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

Colloquium #1

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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 assessment 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 are intended to bring 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, focussed on the role of mode of action information in re-examining and developing new risk assessment approaches. The second colloquium, to be held in early 1998, will be more quantitative in nature and will focus on dose-response considerations, including low-dose extrapolation methods.

The overall goal of the first two colloquia is to provide Agency scientists an opportunity to share perspectives on the role of mode of action in shaping future human health risk assessment approaches. 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

The RAF invited a cross-section of senior Agency scientists (from headquarters, Research Triangle Park, and the regions) to participate in round table discussions (see participant list in Appendix B). Participants engaged in active and open discussions throughout the 2-day colloquium, both in plenary and breakout sessions. The group 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, 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.

Prior to the first colloquium, each participant received the white paper, five case studies (Appendix C), a "charge" (Appendix D), a working definition of "mode of action," and a list of questions developed to guide colloquium discussions (Appendix E). An outside speaker, Rory Conolly from the Chemical Industry Institute of Toxicology (CIIT), was invited to open the colloquium. His presentation, like the white paper, was intended to elicit thought and help initiate group discussion on past, current, and potential future risk assessment approaches.

Melvin Andersen, ICF Kaiser Inc., K.S. Crump Group, served as the colloquium facilitator. He presented an overview of the case studies and throughout the colloquium guided group discussions to ensure that the general and specific questions were deliberated. Each participant was assigned to one of three breakout groups (see group assignments in Appendix F). In making the group assignments, EPA sought to ensure a mix of expertise and Agency representation in each group. A group leader helped to facilitate discussions in each breakout group and a rapporteur captured key discussion points and group consensus.

The colloquium was structured as a series of alternating plenary sessions and small group discussions (see Agenda, Appendix G). Initial 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 full group discussions on "critical harmonization issues" and quantitative dose-response issues to be covered at the next colloquium.

SECTION TWO

OPENING PLENARY SESSION

INTRODUCTORY PRESENTATIONS

Welcoming Remarks

To open the colloquium series, William Wood, Director of the Risk Assessment Forum, welcomed all participants and observers. He thanked all those who helped in developing the colloquium series, including members of the planning committee[1] and authors of the white paper. He emphasized the primary goal of the colloquium series: to provide an opportunity for Agency staff to exchange perspectives on mode of action and harmonization issues in human health risk assessment. He commented that the response to the colloquium series was very positive, noting that at least half of EPA's risk assessment community was represented at this event. The overall expectation is that participants will come away with a general appreciation for the use of mode of action, its limitations, challenges, and utility in the risk assessment arena.

Background/Goals of the Human Health Risk Assessment Framework

Vanessa Vu of EPA's Office of Pollution Prevention and Toxics, and co-chair of the planning committee, provided some background on the colloquium series and an overview of the goals of the human health risk assessment framework (see Section One). Dr. Vu emphasized that, because of evolving science, EPA has been challenged to use mechanistic information in risk assessment instead of the current endpoint-specific approach. The following questions/issues, therefore, need to be examined in light of the newer science:

- Is routine application of nonthreshold and threshold approaches for cancer and noncancer endpoints, respectively, appropriate in all cases?
- How should EPA treat dose-response for the observable range (e.g., benchmark dose, point estimate, 95% lower confidence limit, etc.)?
- How should doses be adjusted across species?
- How should less than lifetime exposures be evaluated?

Dr. Vu explained that the scope of Colloquium #1 was to discuss qualitative aspects of mode of action, with the case studies serving to help focus discussions. Because of the limited

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[1]Planning committee members include Vanessa Vu (co-chair), Gary Kimmel (co-chair), Bill Wood, Kim Hoang, Annie Jarabek, Jennifer Seed, and Wendy Yap.

time available for breakout group discussion, the five case studies could not include all possible areas of interest (e.g., portal of entry considerations, mode of action of certain endpoints), but instead were designed to serve as a stepping off point for discussions on mode of action and the harmonization of human health risk assessment approaches.

