study**

Concentration (ppm):	Incidence of Tumors (Numeric) <sup>a</sup>							
	Males				Females			
	Control	50	200	600	Control	50	200	600
<b>Lungs:</b>	(58)	(59)	(60)	(60)	(60)	(60)	(60)	(59)
Well differentiated adenoma [B]	0	0	0	0	1	0	0	0
<b>Nose:</b>	(59)	(60)	(59)	(59)	(60)	(60)	(60)	(59)
Inverted papilloma [B]	0	0	0	4	0	0	0	0
Squamous cell carcinoma [M]	0	0	0	2	0	0	0	4
Papilloma [B]	0	0	1	0	0	0	0	0
Carcinoma in situ [M]	0	0	0	1	0	0	0	0
Total benign tumors	0	0	1	4	0	0	0	0
Total malignant tumors	0	0	0	3	0	0	0	4
Total nasal tumors	0	0	1	7**	0	0	0	4
<b>Larynx:</b>	(59)	(60)	(60)	(60)	(60)	(60)	(60)	(59)
Squamous cell carcinoma [M]	0	0	0	0	0	0	0	1

<sup>a</sup> Figures in parentheses represent the number of animals from which this tissue was examined microscopically. [B] = benign, [M] = malignant. Significance of differences in pairwise (Fisher's) test between each treatment and control incidence is represented by \*\*( $p < 0.01$ ).

**Table 5**  
**Regional Distribution of Vinyl Acetate-Induced Nasal Tumors in Rats<sup>a</sup>**

Site of Origin	Tumor Type
Olfactory region	2 inverted papillomas 1 exophytic papilloma 1 squamous cell carcinoma 1 carcinoma <i>in situ</i>
Respiratory region	2 inverted papillomas
Cuboidal epithelium of zone B*	2 squamous cell carcinoma
unknown	3 squamous cell carcinoma

<sup>a</sup> The histopathological evaluation of the nasal cavity included four cross-sections as follows: slightly posterior to the upper incisor tooth at the level of the first palatine fold at the level of the incisive papilla, and slightly anterior to the first molar. These cross-sections are approximately equivalent to those described by Mery *et al.* (1994) as follows: section 5, 9, 11, and 19, respectively. The nasal lesions were recorded for five anatomical zones: zone A, maxilloturbinates; zone B, nasolachrimal duct medial/ventral to incisor tooth root; zone C, palatine fold region, with anterior region of olfactory epithelium; zone D, maxillary sinus region, anterior to ostium; and zone E, ethmoturbinate region.

\* Zone B is located within a cross section of the nose at the level of the nasolachrimal duct medial/ventral to the incisor tooth root.

### ***Comparison of Tumorigenic Responses of Vinyl Acetate and Acetaldehyde***

The tumorigenic response of rats to vinyl acetate and acetaldehyde are similar only in that both produce squamous cell carcinomas. The squamous cell carcinomas arise mainly from respiratory regions in rats exposed to high concentrations of acetaldehyde and from both respiratory and olfactory regions in rats exposed to vinyl acetate (Table 6). These two compounds differ in that acetaldehyde also produces adenocarcinoma, with a greater incidence than squamous cell carcinoma, and arising primarily from olfactory regions. It is not entirely surprising that the tumorigenic response produced by the two compounds are not identical. First, acetaldehyde was tested at higher concentrations than vinyl acetate increasing the likelihood that acetaldehyde detoxication pathways (in terms of removal of genotoxic species), such as aldehyde dehydrogenase, are saturated. Second, acetaldehyde differs in its water solubility and reactivity which is likely to affect differences in flow dynamics. Third, acetaldehyde demonstrates genotoxic activity (e.g., clastogenesis) without metabolic activation and, therefore, the active species is available to all nasal mucosal cells. Vinyl acetate on the other hand requires metabolic activation to elicit clastogenic activity and was tested at lower exposure concentrations where aldehyde dehydrogenase activity is less likely to be saturated.

Carboxylesterase liberates acetaldehyde and acetic acid from vinyl acetate. However, carboxylesterase is localized only within specific cell types (Bogdanffy *et al.*, 1987). Studies on the mechanism of action presented below show that acetic acid is responsible for the cytotoxic effects of vinyl acetate. Thus, replicating epithelial and seromucous gland cells of respiratory mucosa and sustentacular cells (these may not be capable of replication), and Bowman's gland cells of olfactory mucosa are apt to be primary sites of metabolic activation of vinyl acetate and targets for toxicity. It is only under high concentration exposure scenarios, when acetaldehyde detoxication capacity is overwhelmed, and subsequent cellular proliferation is induced that a role for acetaldehyde in vinyl acetate carcinogenesis might be expected. Nevertheless, the cellular targets of the two compounds would be expected to differ.

**Table 6**  
**Vinyl Acetate vs. Acetaldehyde**  
**Comparison of Carcinogenic Activities**

	<b>Vinyl Acetate</b>				<b>Acetaldehyde<sup>a</sup></b>				
Species tested	Rat, mouse				Rat, hamster				
Species with positive response	Rat				Rat, hamster				
No observed adverse effect level (NOAEL) for tumors	200 ppm				not determined				
Lowest positive exposure concentration (LOEL)	600 ppm (rat) <sup>b</sup>				750 ppm (rat)				
Response at LOEL <sup>c,d</sup>	9% (11/119)				24% (24/98)				
Types of rat nasal tumors <sup>3</sup>	<u>0</u>	<u>50</u>	<u>200</u>	<u>600</u>	<u>0</u>	<u>750</u>	<u>1500</u>	<u>3000/1000</u>	
	<i>n</i> =	119	120	119	118	99	100	106	102
Adenocarcinoma	-	-	-	-	-	22	57	42	
Papilloma	-	-	1	4	-	1	-	-	
Squamous cell carcinoma	-	-	-	6	1	1	15	32	
Carcinoma <i>in situ</i>	-	-	-	1	-	-	3	6	
Time of earliest tumor	24 Months (Week 103; benign exophytic papilloma in a 200 ppm male). All other tumors observed at terminal sacrifice (weeks 106-107)				≤ 12 Months (malignant adenocarcinoma in a 750 ppm male). Squamous cell carcinomas and carcinoma <i>in situ</i> were also observed at ≤ 12 months of exposure.				

<sup>a</sup>Data from Woutersen *et al.*, 1986

<sup>b</sup>One benign exophytic papilloma was observed in a 200 ppm male

<sup>c</sup>Males and females combined after two years of exposure

<sup>d</sup>Benign and malignant tumors combined

### III. Mode of Action and Determinants of Response

This section provides additional data that provide a basis on which to propose a mode of action.

#### Cytotoxic Activity

The proposed mode of action was investigated in a series of *in vitro* experiments (Kuykendall *et al.*, 1993). The first hypothesis tested is that vinyl acetate-induced cytotoxicity in nasal tissues is a carboxylesterase-dependent process. The second hypothesis is that either acetic acid, acetaldehyde, or both are the primary cytotoxic metabolites. To test these hypotheses, an *in vitro* assay for nasal tissue cytotoxicity was utilized. This assay has been useful in previous studies aimed at elucidating the mechanism of toxic action of dibasic esters (Trela and Bogdanffy, 1991a,b). The assay is based on measurement of cytotoxicant-induced release into the incubation medium of the intracellular enzyme acid phosphatase. Previous research has shown a tight correlation between acid phosphatase and early ultrastructural change in sustentacular cells (Trela *et al.*, 1992).

To study the role of carboxylesterase in the cytotoxic effects of vinyl acetate, rats were pretreated with a nonspecific esterase inhibitor, bis(p-nitrophenyl) phosphate (BNPP) (Heymann and Krisch, 1967). BNPP pretreatment for three days prior to tissue collection had no cytotoxic effect on maxilloturbinate (lined with respiratory epithelium) or endoturbinate-1 (lined with olfactory epithelium) tissues (Table 7). Vinyl acetate (50 nM) induced an approximately 3- to 4-fold increase in acid phosphatase release from both turbinate types. Pretreatment with BNPP attenuated the vinyl acetate-induced cytotoxic response. Following BNPP pretreatment, 50 nM vinyl acetate induced only an approximate 2-fold increase in acid phosphatase release. Vinyl acetate treatments caused a reduction in media pH as made obvious by the visible, time- and concentration-dependent, change in the color of the media pH indicator.

Because BNPP pretreatment attenuated the cytotoxic response in both turbinate types, it was of interest to determine if BNPP pretreatment also inhibited metabolism of vinyl acetate. Administration of BNPP inhibited the release of acetaldehyde into the media approximately 59% or 37% in maxilloturbinate and endoturbinate-1 tissues, respectively (Table 8). The hydrolysis of vinyl acetate produces acetaldehyde and acetic acid. To assess the role of acetaldehyde in the cytotoxic effects of vinyl acetate, turbinates were incubated for 1 hr in media alone or in media containing semicarbazide, an aldehyde scavenger. Semicarbazide alone

was slightly, but not significantly, cytotoxic to both turbinate types (Table 9). Inclusion of semicarbazide in the incubation media offered no protection from vinyl acetate-induced cytotoxicity to either maxilloturbinate or endoturbinate-1 tissues.

**Table 7**  
**Effect of BNPP Pretreatment on Vinyl Acetate-Induced Cytotoxicity:**  
**Release of Acid Phosphatase into Media<sup>a</sup>**

<b>Pretreatment: In vitro Treatment:</b>	<b>Saline Control</b>	<b>Saline Vinyl Acetate</b>	<b>BNPP Control</b>	<b>BNPP Vinyl Acetate</b>
Maxilloturbinate	7.4 ± 0.4 <sup>b</sup>	27.6 ± 3.5 <sup>c</sup>	8.0 ± 0.9	17.2 ± 1.3 <sup>d,e</sup>
Endoturbinate-1	6.8 ± 0.7	21.3 ± 1.9 <sup>c</sup>	6.3 ± 1.5	15.8 ± 1.7 <sup>d,e</sup>

<sup>a</sup> Rats were pretreated with saline, a 5% (maxilloturbinate), or a 10% (endoturbinate-1) suspension of BNPP in saline (refer to Kuykendall *et al.*, 1993). Nasal explants were incubated for 1 hr in media containing 50 mM vinyl acetate and media was assayed for acid phosphatase activity.

<sup>b</sup> Values are expressed as mean percentage of acid phosphatase release (n=4) ± S.E.M.

<sup>c</sup> Statistically different from saline/control (p ≤ 0.05).

<sup>d</sup> Statistically different from BNPP/control (p ≤ 0.05).

<sup>e</sup> Statistically different from saline/vinyl acetate treatment (p ≤ 0.05).

**Table 8**  
**Effect of BNPP Pretreatment on Vinyl Acetate Metabolism:**  
**Acetaldehyde Release into Media<sup>a</sup>**

<b>Pretreatment:</b>	<b>Saline</b>	<b>BNPP</b>
Maxilloturbinate	25.7 ± 4.2 <sup>b</sup>	10.6 ± 1.6 <sup>c</sup>
Endoturbinate-1	34.9 ± 3.0	22.1 ± 0.8 <sup>c</sup>

<sup>a</sup> Rats were pretreated with saline, a 5% (maxilloturbinate), or a 10% (endoturbinate-1) suspension of BNPP in saline (refer to Kuykendall *et al.*, 1993). Nasal explants were incubated for 20 min in WME containing 50 mM vinyl acetate and media was assayed for acetaldehyde.

<sup>b</sup> Values are expressed as mM acetaldehyde; mean ± S.E.M. (n=4).

<sup>c</sup> Statistically different from saline control (p < 0.05).

**Table 9**  
**Effect of Semicarbazide on Vinyl Acetate-Induced Cytotoxicity:**  
**Release of Acid Phosphatase into Media<sup>a</sup>**

<i>In vitro</i> Treatment:	Control	Vinyl Acetate	Semicarbazide	Semicarbazide ± Vinyl Acetate
Maxilloturbinate	5.8 ± 1.1 <sup>b</sup>	17.9 ± 2.8 <sup>c</sup>	9.6 ± 1.2	19.0 ± 2.2
Endoturbinate-1	6.1 ± 1.7	18.3 ± 3.5 <sup>c</sup>	9.9 ± 2.6	29.3 ± 9.0 <sup>d</sup>

<sup>a</sup>Turbinates were isolated from untreated rats (refer to Kurykendall *et al.*, 1993). Nasal explants were incubated for 1 hr in media containing 50 mM vinyl acetate and media was assayed for acid phosphatase activity.

<sup>b</sup> Values are expressed as mean percentage of acid phosphatase release (n=4) ± S.E.M.

<sup>c</sup> Statistically different from control (p ≤ 0.05).

<sup>d</sup> Statistically different from semicarbazide control (p ≤ 0.05).

To the contrary, a slight but not statistically significant increase in acid phosphatase release was noted in endoturbinate-1 tissues incubated with vinyl acetate and semicarbazide relative to those incubated with vinyl acetate alone. To test the cytotoxic potential of acetaldehyde and acetic acid in nasal turbinates, maxilloturbinate and endoturbinate-1 tissues were incubated for 1 hour in media with or without 50 mM acetaldehyde (Table 10) or acetic acid (Table 11). Acetic acid, but not acetaldehyde, was cytotoxic at this concentration. These studies demonstrate that vinyl acetate is cytotoxic to nasal turbinates, that the carboxylesterase-mediated metabolism of vinyl acetate is necessary for cytotoxicity, and that acetic acid, not acetaldehyde is the principal cytotoxic metabolite.

### Genotoxic Activity

*In vivo* mutagenesis studies with vinyl acetate have been, in general, negative especially when tested at nonlethal levels by the inhalation route (Table 12). Induction of erythrocyte micronuclei has been demonstrated only when tested by the oral and i.p. routes at lethal levels. *In vitro* mutagenesis assays with prokaryotes are also generally negative. However, vinyl acetate has been reported to be mutagenic or clastogenic with or without an exogenous source of enzymatic metabolism in a number of *in vitro* assays employing cultured human lymphocytes, mouse L5178Y lymphoma cells, or Chinese hamster ovary cells (Jantunen *et al.*, 1986; He and Lambert, 1985; Kirby, 1983; Mäki-Paakkanen and Norppa, 1987). These studies suggested a clastogenic effect possibly similar to that induced by acetaldehyde. Alkaline elution studies with human leukocytes show some DNA-crosslinking activity (He and Lambert, 1985). Oral administration of [vinyl-U-<sup>14</sup>C] vinyl acetate showed



the association of radioactivity with hepatic nucleic acid and nuclear proteins but no specific adducts could be identified (Simon *et al.*, 1985b).

### ***Role of Reduced Intracellular pH***

The clastogenic effects observed in *in vitro* studies with mammalian cells could be, at least in part, the result of reduced intracellular pH that results from the liberation of acetic acid from vinyl acetate. Morita (1995) has shown that low pH (pH 6.6) leads to chromosomal aberrations and sister chromatid exchanges in Chinese hamster ovary cells and that these effects are S- phase dependent. Thus, cells in a highly replicating population might be extra sensitive to low pH-induced clastogenesis. Neutralization of the media abolished the clastogenic activity (Morita *et al.*, 1990). These observations supported the work of Sipi *et al.* (1992) who showed that addition of organic acid metabolites of a variety of vinyl esters to the culture media reduced the pH of the media 0.5-1.0 units and facilitated induction of sister chromatid exchanges in whole blood human lymphocytes. However, Sipi *et al.* (1992) who studied vinyl acetate specifically also noted that vinyl acetate-induced sister chromatid exchange results could not be explained solely by the acetic acid-induced reduction in media pH. Since the studies by Morita, Sipi, and colleagues relied on measures of extracellular (media) pH, it can be inferred that the effect on clastogenic activity from reductions in intracellular pH would likely be at least as pronounced, if not more pronounced. It is readily envisaged that vinyl acetate could cross the plasma membrane and be hydrolyzed intracellularly leading to the trapping and intracellular build up of acetic acid. Acetic acid would be extensively ionized under physiological conditions.

**Table 10**  
**Effect of Acetaldehyde on Nasal Explant Cytotoxicity:**  
**Release of Acid Phosphatase into Media<sup>a</sup>**

<b><i>In vitro</i> Treatment:</b>	<b>Control</b>	<b>Acetaldehyde</b>
Maxilloturbinate	8.6 ± 2.1 <sup>b</sup>	8.3 ± 0.7
Endoturbinate-1	9.1 ± 1.7	8.7 ± 1.4

<sup>a</sup> Turbinates were isolated from untreated rats (refer to Kuykendall *et al.*, 1993). Nasal explants were incubated for 1 hour in media containing 50 mM acetaldehyde and media was assayed for acid phosphatase activity.

<sup>b</sup> Values are expressed as mean percentage of acid phosphatase release (n=4) + S.E.M.

**Table 11**  
**Cytotoxic Effects of Acetic Acid on Nasal Explant**  
**Release of Acid Phosphatase into Media<sup>a</sup>**

<b><i>In vitro</i> Treatment:</b>	<b>Control</b>	<b>Acetic Acid</b>
Maxilloturbinate	6.1 ± 0.7 <sup>b</sup>	24.0 ± 1.3
Endoturbinate-1	7.4 ± 1.1	25.9 ± 1.8

<sup>a</sup> Turbinates were isolated from untreated rats (refer to Kuykendall *et al.*, 1993). Nasal explants were incubated for 1 hour in media containing 50 mM acetaldehyde and media was assayed for acid phosphatase activity.

<sup>b</sup> Values are expressed as mean percentage of acid phosphatase release (n=4) + S.E.M.

<sup>c</sup> Statistically different from control (p< 0.05).