Keynote Speaker

Rory Conolly, a Senior Scientist at CIIT, provided the group with his views on risk assessment approaches, speaking about the relevance of mode of action and dose-response modeling in shaping future risk assessments. The central question he addressed in his presentation, entitled "Evolution of Human Health Risk Assessment: Using Biological Information to Define Modes of Action, Develop Exposure-Response Models, and Refine Default Assumptions," was, How do we move forward and bring the newer science into risk assessment at a reasonable and responsible rate? Highlights of the presentation are provided below; a copy of the speaker handout is presented in Appendix H.

Historical Perspective

- In the 1970s, only a limited understanding of mechanism of action existed. Default methods and models were based on state of the science at that time and were therefore appropriate.

- In deriving risk assessment methodologies, regulators have strived to minimize uncertainty and derive reasonable risk estimates, balancing the desire not to miss any risks with the desire not to overestimate risk and incur unnecessary compliance costs. Looking to the future, risk assessors should continue to seek to reduce uncertainty in risk assessment, using mechanistic data where possible to improve predictions of risk.

Where Are We Today?

- Today we have a larger data base and a better understanding of mode of action in cancer and noncancer response. It is, therefore, appropriate to use the latest science and to update risk assessment practices.

- A lag time between availability of new science and acceptance and use in practice is inevitable. Moving forward requires reaching consensus, which involves working out the details and developing methodology to use the new science.

How to Get Science Into Risk Assessments

- As understanding improves, risk assessment policies need to be re-evaluated; EPA's new cancer guidelines show how the Agency is starting to do this.

- More sophisticated validated models (PBPK and biologically based) for dose-response need to be developed. Ideally, we need models to describe the whole exposure response process. Exposure-response models are available but are not as well developed as PBPK models; they have been used for dioxin, 5-fluorouracil, chloroform, and formaldehyde.

- When are models mature enough for widespread use? To be used in risk assessment, a model needs to be validated against animal and human data; receive adequate quality control; and be peer-accepted.

Challenges for Regulators/Where Is Risk Assessment Going?

- Regulators face the challenge of incorporating evolving and more sophisticated approaches into risk assessment methodology. Guidelines need to be developed to identify acceptable models for use in risk assessment; criteria can be qualitative (e.g., taking component parts of a model, comparing it to the default approach, and deciding whether uncertainty is increasing or decreasing).

- Evaluation of mechanistic data will not be easy. A wide spectrum of interactions/mechanisms exist; some do not result in toxic effects. Therefore, substantive questions exist concerning how one evaluates various biomarkers and relates them to toxicity.

- Additional data are needed to fully understand the shape of the dose-response curve at low levels of exposure and to effectively incorporate these data into the quantitative risk assessment. Experimental work could be performed to address this knowledge gap.

- Computer models have become cheaper, faster, and increasingly sophisticated. We can now incorporate biology into models (e.g., models showing airflow through the nasal passages of rats and humans)—something that could not be done 10 years ago. The nasal passage models demonstrate that detailed anatomical modeling can make a difference in risk predictions; this is a lesson for any organ with anatomical complexity—we should continue to develop such models and use them in risk assessment.

- Defaults will continue to be important in risk assessment, but we need to keep up with the science. Some of yesterday's defaults will not be good enough for tomorrow. Most chemicals will not have rich data sets (may have limited but targeted data collection [e.g., PBPK models, short-term assays, predictive computer model]). Defaults will still need to be used in the future, but they will be enriched by the newer science and modeling technologies.

- Well-articulated risk assessment strategy can motivate research, specifically in terms of how biologically based modeling is incorporated into risk assessment. EPA could take the lead in specifying data needs (e.g., the kind of descriptive data needed, the criteria needed for validation of models, and the role human models should play in model validation). Promulgation of new risk assessment guidance using mechanistic data is needed to encourage industry to pay for research. Industry needs to be sensitive to the fact that some lag time is inevitable, but regulators should not let the lag time get too long.