Table 12  
Summary of the Genotoxicity Data on Vinyl Acetate\*

Test System	Result <sup>a</sup>		Dose <sup>b</sup> (LED/HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SOS Chromotest, E. Coli, PQ37	-	-	8.6 mg/ml	Brams <i>et al.</i> (1980)
DNA-protein crosslink, E. coli HB 101pUC13, filter binding	-	+	1-100 mM	Kuykendall & Bogdanffy (1992b)
<i>S. typhimurium</i> TA100, reverse mutation	-	-	1,000 µg/pl	Linjinsky & Andrews (1980)
<i>S. typhimurium</i> TA100, reverse mutation	-	-	10,000 µ/pl	McCann <i>et al.</i> (1975)
<i>S. typhimurium</i> TA100, reverse mutation	-	-	500 µ/pl	Brams <i>et al.</i> (1987)
<i>S. typhimurium</i> TA100, reverse mutation	-	-	30 µmol/pl	Florin <i>et al.</i> (1980)
<i>S. typhimurium</i> TA100, reverse mutation	-	-	> 4 x 10 <sup>3</sup> M/pl	Bartsch <i>et al.</i> (1979)
<i>S. typhimurium</i> TA100, reverse mutation	-	-	Vapour	Bartsch <i>et al.</i> (1979)
<i>S. typhimurium</i> TA1535, reverse mutation	-	-	1000 µg/pl	Linjinsky & Andrews (1980)
<i>S. typhimurium</i> TA1535, reverse mutation	-	-	10,000 µg/pl	McCann <i>et al.</i> (1975)
<i>S. typhimurium</i> TA1535, reverse mutation	-	-	30 µmol/pl	Florin <i>et al.</i> (1980)
<i>S. typhimurium</i> TA1537, reverse mutation	-	-	1000 µg/pl	Linjinsky & Andrews (1980)
<i>S. typhimurium</i> TA1537, reverse mutation	-	-	10,000 µg/pl	McCann <i>et al.</i> (1975)
<i>S. typhimurium</i> TA1537, reverse mutation	-	-	30 µmol/pl	Florin <i>et al.</i> (1980)
<i>S. typhimurium</i> TA1538, reverse mutation	-	-	1000 µg/pl	Linjinsky & Andrews (1980)
<i>S. typhimurium</i> TA1530, reverse mutation	-	-	> 4 x 10 <sup>3</sup> M/pl	Bartsch <i>et al.</i> (1979)
<i>S. typhimurium</i> TA1530, reverse mutation	-	-	Vapour	Bartsch <i>et al.</i> (1979)
<i>S. typhimurium</i> TA98, reverse mutation	-	-	1000 µg/pl	Linjinsky & Andrews (1980)
<i>S. typhimurium</i> TA98, reverse mutation	-	-	10,000 µg/pl	McCann <i>et al.</i> (1975)
<i>S. typhimurium</i> TA98, reverse mutation	-	-	500 µg/pl	Brams <i>et al.</i> (1987)
<i>S. typhimurium</i> TA98, reverse mutation	-	-	30 µmol/pl	Florin <i>et al.</i> (1980)
DNA-protein crosslink, nasal epithelial cells, <i>in vitro</i>	+	0	5 mM	Kuykendall <i>et al.</i> (1993)
DNA crosslink, alkaline elution, purified human lymphocytes, <i>in vitro</i>	+	0	10 mM	Lambert <i>et al.</i> (1985)
Cell transformation SA7/Syrian hamster embryo cells	+	0	500 µg/ml	Castro (1981)
Gene mutations, mouse lymphoma L5178Y cells	+	0		Kirby (1983)
Sister chromatid exchange, Chinese hamster ovary cells, <i>in vitro</i>	+	+	0.125 mM	Norppa <i>et al.</i> (1985)
Sister chromatid exchange, human lymphocytes, <i>in vitro</i>	+	0	0.05 mM	Norppa <i>et al.</i> (1985)
Sister chromatid exchange, human isolated lymphocytes <i>in vitro</i>	+	0	0.1 mM	He and Lambert (1985)
Sister chromatid exchange, human lymphocytes <i>in vitro</i>	+	0	0.25 mM	Sipi <i>et al.</i> (1992)
Micronucleus test, human lymphocytes, <i>in vitro</i>	+	0	0.5 mM	Maki-Paakkanen & Norppa (1987)
Chromosomal aberrations, human lymphocytes, <i>in vitro</i>	+	0	0.2 mM	Norppa <i>et al.</i> (1985)
Chromosomal aberrations, human lymphocytes, <i>in vitro</i>	+	0	0.25 mM	Jantunen <i>et al.</i> (1986)
Sister chromatid exchange, mice cells, <i>in vivo</i>	+		370-470 mg/kg i.p. injection	Takeshita <i>et al.</i> (1986)
Micronucleus test, mouse bone marrow, <i>in vivo</i>	+		1000 mg/kg i.p. injection	Maki-Paakkanen & Norppa (1987)
Micronucleus test, mouse bone marrow, <i>in vivo</i>	-		1000 ppm	Owen, 1980b
Micronucleus test, rat bone marrow, <i>in vivo</i>	-		1000 ppm	Owen, 1980a
Meiotic micronucleus test, mice, <i>in vivo</i>	-		1000 mg/kg i.p. injection	Lahdetie (1988)
DNA binding, rat hepatocytes, <i>in vivo</i> ( <sup>14</sup> C-label)	-		1mCi/ml oral	Simon <i>et al.</i> (1985b)
DNA binding, rat hepatocytes, <i>in vivo</i> ( <sup>14</sup> C-label)	-		1200-1800 ppm inhalation	Simon <i>et al.</i> (1985b)
Sperm morphology, F1 mice, <i>in vivo</i>	+		500 mg/kg i.p. injection	Lahdetie (1988)

\* Adapted from IARC No. 11. (1995)

<sup>a</sup> +, considered to be positive; (+), considered to be weakly positive in an inadequate study; -, considered to be negative; 0, not tested

<sup>b</sup> LED, lowest effective dose; HID, highest effective dose. *In vitro* tests, µ/ml; *in vivo* tests, mg/kg bw.

The potential role for acetaldehyde in the mechanism of vinyl acetate carcinogenesis is summarized in Table 13 and discussed in the following section. Like vinyl acetate,

acetaldehyde is not mutagenic in the Ames test (Rosenkranz, 1978; Sasaki and Endo, 1978), but shows activity in a DNA-repair test in *E.coli* (Rosenkranz, 1978), is mutagenic in the mouse lymphoma test (Wangenheim and Bolcsfoldi, 1988) and induces chromosomal aberrations and sister chromatid exchanges in mammalian cells (Bird *et al.*, 1982; He and Lambert, 1985; Obe and Ristow, 1977). Unlike vinyl acetate, acetaldehyde has been reported to induce gene mutations at the HPRT locus in human lymphocytes (He and Lambert, 1990). Acetaldehyde was weakly mutagenic in this system as concentrations ranging from 200  $\mu\text{M}$  to 2400  $\mu\text{M}$  were necessary to produce a positive response. Sequencing of the HPRT gene in the mutant clones suggested that the majority of mutations were partial deletions or rearrangements. These results are consistent with the conclusion that acetaldehyde is clastogenic and that DNA-protein crosslinks (DPXL) are the putative genotoxic lesion. The possibility that acetaldehyde is the active genotoxic metabolite of vinyl acetate is supported by the studies of He and Lambert (1985), which showed a striking similarity in the time- and concentration-dependent effects of the SCE-frequency with the two substances. The authors assumed that ester hydrolysis of vinyl acetate occurs within the cell, because the addition of purified carboxylesterases to the extracellular environment had no effect on the SCE-frequency caused by vinyl acetate *in vitro*.

### **Role of Acetaldehyde**

To further study the possibility that the genotoxic activity of vinyl acetate can be ascribed to the release of acetaldehyde following carboxylesterase-mediated activation, Kuykendall *et al.* (1993) evaluated the effect of carboxylesterase inhibition by BNPP on the induction of DPXL in rat nasal epithelial cells. Preincubation of cells with 0.1, 0.5, and 1 mM BNPP inhibited vinyl acetate-induced crosslink formation in a dose-dependent manner in epithelial cells from both respiratory and olfactory tissues. There was a 76% and 78% reduction of 25 mM vinyl acetate-induced crosslink formation by 1 mM BNPP in treated respiratory and olfactory cells, respectively, confirming the dependence of crosslink formation on carboxylesterase-mediated hydrolysis.

**Table 13**  
**Summary of Factors to Consider in Evaluating the**  
**Role of Acetaldehyde in Vinyl Acetate Carcinogenesis**

- + Role of acetaldehyde in the mechanism of vinyl acetate clastogenic action is supported by a similar clastogenic pattern of acetaldehyde.
- + Acetaldehyde and vinyl acetate have similar time- and concentration-dependent effects on SCE frequency.
- + Work of Kuykendall *et al.* (1993) shows that DNA protein crosslinks (DPXL) in nasal cells are inhibited by BNPP pretreatment suggesting that vinyl acetate-induced DPXLs are related to intracellular formation of acetaldehyde.
- In vitro experiments by Kuykendall *et al.* show that acetaldehyde-DPXL are unstable ( $t_{1/2} = 6.5$  hours)
- Lam and Heck data show significant increase in acetaldehyde-DPXL at 1000 ppm (no significant DPXL at 100 or 303 ppm)
- Rigorous search for adducts by Hemminki and Suni (1984) show only reversible Schiff's base reactions

*Conclusion:* Role of metabolically-liberated acetaldehyde becomes significant only at high vinyl acetate exposure concentrations where DPXLs contribute to cytotoxicity, and possibly some mutagenicity that is not clearly supported by data but only under conditions of induced cell proliferation.

Lam *et al.* (1986) studied the effects of inhalation exposure of rats to acetaldehyde on the induction of DPXL. In this study, there was no significant increase in the amount of DNA crosslinked to protein at  $\leq 303$  ppm acetaldehyde in either respiratory or olfactory mucosa following a single 6 hr exposure or 5 days of exposure. DPXL levels were significant in respiratory mucosa after a single 6 hour exposure to 1000 ppm acetaldehyde. The DPXL levels in respiratory mucosa were similar after 5 days of exposure. DPXL levels were significant in olfactory mucosa only after 5 days of repeated exposure to 1000 ppm. Thus, the respiratory epithelium appears to be considerably more resistant to the cytotoxic and other effects of DPXL than the olfactory mucosa (Lam *et al.* (1986), Appelman *et al.*, 1982; Woutersen *et al.*, 1984). The comparison further suggests that cytotoxicity, rather than DPXL levels, may be the primary determinant in the mechanism of action of acetaldehyde with some secondary contribution to genetic damage (in particular, clastogenicity).

The efficiency of formation of the acetaldehyde-DNA crosslink, and its stability was compared

to that of formaldehyde. Using an *in vitro* system composed of plasmid DNA and calf thymus histone as a model, formaldehyde-induced DPXL were formed with an efficiency 14 times greater than that of acetaldehyde (Kuykendall and Bogdanffy, 1992a). Furthermore, the acetaldehyde crosslinks were unstable under physiological conditions decaying with a half-life of approximately 6.5 hours (Kuykendall and Bogdanffy, 1992b). That is, approximately  $5 \times 10^{-4}$  of the original amount of crosslinks formed would be present at the time of replication. Interestingly, low pH facilitated the acetaldehyde-induced crosslinks which is probably a consequence of a tighter association of the histone proteins with DNA (Kuykendall and Bogdanffy, 1992b). Free amino groups on histones would be more extensively ionized at low pH facilitating their association with negatively charged phosphate groups on DNA. These observations support those of Morita (1995) discussed above which show increased clastogenic activity in Chinese hamster ovary cells at low pH.

In general, the results of functional assays for mutagenic activity of acetaldehyde are not consistent with the induction of point mutation, which might be expected from some type of DNA adduct of acetaldehyde, but suggest clastogenicity related to DNA-DNA and DPXL (Dellarco, 1988). A recent report showed the induction of 6-thioguanine resistance in normal human fibroblasts following 5 hr cultures in the presence of 5 mM acetaldehyde (Grafstrom *et al.*, 1994). However, neither 1, 2.5, 8, nor 10 mM acetaldehyde produced this response under the same conditions. There are only two reports in the literature investigating the potential for acetaldehyde to form DNA adducts *in vitro*. The first work was conducted by Hemminki and Suni (1984) in which nucleosides were incubated for 20 hours in pure acetaldehyde. Acetaldehyde was found to bind and form an unstable, reversible reaction product, principally with guanosine, which is believed to be a Schiff base condensation product on the N<sup>2</sup> exocyclic amino group. This product was stabilized by addition of sodium borohydride and the reduction product was identified as N<sup>2</sup>-ethylguanosine. These results have been confirmed by Vaca *et al.* (1995) who demonstrated the low rate of reactivity and stability of the acetaldehyde adducts and that only the reduced form of the adduct is somewhat stable under reducing conditions. More recently, Fang and Vaca (1997) presented evidence of low levels (approximately 2-3 adducts per 10<sup>7</sup> nucleotides) of N<sup>2</sup>-ethyl-3' -deoxyguanosine monophosphate in peripheral blood cells (granulocytes and lymphocytes) of alcohol-intoxicated humans.

Formation of Schiff base intermediates with primary amines is a well characterized reaction of aldehydes and is believed to be the first step in DPXL formation (Feldman 1979; Ohba *et al.*, 1979). *In vitro* studies have shown that the first reaction product of acetaldehyde, in the

sequence of steps leading to DPXL formation, is not with DNA but with amino acid residues, principally  $\epsilon$ -amino groups of lysine, which is then followed by condensation with free amino groups of DNA principally guanine (Kuykendall and Bogdanffy, 1992b, 1994).

These data suggest that the role of acetaldehyde in vinyl acetate carcinogenesis becomes significant only at high concentrations where DPXLs contribute to cytotoxicity and possibly a clastogenic effect, and only under conditions of induced cell proliferation.

### ***Role of Epoxide Intermediates***

Theoretically, oxidation of vinyl acetate by mono-oxygenases could lead to acetoxy oxirane, the epoxide of vinyl acetate, a substance which is mutagenic in the Ames test without metabolic activation (Simon *et al.*, 1986). However, several lines of evidence lead to the conclusion that an epoxide is not formed in appreciable quantities to have a role in vinyl acetate carcinogenesis. First, unlike acetoxy oxirane, vinyl acetate is not genotoxic in the Ames test. Second, studies by Norppa *et al.* (1985), Laib and Bolt (1986) Simon *et al.* (1986) and Fedtke and Wiegand (1990) support the idea that vinyl acetate is rapidly split by esterases and is therefore not readily available for epoxidation. Moreover, the half-life of acetoxy oxirane in phosphate buffer (pH 7.8, at 37°C) is only 2.8 min and its mutagenicity is abolished completely by S9 mix (Simon *et al.*, 1986). Third, vinyl acetate did not induce hepatic foci of cellular alteration (ATPase, GGTase) when administered intraperitoneally to neonatal rats either with or without phenobarbital promotion (Laib, and Bolt, 1986). Other vinyl compounds which undergo oxidation at the  $\pi$  bond to form a reactive epoxide metabolite, such as vinyl chloride and vinyl carbamate, are positive in this test.

### **Cell Proliferation Effects**

The effects of vinyl acetate exposure on nasal epithelial cell proliferation were evaluated in rats exposed for 1, 5, or 20 days to 0, 50, 200, 600, or 1000 ppm (Bogdanffy *et al.*, 1997a). Exposure to vinyl acetate produced lesions in the olfactory epithelium of rats exposed to 600 or 1000 ppm (Table 14). The severity of olfactory epithelial lesions was concentration-related and decreased along an anterior-posterior gradient. The severity of the lesions increased with extended durations of exposure. Following one exposure, lesions were characterized by degeneration, necrosis, and exfoliation of olfactory epithelial cells. Areas of the olfactory mucosa most severely effected were the dorsal one-third of the nasal septum and dorsolateral wall (i.e. areas just distal to the dorsal arch), Masera's organ and the medial most extent of the ethmoid turbinates.

Olfactory mucosal lesions following 5 or 20 exposures were primarily those of post-necrotic repair and adaptation. Lesions were characterized by regenerative hyperplasia of olfactory epithelium along with attenuation and/or disorganization of the olfactory mucosa. Occasional areas of squamous metaplasia were also present. Degeneration and atrophy of olfactory nervebundles in the olfactory lamina propria were discernible following 20 exposures.

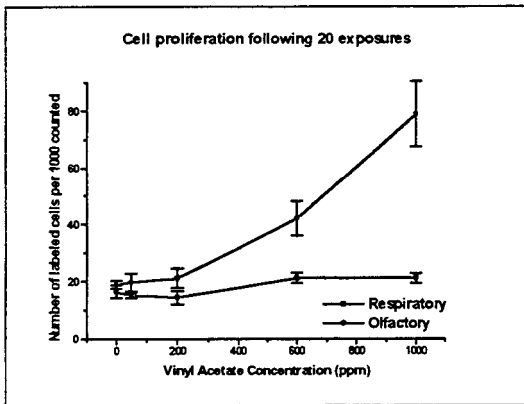
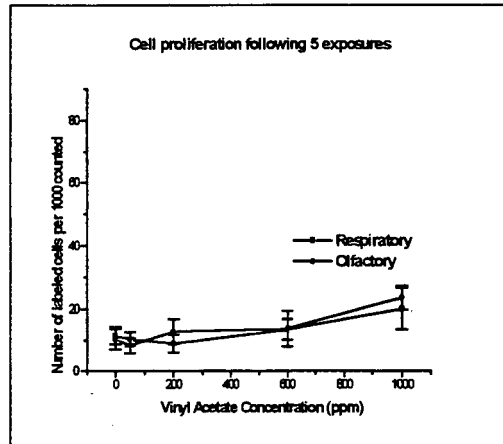
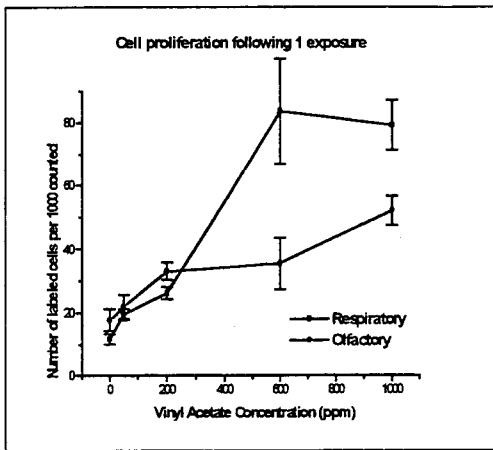
Cell proliferation responses were observed in both respiratory and olfactory epithelia at 1 day of exposure (Figure 3). The responses were statistically significant in the 600 and 1000 ppm groups at these times. Following 5 days of exposure, the proliferation responses subsided with only slight increases noted at the 1000 ppm level. Following 20 days of exposure, the respiratory epithelium appeared to have reached a point of adaptation to exposure while the olfactory epithelium rebounded with a second wave of proliferation. This unusual time course illustrates the distinctiveness of the responses of respiratory and olfactory epithelia and highlights the concept that these tissues are separate organs. Transient cell proliferation responses have also been observed in respiratory epithelium of rats exposed to 6 ppm formaldehyde for up to six weeks (Monticello and Morgan 1994).



**Table 14**  
**Histopathological Observations of Rats Exposed to Vinyl Acetate**  
**for up to Four Weeks<sup>a</sup>**

	Exposure Concentration				
1 Exposure	0	50	200	600	1000
Olfactory Epithelial Degeneration	0	0	0	5	5
Respiratory Epithelial Degeneration	0	0	0	0	1
5 Exposures					
Olfactory Epithelial Degeneration	0	0	0	3	5
Olfactory Epithelial Regenerative Hyperplasia	0	0	0	5	5
Respiratory Epithelial Regenerative Hyperplasia	0	0	0	0	2
20 Exposures					
Olfactory Epithelial Degeneration	0	0	0	5	5
Olfactory Epithelial Regenerative Hyperplasia	0	0	0	5	5
Respiratory Epithelial Regenerative Hyperplasia	0	0	0	0	1
Squamous metaplasia	0	0	0	0	1

<sup>a</sup> Five male rats were exposed nose-only for periods of 6 hrs per day, 5 days per week



**Figure 3.** Cell proliferation responses in respiratory and olfactory epithelium following 1, 5, or 20 exposures to vinyl acetate. Respiratory regions included the medial and lateral aspects of the nasoturbinate at level II (Young, 1981). Olfactory regions included the dorsal meatus and posterior portion of the nasal septum at level IV.

The cell proliferation responses could be interpreted as a two phase reaction to exposure. The first is characterized as chemical insult followed by early regenerative repair (exposure days 1-5). Early proliferative responses of respiratory and olfactory epithelium have been noted for nasal irritants such as formaldehyde and acrylic acid (Swenberg *et al.*, 1986). With regard to effects on respiratory epithelium, only one case of minimal degeneration in five rats exposed was noted at 1000 ppm following 1 six hour exposure, and two cases of regenerative hyperplasia were observed at 1000 ppm following 5 days of exposure. Complete recovery of the respiratory epithelium was observed following 20 days of exposure. The minimal response and recovery in respiratory epithelium is consistent with the lack of non-neoplastic effects in respiratory epithelium following 2 years of exposure. The lack of histopathology and cell proliferation responses following 5 or 20 exposures suggests that the second phase of reaction to exposure includes biochemical adaptation. In olfactory epithelium, the rebound response

observed after 20 exposures coupled with expressions of active degeneration suggest adaptive response apparent in respiratory epithelium is either not possible or not extensive enough in olfactory epithelium.