Introduction to Case Studies and Colloquium Issues and Charge to the Breakout Groups

Melvin Andersen of ICF Kaiser, Inc. facilitated the colloquium. In his introductory remarks he encouraged the group to engage in active discussions on how to use mode of action information wisely. To open discussions, Dr. Andersen reviewed the definition of mode of action developed for the purposes of this colloquium.

> *Mode of action is defined as those key biological events that are directly linked to the occurrence of toxic responses. These events include absorption and entry into the body up to the final manifestation of toxicity.*

Dr. Andersen suggested that the group keep the following questions/issues in mind when thinking about mode of action.

- What is the nature of the chemical causing the effect?

- What are the initial interactions that a chemical has with macromolecules or cellular components?

- All details may not be necessary, but the challenge lies in deciding on how to incorporate available new information.

- Information on what is happening at the molecular level will continue to grow. New guidance emphasizes mode of action (e.g., IARC, NRC, EPA). Our choices are either to continue to be proactive or be reactive later.

Dr. Andersen charged the group to begin exchanging ideas and perspectives in the first breakout session, specifically discussing the definition of mode of action and general questions pertaining to mode of action and risk assessment (see Section Three).

Dr. Andersen then provided the group with a brief overview of the case studies, explaining that the case studies were developed to emphasize diverse issues and that the nine general questions (see Section Four) provided to participants were intended to guide discussions. The first four questions are somewhat generic in nature, while the remaining questions focus

more on mode of action and how information can be used to influence decisions on risk assessment approaches.

Questions/Comments

As summarized below, a brief group discussion followed the keynote address and introductory remarks by EPA and the facilitator.

- One attendee questioned how feasible it might be to apply work done in the pharmaceutical industry (where a significant amount of human data and mode of action information are available) to developing PBPK models and validating existing animal models for the chemicals of interest to EPA, FDA, etc. Responses indicated that while some of this information is available for the therapeutic effects of anticancer drugs and has been used in the development of fundamental pharmacokinetic models, data are largely unavailable for the toxic effects of those drugs. The goal of most pharmacokinetic studies in the pharmaceutical industry is to get information on the therapeutic dose range, not to learn specifically how the chemical acts. PBPK models for pharmaceutical drugs have not been widely used for the type of extrapolations used by EPA in studying toxic chemicals (e.g., species or high to low dose extrapolations). The industry is beginning to see biologically based models as a good adjunct to human data. Such models may be used by the industry to evaluate developmental/reproductive effects where little human data are available. A few participants noted that acquiring any available data may be difficult because of confidentiality issues and the existence of a great deal of chemical-blind data.

- Another participant commented that we may never have low-dose information for the endpoints EPA is currently studying, but emphasized the importance of starting to study mechanistic effects in the low-dose range and linking those events to the observed effect of regulatory interest.

SECTION THREE

BREAKOUT GROUP DISCUSSIONS ON GENERAL QUESTIONS

The opening plenary session was followed by a breakout session designed to give the colloquium participants an opportunity to open discussions on the role of mode of action in risk assessment. The three breakout groups were charged with discussing the following three questions:

Question #1 What are the variety of different purposes for which EPA conducts risk assessments?

Question #2 How has mode of action information been used in risk assessment to date?

Question #3 Are there differences in the importance of mode of action information for conducting risk assessments for different human health endpoints/toxicities?

When participants reconvened in plenary session, Vicki Dellarco, Carole Braverman, and Mark Stanton summarized the discussions from Breakout Groups 1, 2, and 3, as presented below.

What Are the Variety of Different Purposes for Which EPA Conducts Risk Assessments?