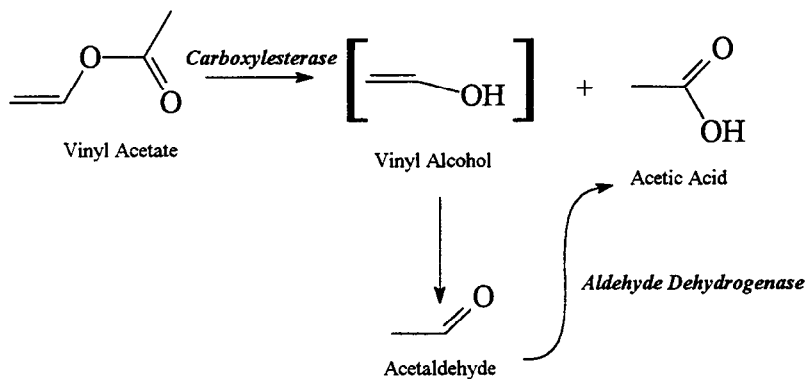
The data from these experiments are instructive in several ways. First, the histopathology demonstrates a strong anterior-to-posterior gradient. Such a gradient would be expected for water soluble or reactive (i.e., metabolized) materials. Second, the gradient of response moves anterior-to-posterior with increasing concentration. Such a gradient would be expected for materials in which deposition is metabolically dependent. As vinyl acetate concentration increases, fractional deposition decreases (i.e. greater distal penetration in the airway) due, in part, to saturation of the metabolism-dependent component of deposition (Morris, 1995). Finally, the data suggest that strong proliferative effects in the nasal cavity are observed early in the sequelae of toxic responses to vinyl acetate exposure. These responses are likely to have influence on the subsequent generations of epithelial cells that populate the nasal cavity during the lifespan of the animal, and support a mode of action that is strongly dependent on induced cellular proliferation.

#### **IV. Determinants of Uptake and Tissue Dose**

One of the key determinants of uptake for vinyl acetate is carboxylesterase metabolism located in the olfactory epithelium of the URT.. This metabolism accounts for the proximal to distal scrubbing of the chemical from the inspired airstream as well as the distribution pattern of lesions observed in respiratory tract. A PB-PK model of vinyl acetate uptake and metabolism in rats and humans has been developed using data derived from *in vitro* (rats and humans) and *in vivo* (rats) kinetic analyses of deposition, uptake, and metabolism.

##### ***Carboxylesterase***

The presumed mechanism of vinyl acetate nasal toxicity includes a significant role for the carboxylesterase-mediated metabolism to acetic acid. The metabolism of vinyl acetate is presented in Figure 4:



**Figure 4** Established metabolic pathways for vinyl acetate.

Nasal mucosa of several species possesses active xenobiotic metabolizing systems that include oxidative cytochrome P-450 systems, reductases, alcohol and aldehyde dehydrogenases, phase II hydrolytic and conjugation enzymes, flavin monooxygenases, and carboxylesterases (Dahl and Hadley, 1991). Several of these enzymes have been localized histochemically and are found in discrete cell types within respiratory and olfactory mucosae (Bogdanffy, 1990).

Simon *et al.* (1985a) showed that vinyl acetate is hydrolyzed in preparations from rat and human plasma, and rat liver and lung. The rate of hydrolysis of vinyl acetate has also been measured in homogenates of nasal respiratory and olfactory mucosa (Bogdanffy and Taylor, 1993). Rat nasal carboxylesterase catalyzes the hydrolysis of vinyl acetate with great efficiency.  $V_{\max}$  values for rat and mouse respiratory tissue ranged from 22 to 46 mmoles/min/mg. This is about the same as, or somewhat higher than that obtained from rat liver microsomes (23 mmoles/min/mg), and significantly greater than rat lung microsomes (6.2 mmoles/min/mg) and rat and human plasma (0.56, and 0.69 mmoles/min/mg, respectively).  $V_{\max}$  values for rat and mouse olfactory tissue were considerably higher than other tissues and ranged from 95 to 254 mmoles/min/mg.

$K_m$  values obtained for rat and mouse nasal tissues ranged from 0.30 to 1.07 mM.  $K_m$  values obtained by Simon *et al.* (1985a) were about the same or higher than nasal tissue: rat liver microsomes, 0.73 mM; rat lung microsomes, 6.1 mM; rat plasma, 4.0 mM; and human plasma, 7.1 mM. Therefore, the increased capacity of rat nasal tissue to catalyze the metabolism of vinyl acetate, coupled with the lower  $K_m$  of nasal tissue relative to other tissues, yields a highly efficient system for the *in situ* production of acetic acid and acetaldehyde. The efficiency of hydrolysis of vinyl acetate in rat nasal tissue can be compared by considering the intrinsic metabolic clearance,  $V/K$ . In rat and mouse respiratory tissue,  $V/K$  ranged from 52 to 79

L/min/mg. In olfactory tissue, V/K ranged from 270 to 469 L/min/mg. Calculating this ratio from the data of Simon *et al.* (1985a) yields the following values: rat liver microsomes, 32 L/min/mg; rat lung microsomes, 1.02 L/min/mg; rat plasma, 0.14 L/min/mg; and human plasma, 0.10 L/min/mg. Since the liver data of Simon *et al.* (1985a) was collected using an isolated microsomal fraction while the data reported here was derived from whole homogenates, the differences between nose and liver carboxylesterase are likely to be even greater. Thus rat olfactory tissue is the most efficient tissue type catalyzing the hydrolysis of vinyl acetate.

The results of the kinetic studies with tissue homogenates do not offer an explanation for the species difference in sensitivity to the carcinogenic effects of vinyl acetate. Vinyl acetate was hydrolyzed with approximately equal efficiency in respiratory or olfactory tissue of both rats and mice. An explanation for the species difference in carcinogenic susceptibility must depend on other factors such as differential rates of DNA damage and repair, cell proliferation kinetics, and respiratory physiological factors influencing deposition of vinyl acetate vapor in upper airway tissues.

### *Aldehyde Dehydrogenase*

Nasal aldehyde dehydrogenase is present in specific cell types of both respiratory and olfactory mucosa although the activity is greater in respiratory mucosa (Bogdanffy *et al.*, 1986; Casanova-Schmitz *et al.*, 1984). Thus, acetaldehyde produced from vinyl acetate would also be expected to be oxidized to acetic acid provided the cell type containing carboxylesterase also contains aldehyde dehydrogenase. Casanova-Schmitz *et al.* (1984) studied the kinetics of aldehyde dehydrogenase in rat mucosal homogenates. The authors observed the presence of two isoforms in both the respiratory and olfactory mucosa, one of which may catalyze the oxidation of both formaldehyde and acetaldehyde. The higher  $K_m$  isozyme, responsible for the oxidation of acetaldehyde had a specific activity approximately five to eight times greater in homogenates of respiratory (128 nmoles/min/mg protein) than olfactory tissue (28 nmoles/min/mg protein).  $K_m$  values obtained for the two tissues were similar ( $20 \pm 3$ , and  $22 \pm 7$  mM for respiratory and olfactory, respectively). Therefore, the respiratory mucosa appears to be better equipped to detoxify acetaldehyde produced from the hydrolysis of vinyl acetate. This is an important observation because it suggests that as vinyl acetate concentration is reduced from high experimental levels to low ambient levels, and the deposition pattern moves progressively towards the anterior of the nasal cavity (consistent with the concentration-related gradient in nasal lesion formation noted above and the anticipated deposition pattern which will be discussed below), an increasing fraction of vinyl acetate is deposited in respiratory epithelium

where acetaldehyde metabolites are more readily detoxified.

### **Histochemical Distribution of Vinyl Acetate-Metabolizing Enzymes**

Nasal carboxylesterase and aldehyde dehydrogenase activities are critical enzymes in the proposed mechanism of metabolic activation and detoxication of vinyl acetate. A comparison of the cellular distributions of carboxylesterase and aldehyde dehydrogenase helps in understanding uptake and possibly tissue sensitivity. Carboxylesterase activity is histochemically detectable in all epithelial cells and seromucous glands of respiratory mucosa (Bogdanffy *et al.*, 1987). In olfactory mucosa, carboxylesterase is present in sustentacular cells, basal cells, and Bowman's glands. Aldehyde dehydrogenase is present in all epithelial cells of respiratory mucosa, but is present in only basal cells and Bowman's glands of the olfactory mucosa and only at minimally detectable levels (Bogdanffy *et al.*, 1986). This comparison suggests acetaldehyde metabolites produced in the surface epithelium will be converted to acetic acid to a greater extent in respiratory epithelium than olfactory epithelium. Further, the mode of action studies presented above showed that acetaldehyde was not cytotoxic, indicating little contribution of acetic acid derived from acetaldehyde oxidation to the overall mechanism of vinyl acetate-induced cytotoxicity.

Further support for this conclusion comes from a comparative analysis of the V/K ratios of carboxylesterase vs. aldehyde dehydrogenase derived from the respiratory tissue homogenate experiments. The former is in the range of 50 L/min/mg protein while the latter is approximately  $6 \times 10^{-6}$  L/min/mg protein. Thus, the contribution of carboxylesterase to the total amount of acetic acid generated intracellularly is significantly greater than that of aldehyde dehydrogenase oxidation of acetaldehyde.