The breakout groups identified multiple ways in which EPA uses risk assessment. With the protection of human health and the environment stated as the primary purpose, the groups identified the following specific examples of why and when EPA conducts risk assessments:

- Setting regulatory standards
- Educational purposes
- Screening level analyses
- Measuring public health impact
- Determining important toxic endpoints
- Identifying susceptible receptors
- Evaluating site remediation options
- New product and pesticide registration/cancellation
- Setting acceptable exposure levels
- Evaluating the need for emergency actions
- Evaluating residual risk
- Deriving drinking water standards
- Permitting
- Supporting state regulations
- Coperating with the international community
- Evaluating pollution prevention approaches
- Setting ambient water quality criteria
- Responding to public and Congressional requests
- Reporting toxic release inventory
- Ranking/prioritizing chemicals
- Identifying data needs
- Setting priorities for research

How Has Mode of Action Information Been Used in Risk Assessment to Date?

The groups identified the following ways in which mode of action information has been used to date in the risk assessment process.

- PBPK modeling.
- Distinguishing between noncancer and cancer effects (threshold versus nonthreshold).
- Identifying endpoints by looking at precursor events.
- Identifying the hazard expression, emphasizing early life risk (e.g., vinyl chloride).
- Evaluating species differences and the relevance of animal data to humans (e.g., 1,3-butadiene, alpha-2μ globulin).
- Integrating data, evaluating anatomical precursor events (e.g., ozone).
- Identifying endpoints of concern by looking at important precursor events (e.g., vinyl chloride, butadiene).
- Grouping compounds by a common mechanism of toxicity (e.g., antithyroid compounds or cholinesterase inhibitors).
- Strengthening the basis for certain hazard calls and the justification for certain quantitative approaches (e.g., melamine—strayed from low-dose linear approach).
- Influencing the endpoint used to choose an RfC/RfD (e.g., vinclozaline as an anti-androgen).
- Excluding a particular animal model because it does not capture human risk.
- Adding risks for common mode of action (e.g., noncancer effects)

Are There Differences in the Importance of Mode of Action Information for Conducting Risk Assessments for Different Human Health Endpoints/Toxicities?

The consensus reached on this question was that no differences exist; mechanistic information is applicable to both cancer and noncancer assessments. Mode of action is important in both cancer and noncancer risk assessment and we therefore need to consider biology and route differences for all endpoints.

During the discussion of the general questions, several points were made regarding the utility and limitations of mode of action in risk assessment:

- While there has been a philosophical desire to use mode of action in risk assessment, its use has been limited by the lack of available data and time. Historically, mode of action has generally been used more qualitatively in hazard identification versus quantitatively in dose-response assessment. Interest in looking more closely at mode of action exists; linking the limited amount of information to the endpoint will be the challenge.

- At an international meeting held in the summer of 1997 to discuss mechanistic data in risk assessment, a number of participants reportedly were not fully convinced that using mode of action data would allow for better risk assessments.

- Interspecies differences do not appear to get the attention of regulators (i.e., using mechanistic data in animals to predict human toxicity).

- With limited resources in the regions, risk assessors/managers may not be able to consider comprehensive chemical-specific toxicity evaluations. While the benefit of this type of exercise is recognized by the regions, regional staff still look for numbers/bottom lines. In addition, from a regional perspective, improving fate and transport understanding is equally important to improving toxicity assessment.

- Several participants noted that scientists are developing new gene tests and building data banks, but may not necessarily have risk assessment in mind. It is not clear exactly how this information may be used or integrated into regulations or guidelines.

- It is hoped that, within the next decade or so, toxicity tests will be designed to provide both a less expensive and more informative test system (with mode of action in mind). Scientists and risk managers will need to look at new science and develop a process to use the data. Participant input at these colloquia will help shape that process.

- Overall, participants recognized the advantages of using mode of action, but acknowledged that the process of systemizing the information will be difficult. A clearer definition of how mode of action will/can be used is needed. To date, mode of action has been used on a case-by-case basis, but a growing body of data is being developed from which we can learn and shape future efforts. No unifying systematic approach exists now, however. In developing existing toxicity values (e.g., RfDs, slope factors), numerous chemicals were studied; going back and re-examining mode of action for all of these chemicals is a daunting task; a way to streamline research efforts is therefore critical.

- Participants recognized that as the process of evaluating the role of mode of action in risk assessment continues, caution must be taken not to fall into the "paralysis by analysis" trap. Several participants emphasized the importance of having a process in place so that scientists do not become bogged down with too much information. Understanding every molecular event is not necessary to make a decision on activity (e.g., we do not understand all events in mutagenicity, but we still consider it a precursor to cancer).

- The group raised the following questions: 1) Will mode of action evaluation result in more confusion? 2) When will mode of action be ready for "prime time?" 3) When will the scientific community be ready to accept the process?

- The recently enacted Food Quality Protection Act mandates that a screening process for estrogens and other hormonally active mechanisms be established. Data will be collected for thousands of chemicals; the screening will look for estrogen or anti-androgen action in these chemicals. This example illustrates how mode of action considerations have helped determine endpoints.

- Participants again emphasized that working through new approaches does not mean past approaches are being criticized; EPA is merely trying to evaluate how new data sets can be used to improve human health risk assessment.

SECTION FOUR

BREAKOUT GROUP DISCUSSIONS ON CASE STUDIES

More than half of the 2-day colloquium was devoted to discussing the five case studies (see Appendix C). The case studies, "loosely designed" after real chemicals, were developed to help foster group discussions on qualitative issues critical to re-evaluating risk assessment approaches, such as selecting endpoints of interest, considering the influence of mode of action, identifying common critical events, and evaluating whether a data set supports using an alternative approach to the default dose-response analysis. Each case study included five sections: 1) a brief introductory section highlighting general compound properties/characteristics; 2) toxicokinetics; 3) effects in humans; 4) effects in animals; and 5) additional data relevant to mode of action.

Case study discussions focussed on the questions listed below. Participants also were encouraged to consider the broader questions of where mode of action and harmonized approaches were most evident.

- What are the toxic effects associated with the compound?
- How similar are the effects in studies of animals and humans?
- How consistent are the data across species and routes of exposure?
- At what administered doses or exposure concentrations are the effects observed?
- What do we know about mode of action for the different toxicities?
- Is mode of action influenced by dose (i.e., administered dose or exposure concentration)?
- Are there commonalities in mode of action for the various toxicities?
- Do we have enough information to determine a common critical event that leads to all subsequent toxicities for the compound? Is such a common precursor effect expected as a general rule?
- Qualitatively, how does mode of action information influence decisions about choice of risk assessment models for the dose-response analysis?

All three breakout groups reviewed and discussed Case Study A and Case Study B, both in individual breakout sessions and in plenary sessions. Each of the three breakout groups also examined and discussed one of the remaining case studies (i.e., Case Studies C, D, and E).

Breakout session discussions were open and lively, with active participation by all group members. While in some cases participants expressed frustration with the task of sorting through sometimes limited case study data, the exercise served its purpose in fostering discussions on the role of mode of action in the risk assessment process. The groups worked through the case studies, formulating hypotheses, where possible, regarding mode of action and evaluating whether any consistency existed across endpoints. (Some participants noted that the information in most of the case studies only enabled limited discussions on harmonization across endpoints.)

The case studies served to highlight the challenges and limitations of working with available data sets, sometimes making mode of action and harmonization decisions difficult. The groups explored additional data needs, focussing on data that would help support a decision on the appropriate low-dose model to be used. Some of the requested data will be important for the second colloquium, where the group will examine quantitative issues and low-dose extrapolation models.

The sections below summarize the main points discussed during the breakout sessions, captured by the group rapporteurs, and presented in the plenary sessions. Vicki Dellarco, James Rowe, and Mark Stanton presented Case Study A for Groups 1, 2, and 3, respectively. For Case Study B, Vicki Dellarco, Jane Caldwell, and Rita Schoeny presented for Groups 1, 2, and 3, respectively. Annie Jarabek, Oscar Hernandez, and Mark Stanton presented breakout group findings for Case Studies C, D, and E, respectively. Other group members contributed to the presentations and subsequent discussions on an ad hoc basis. The group presentations for Case Studies A and B were structured more strictly around the general questions listed above. While the presentations for the last three case studies captured the essence of the general questions, the presentations focussed more on summarizing the study and presenting mode of action and model hypotheses.