Ultimately, the complete expression of toxicity and neoplasia will be critically dependent on the balance between several competing mechanisms in the different mucosae. The first is cytotoxicity induced by acetic acid from vinyl acetate hydrolysis. The second is metabolic incorporation and general detoxication of acetic acid. The third is detoxication of acetaldehyde. Acetaldehyde is detoxified through both aldehyde dehydrogenase oxidation and through binding to cellular macromolecules and thiols, such as glutathione. Glutathione has been shown histochemically to be present in all epithelial cells of respiratory and olfactory mucosae (Keller *et al.*, 1990).

With regard to the histochemical distribution of the various enzymes involved in the

mechanisms of activation and detoxication of vinyl acetate in human nasal tissue, Lewis *et al.* (1994) have studied the distribution of carboxylesterase activity immunohistochemically in respiratory mucosa. The cellular pattern of activity was similar to that of rat respiratory epithelium with diffuse reactivity noted in ciliated and secretory cells of the luminal respiratory epithelium. Histochemical staining in human olfactory tissues for carboxylesterase activity, or for aldehyde dehydrogenase in any human nasal tissue, has not been reported.

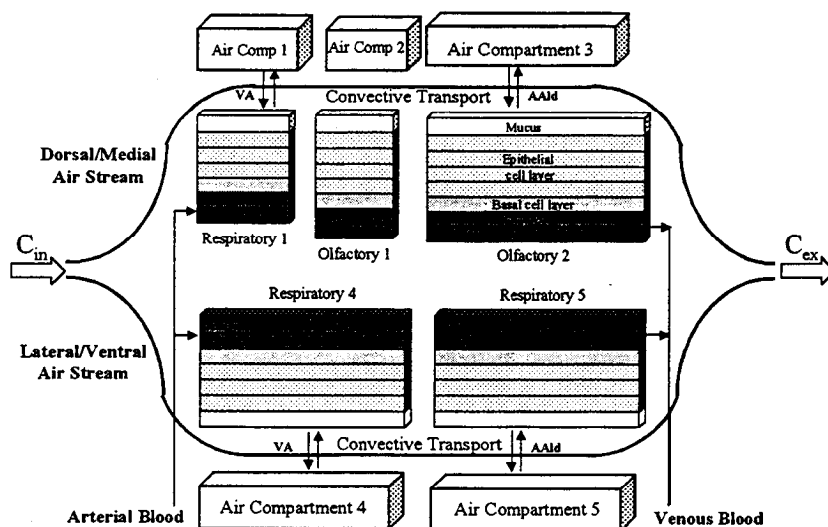
### **Physiologically-Based Modeling of Vinyl Acetate Uptake and Metabolism**

Inhaled chemicals can be extracted in the nasal cavity where they are then metabolized and absorbed into the systemic circulation. Inspired air follows distinct paths in the URT, resulting in asymmetric ventilation to various regions, and the nasal mucosa consists of a variety of cell types each having a different metabolic activity toward the inhaled chemical. Vinyl acetate exposure induces non-neoplastic lesions in the rat nasal cavity with degeneration of the olfactory epithelium as the critical response. *In vivo* experiments show that the severity of olfactory epithelial lesions decreases along an anterior-posterior gradient. Dividing the olfactory region into numerous compartments in the axial direction should allow a better approximation of the proximal to distal scrubbing of the chemical from the inspired air stream and thus account for the distribution of the lesions observed in the nasal cavity.

To accurately capture the flux to the sensitive regions of the nasal mucosa that are at a higher risk of tissue damage due to vinyl acetate, requires a high degree of compartmentalization of the URT. The model of Plowchalk *et al.* (1997) was extended by constructing a five-compartment model of the rat nasal cavity and a four-compartment model of the human nasal cavity. The airflow is split into the two demonstrated distinct pathways in the URT: lateral/ventral and dorsal/medial (Kimbell *et al.*, 1993). To better characterize the vinyl acetate flux to the apical regions of the olfactory tissue, the five-compartment model divides the olfactory region into two compartments; a small dorsal anterior compartment and a larger posterior compartment. The respiratory mucosa on the ventral side is also divided into an anterior and a posterior compartment, resulting in five tissue compartments, similar to the model structure proposed by Frederick *et al.* (Fig. 5). Since the human nasal cavity has only a small area covered with olfactory mucosa, one olfactory compartment on the dorsal side is used in the equivalent human PB-PK model. In addition to representing the nasal mucosa using more compartments the current model also incorporates air phase resistance to mass transfer from the lumen to the air:mucus interface. This is an improvement over the previous model by Plowchalk *et al.* (1997) that assumed equilibrium between the air and the mucus phase.

Nasal carboxylesterase and aldehyde dehydrogenases are critical enzymes in the mechanism of metabolic activation and detoxification of vinyl acetate. These enzymes are located in specific cell and tissue types within the nasal cavity. The extent to which vinyl acetate is extracted from the air stream and metabolized is dependent upon the compliment of enzyme activity in the various tissue sub-compartments. Each of the tissue compartments in the model are further subdivided into a number of subcompartments to represent the mucus layer and the various cell types of the tissue. The histochemical localization described earlier was used to distribute the enzymatic activities of carboxylesterase and aldehyde dehydrogenase within the compartments of each tissue stack.

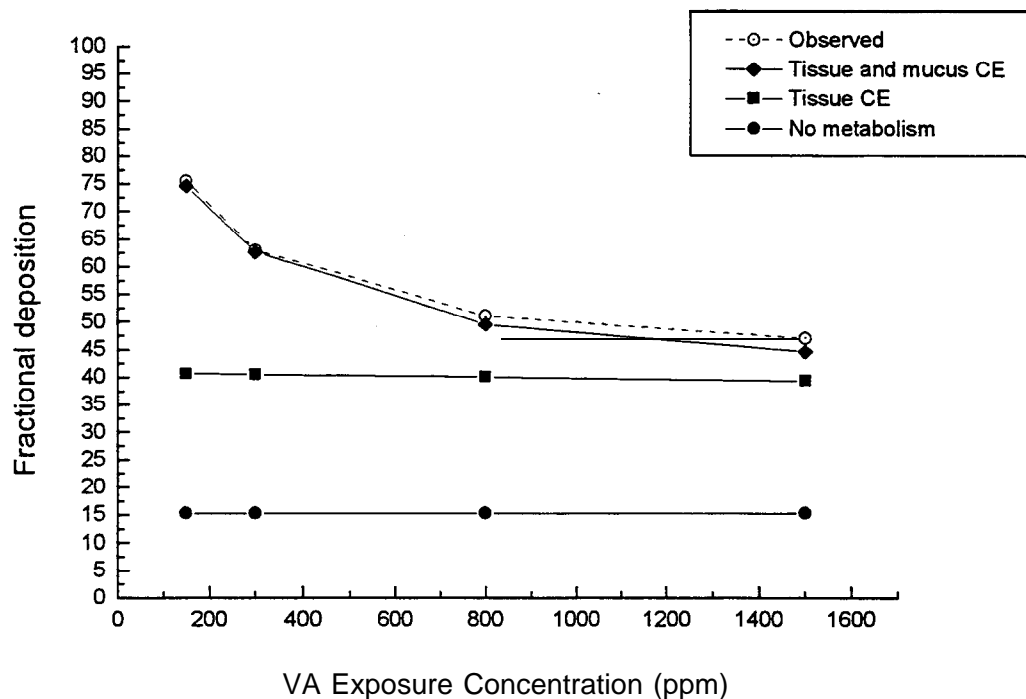




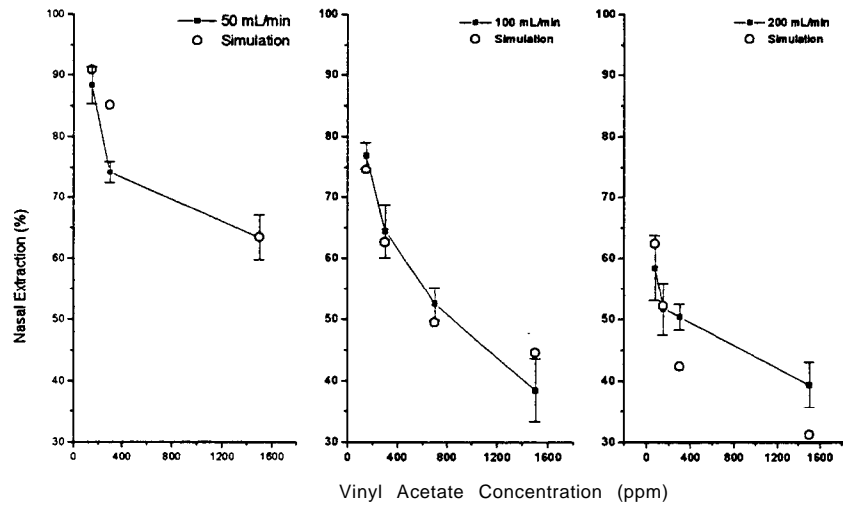
**Figure 5.** Schematic representation of the PB-PK model for vinyl acetate extraction and metabolism in the nasal cavity. The basic model structure is adapted from Morris (1993). Regional gas phase mass transfer coefficients and compartment sizes are as defined in Frederick et al (1998). Tissue enzyme distribution and estimation of intracellular pH are as in Plowchalk et al. (1997). The basic model structure is similar for rats and humans except that the human model does not contain an olfactory 2 compartment. Physiological and metabolic constants for each model are species-specific.  $C_m$  = concentration entering the nose,  $C_{ex}$  = concentration entering the nasopharynx; CE = carboxylesterase; AldH = aldehyde dehydrogenase; AAld = acetaldehyde; AA = acetic acid.

A high-affinity/low-capacity carboxylesterase pathway was included in the model. The kinetic constants for this pathway were obtained by numerical optimization against deposition data collected at a flow rate of 100 mL/min. Model estimates of fractional deposition (Figures 6 and 7), absolute deposition, and expired vinyl acetate and acetaldehyde concentrations (Figure 8) were in good agreement with the experimental data as a result. The presence of both high- and low-affinity isoforms of carboxylesterase in various species and tissues is common (Morgan *et al.*, 1994).  $K_m$  values of approximately 25 and 400  $\mu\text{M}$  for the high- and low-affinity carboxylesterase have been reported for rat liver and are consistent with those predicted from the PB-PK model for nasal epithelium (55  $\mu\text{M}$ ). The optimized values for the respiratory and

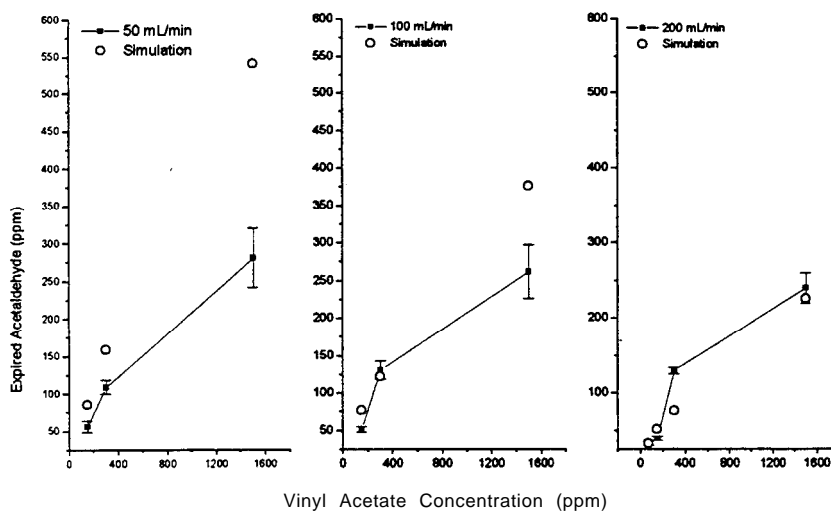
olfactory high affinity/low capacity term are:  $V_{max}$  2.6 mg/hr;  $K_m$ ,  $4.7 \times 10^{-3}$  mg/mL (55  $\mu$ M). For expired acetaldehyde, systematic departures from the observations were evident at the highest inspired vinyl acetate concentrations (Figure 8). However, model predictions were consistently reasonable at the lower vinyl acetate concentrations. Thus, the model was considered acceptable for low exposure dosimetry extrapolation.



**Figure 6.** Fractional deposition of vinyl acetate in the nasal cavity is nonlinear with exposure concentration. Simulation without nasal carboxylesterase (Tilled circles) indicate blood flow has little impact on vinyl acetate deposition. With carboxylesterase present (squares) deposition is increased and addition of a high-affinity/low-capacity carboxylesterase pathway provides deposition estimates (diamonds) similar to the experimental data (open circles).



**Figure 7.** Model simulations versus experimental observations of nasal extraction of vinyl acetate. Nasal extraction studies were carried out at three flow rates. The studies conducted at a flow rate of 100 mL/min were used to optimize the high affinity/low capacity metabolic pathway used in the whole nose model. Model simulations of the experiment (open circles) were run for each exposure concentration and flow rate. Experimental observations (filled squares) are the mean  $\pm$  S.E. for an n=4 or 5.



**Figure 8.** Model simulations versus experimental observations of acetaldehyde release into expired air during the nasal extraction studies. Nasal extraction studies were carried out at three flow rates. The studies conducted at a flow rate of 100 mL/min were used to optimize the high affinity/low capacity metabolic pathway used in the whole nose model. Model simulations of the experiment (open circles) were run for each exposure concentration and flow rate. Experimental observations (filled squares) are the mean  $\pm$  S.E. for an  $n = 4$  or  $5$ .

## Dosimeters Based on Mode of Action

### *Rat*

Potential dosimeters for olfactory mucosa exposure to vinyl acetate, acetaldehyde, and acetic acid include peak tissue concentrations, area under the concentration-time curve (AUC) and total amount of metabolites formed. Simulations of 6-hour inhalation exposure to vinyl acetate at concentrations of 50, 200, and 1000 ppm indicate olfactory tissue exposure of rats to vinyl acetate is minimal compared to both acetaldehyde and acetic acid (Table 15). This is due to rapid hydrolysis of vinyl acetate by carboxylesterase and is also the reason for the steep vinyl acetate concentration gradient (6 orders of magnitude) predicted for olfactory tissue.

**Table 15**  
**Olfactory 1 tissue dosimeters predicted after simulations of a 1-hour inhalation exposure of rats to 50-600 ppm vinyl acetate<sup>a</sup>**

Exposure Concentration (ppm)	Steady-state Tissue Concentration (µg/mL)			AUC (µg x hr/mL)			Amount (mg) formed per mL tissue	
	VA	AAld	AA	VA	AAld	AA	AAld	AA
50	0.22	1.9	56.5	1.33	12.0	339	23	31
200	1.11	6.3	200.8	6.67	38.0	1203	112	153
600	4.08	14.1	411.7	24.5	84.6	2467	390	531

<sup>a</sup> Simulations were run at an inspiratory flow rate of 197 mL/min.

VA = vinyl acetate

AAld = acetaldehyde

AA = acetic acid

Dosimeters from model predictions of vinyl acetate-induced intracellular acidification of olfactory epithelium are presented in Table 16. The predicted  $pH_i$  decreases and reaches a steady-state which is a function of the predicted rate of  $H^+$  formation by metabolism and predicted rate of  $H^+$  extrusion by the  $Na^+/H^+$  antiport. Thus, a 6-hr exposure to 50 ppm is not expected to cause a significant increase in  $H^+$  exposure or decreased  $pH_i$  ( $\Delta pH_i < 0.1$ ). This modeling prediction is in accordance with the observed NOAEL. A high incidence of olfactory lesions were observed in rats after one 6 hour exposure to vinyl acetate at 600 ppm (Bogdanffy *et al.*, 1997), which is consistent with the predicted  $pH_i$  reduction ( $pH_i = 6.91$ ).

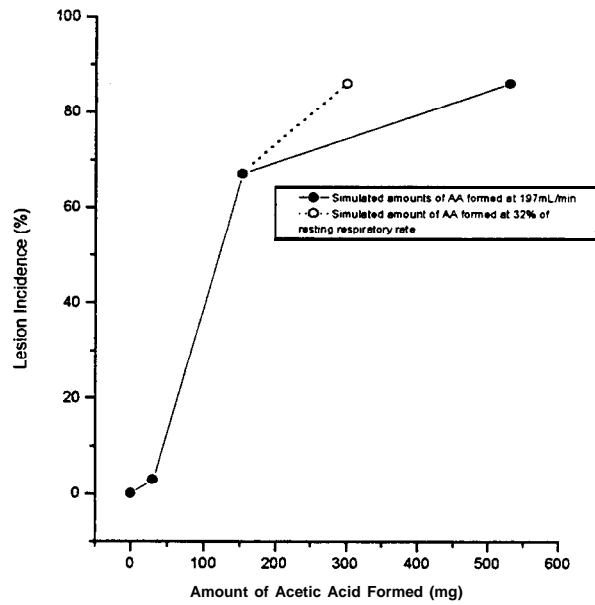
1 death is a function of both the degree and duration of cellular acidification (Nedergaard *et al.*, 1991). Exposures of neuronal and glial cells, cells which can be considered analogous to olfactory sensory and sustentacular cells to  $\text{pH}_i$  6.7 for less than 4 hours is not cytotoxic, whereas 6-hr exposures to the same pH will cause cell death (10-20%). A single 6-hr exposure of rats to 200 ppm was without effect, but chronic exposure of rats to 200 ppm produced a significant incidence of olfactory degeneration. Therefore it appears from the intracellular pH model that a  $\text{pH}_i$  of 7.15, predicted by the model for a 200 ppm exposure is tolerated for only short durations, but not over a lifetime of exposures.

**Table 16**  
**Dosimeters of intracellular acidification in olfactory epithelium of the rat after a 6-hour exposure to vinyl acetate**

<b>Exposure Conc. (ppm)</b>	<b>Final Proton Conc. (mM)</b>	<b><math>\Delta</math> Final Proton Conc. (mM)</b>	<b>Proton AUC (mmole x hr/L)</b>	<b>D Proton AUC (mmole x hr/L)</b>	<b>Final <math>\text{pH}_i</math></b>	<b><math>\Delta</math> Final <math>\text{pH}_i</math></b>
0	$3.98 \times 10^{-8}$	0	$2.39 \times 10^{-7}$	0	7.40	0
50	$4.79 \times 10^{-8}$	$0.8 \times 10^{-8}$	$2.84 \times 10^{-7}$	$+0.45 \times 10^{-7}$	7.32	-0.08
200	$7.08 \times 10^{-8}$	$+3.1 \times 10^{-8}$	$4.21 \times 10^{-7}$	$+1.83 \times 10^{-7}$	7.15	-0.25
600	$1.23 \times 10^{-7}$	$+8.3 \times 10^{-8}$	$7.31 \times 10^{-7}$	$+4.92 \times 10^{-7}$	6.91	-0.49

An interesting aspect of the dose-response curve for olfactory degeneration (Figure 1) is the small rise in the response between 200 ppm and 600 ppm. Nasal air flow rate is an important determinant of vinyl acetate delivery to nasal tissue and, hence, the degree of cellular acidification. Factors influencing ventilation rate, such as respiratory depression induced by exposure to high concentrations of irritating vapors may confound the interpretation of exposure-response data. Respiratory rate depression was a determinant in the interspecies differences in response to formaldehyde-induced nasal tumors (Barrow, *et al.*, 1986). Recently, the sensory irritation responses of mice to vinyl acetate exposure were measured (Dudek, 1996). The measured  $\text{RD}_{50}$  for vinyl acetate was 380 ppm. From these data, the respiratory rate was predicted to be depressed approximately 68% at 600 ppm. Assuming a similar response of rats to vinyl acetate, simulations of the 600 ppm exposure were conducted with a 68% reduction in minute volume. Replotting lesion incidence with the new dosimeter for the 600 ppm exposure reduced the sigmoidicity of the response curve. Therefore, depression of

respiratory rate at high concentrations provides a possible explanation for the observed nonlinearities in the response data in the high exposure concentration range (Figure 9).



**Figure 9.** Simulations with respiratory rate reduced by 68% at only the 600 ppm exposure concentration (open circles) reduced the total amount of acetic acid formed in olfactory tissue and resulted in a more linear correlation between response and external exposures of 50 ppm (3% response) and 600 ppm (86% response). Solid circles represent acetic acid estimates based on normal respiratory rate at all exposure concentrations.

## Human

Estimates of dosimeters for human olfactory epithelium at 50 and 600 ppm are consistent with the rat in that tissue exposure to vinyl acetate is minimal, while the greatest exposure is to acetic acid (Table 17). Both predicted tissue concentration and AUC are greater in humans compared to the rat when exposed to the same vinyl acetate concentrations suggesting that, relative to the rat, human olfactory tissue may receive a greater dose of metabolites per unit vinyl acetate exposure. However, predictions of  $pH_i$ ,  $H^+$  concentration and  $H^+$  AUC all indicate similar exposure of nasal tissue to reduced  $pH_i$  in humans compared to rats when exposed to the same external exposure concentration. Simulation of a 6-hour, 50 ppm exposure to vinyl acetate resulted in a predicted final  $pH_i$  of 7.32 in the rat compared to 7.35 in the human.

Table 17

Dosimeters of olfactory epithelium exposure in humans after a simulated 6-hour inhalation exposure to vinyl acetate

Exposure Concentration (ppm)	Steady-state Tissue Concentration ( $\mu\text{g/mL}$ )			AUC ( $\mu\text{g} \times \text{hr/mL}$ )			Amount (mg) formed per mL tissue	
	VA	AAld	AA	VA	AAld	AA	AAld	AA
50	0.2	10.6	82.7	1.35	63.6	495	15.7	21.4
200	1.7	38.9	345.6	10.1	233.3	2067.6	114.2	155.8
600	13.8	77.8	655.6	82.4	466.3	3921.6	752.2	1025.5

<sup>a</sup> Simulations were run at an inspiratory flow rate of 7.5 L/min

VA = vinyl acetate

AAld = acetaldehyde

AA = acetic acid



Table 18

**Dosimeters of intracellular acidification in olfactory epithelium of humans after a simulated 6-hour exposure to vinyl acetate**

<b>Exposure Conc. (ppm)</b>	<b>Final Proton Conc. (mM)</b>	<b>D Final Proton Conc. (mM)</b>	<b>Proton AUC (mmole x hr/L)</b>	<b>A Proton AUC (mmole x hr/L)</b>	<b>Final pH<sub>i</sub></b>	<b>D Final pH<sub>i</sub></b>
0	$3.98 \times 10^{-8}$	0	$2.39 \times 10^{-7}$	0	7.40	0
50	$4.72 \times 10^{-8}$	$0.74 \times 10^{-8}$	$2.79 \times 10^{-7}$	$0.26 \times 10^{-7}$	7.33	-0.07
600	$8.13 \times 10^{-8}$	$4.15 \times 10^{-8}$	$4.72 \times 10^{-7}$	$1.51 \times 10^{-7}$	7.09	-0.31
	$2.40 \times 10^{-7}$	$2.00 \times 10^{-7}$	$1.40 \times 10^{-6}$	$8.68 \times 10^{-7}$	6.62	-0.71

# Framework for Human Health Risk Assessment Colloquia Series

## Colloquium #2

### CHARGE TO THE PARTICIPANTS

#### Background

There is a recognized need for the development of a framework for human health risk assessment that puts a perspective on the approaches that are currently being practiced throughout the Agency. In its 1994 report entitled *Science and Judgement in Risk Assessment* (NRC, 1994), the NRC noted the importance of an approach that is less fragmented, more consistent in application of similar concepts, and more holistic than endpoint-specific guidelines. Both the NRC and the Agency's Science Advisory Board have raised a number of issues for both cancer and noncancer risk assessment, that should be reconsidered in light of recent scientific progress. In response to these needs, the Agency's Risk Assessment Forum is beginning the long-term development of a human health risk assessment framework. As part of this effort, the Risk Assessment Forum has invited you to participate in the second of two colloquia, which are intended to bring together EPA risk assessors for a dialogue on various scientific and policy issues pertaining to EPA's cancer and noncancer risk assessment approaches. The second colloquium will focus on the role of mode of action information in developing descriptive quantitative models, applicable to a variety of needs for carrying out a risk assessment.

#### Charge to the Participants

Prior to the second colloquium, each participant is receiving a single case study, a list of general questions, and a list of the breakout groups. As in the first colloquium, the case studies and accompanying questions will guide the discussions. The participants will spend the bulk of the first day discussing their assigned case studies with specific focus on the case study questions and the general questions for the plenary session. It is important that each participant review their work group's case study and be prepared to add their scientific and regulatory expertise to the work group discussion.

Each participant has been assigned to a specific breakout group. In making the group assignments, EPA sought to ensure a mix of expertise and Agency representation in each group. Each breakout group will have a chair to facilitate the discussion and a rapporteur to capture the consensus of the group. It is important that each of you participate in the breakout group to which you have been assigned.

# Framework for Human Health Risk Assessment Colloquia Series

## Colloquium #2

### GENERAL QUESTIONS FOR PLENARY SESSION

- Should a common quantitative analysis be conducted when there are commonalities among toxicities?
- In the absence of case-specific PBPK models, is there a common approach for dose adjustment for interspecies extrapolation for all responses? Does this differ for different routes of exposure?

In the presence of PBPK models, how does mode of action (MOA) information influence the dose surrogate in characterizing toxicity? Can it be different for different responses?

- In the absence of BBDR models, how does MOA information influence the default approach(es) to characterize in quantitative terms the potential risk of toxicities at low levels of exposure (i.e., beyond the range of observation)? Are there common default approaches?
- The *1996 Proposed Guidelines for Carcinogen Risk Assessment* have recommended that five factors be considered when determining the margin of exposure (MOE). These include intraspecies variation, interspecies variation, nature of the response, steepness of the dose-response curve, and biopersistence.

The current quantitative approach for noncancer effects generally involves development of a single RfD/RfC for a “critical effect”. Factors used include intraspecies variation, interspecies variation, subchronic to chronic extrapolation, LOAEL to NOAEL extrapolation, and completeness of the data base. An additional factor may be applied to account for scientific uncertainties in the study selected for derivation of the RfD/RfC.

If the goal is to harmonize across toxicities, can a consistent set of factors be identified? How does MOA information influence the choice of these factors?

# Framework for Human Health Risk Assessment Colloquia Series

## Colloquium #2

### Breakout Group Assignments

Wednesday and Thursday, June 3-4, 1998

**Breakout Group 1**  
**Versailles I Room**  
**Ethylene Thiourea**  
*Chair: Jennifer Seed*  
*Rapporteur: Vicki Dellarco*

# Barbara Abbott  
# Charles Abernathy  
# Kevin Crofton  
# Julie Du  
# Gary Foureman  
# Jennifer Jinot  
# Carole Kimmel  
# Jim Rowe  
# Gino Louis Scarano  
# Bill Sette

**Breakout Group 2**  
**Georgia Room**  
**Ethylene Oxide**  
*Chair: Gary Kimmel*  
*Rapporteur: Kerry Dearfield*

# Eric Clegg  
# Jim Cogliano  
# Marion Copley  
# Penny Fenner-Crisp  
# Chris Lau  
# Bob Luebke  
# Cheryl Scott  
# Woodrow Setzer  
# Mark Stanton  
# Yin-Tak Woo  
# William Wood

**Breakout Group 3**  
**Connecticut Room**  
**Trichloroethylene**  
*Chair: Vanessa Vu*  
*Rapporteur: Kim Hoang*

# Bob Benson  
# Carole Braverman  
# Chao Chen  
# Oscar Hernandez  
# Lee Hoffman  
# Aparna Koppikar  
# David Lai  
# Marc Mass  
# Bob McGaughy  
# Edward Ohanian  
# Gina Pastino

**Breakout Group 4**  
**Gallery Room**  
**Vinyl Acetate**  
*Chair: Annie Jarabek*  
*Rapporteur: Arnold Kuzmack*

# Donald Barnes  
# Karl Baetcke  
# Anne-Marie Burke  
# Terry Harvey  
# Richard Hill  
# Elizabeth Margosches  
# William Pepelko  
# Rita Schoeny  
# John Whalan  
# Paul White



**W E D N E S D A Y , J U N E 3 , 1 9 9 8 ( c o n t i n u e d )**

- 1:00PM **Continue Breakout Group Discussions**
- 2:30PM **Status Report of Breakout Group Discussions**
- 3:00PM B R E A K
- 3:15PM **Continue Breakout Group Discussions**
- 5:00PM A D J O U R N

**T H U R S D A Y , J U N E 4 , 1 9 9 8**

- 8:30AM **Review of Day Two Charge**
- 8:35AM **Breakout Group Reports and Discussions: Groups 1 and 2**
- 10:00AM B R E A K
- 10:15AM **Breakout Group Reports and Discussions: Groups 3 and 4**
- 12:00PM L U N C H (on your own)
- 1:00PM **Plenary Session: Lessons learned and their applications to the development of a Human Health Risk Assessment Framework**  
Moderators: Vanessa Vu and Gary Kimmel
- 3:30PM A D J O U R N