Case Study A

Compound A, as described in the case study, is a relatively stable, low molecular weight halogenated compound. It is used as a solvent and is a common byproduct of chlorination. Compound A is readily absorbed via inhalation and oral exposures and requires enzymatic catalysis in the body. The case study focusses on the toxic and carcinogenic actions on the nasal passage, kidney, and liver in chronically exposed animals.

Case Study A proved to be the most difficult case study, largely because it was the first, but also because of the nature of Compound A and the multiple issues associated with it. Participants identified additional information that would be needed before they could seriously consider nondefault approaches. General points and responses to the case study questions are presented below.

- *Toxic Effects*: Toxic effects associated with Compound A include nasal toxicity; cancer and noncancer effects in the liver and kidney; central nervous system depression; and cardiac arrhythmias.

- *Differences Between Animals and Humans*: Human data are limited. Effects appear to be similar in the liver in animals and humans.

- *Consistency Across Species/Exposure Routes*: Effects are similar in the liver.

- *Administered/Exposure Dose at Which Effect Is Observed*: Insufficient data are available for humans. Strong dose relationship observed in animals, but difficult to extrapolate to low doses.

- *Mode of Action for Different Toxicities*: Response is observed primarily in high metabolic tissues (i.e., the liver, kidney, and nasal passage) where high localized patterns of P450 are observed. Kidney response is related to cell proliferation. The data suggest that the action of Compound A is systemic, based on the results of the gavage and drinking water studies, but the data do not clearly support a commonality in mode of action across endpoints.

  Mode of action hypotheses presented by the breakout groups included 1) glutathione depletion resulting in cytotoxic response; and 2) oxidative metabolism, forming the unstable ketohalogen.

- *Influence of Dose on Mode of Action*: Differences seen in effects resulting from corn oil versus drinking water are a function of dose, not route of exposure. Dose appears to affect oxidative metabolism, but not glutathione depletion.

- *Common Critical Event for All Toxicities*: Based on available data, the group could not reach a consensus on a common critical event, although the requirement for metabolic transformation for all effects was noted.

- *Influence of Mode of Action on Risk Assessment Dose Model*: Based on the information presented in the case study, the group concurred that the default linear approach should be used for cancer effects. Because tumor development was determined to be secondary to cytotoxicity, the use of the margin of exposure (MOE) approach was suggested; multiple models, however, would need to be used (perhaps different models for different dose ranges). The group agreed that additional data are needed to support decisions regarding linear and MOE approaches.

- *Additional Data Needs*: The groups identified data gaps that limited the ability to answer certain questions. Suggested data needs include study design information; time-dependency data on cell proliferation; documentation of experimental dose levels for all key studies; information on all target organs (or an indication that information for certain target organs <u>is not</u> available); additional human data, including clinical observations and molecular epidemiology data (e.g., changes in genes/biomarkers); and data to better evaluate site concordance.

Participants tended to fall back on the cancer/noncancer default approaches in the absence of certain data. More discussion of noncancer effects was recommended.

Case Study B

Compound B, widely used as an intermediate in chemical synthesis, dissolves easily in water, and is a gas under ambient conditions. Inhalation of Compound B is considered the most important route of exposure. The case study focussed on the cancer and reproductive/developmental effects associated with Compound B.

Compound B is well absorbed, but reacts at the exposure site. It is very reactive and alkylates critical macromolecules. Its metabolism leads to decreased activity. Genotoxic data show mutagenicity *in vitro* and *in vivo*, formation of DNA adducts, and an increase in micronuclei and sister chromatid exchange. Both cancer and reproductive/developmental effects are observed. All groups agreed that a common mechanism across endpoints was evident (i.e., alkylation of DNA) and that the low-dose extrapolation (based on genetic effects) should be similar for all endpoints, although different dose metrics may be needed to assess cancer versus developmental/reproductive outcomes because relatively short exposures may elicit developmental/reproductive effects.

The facts that support the groups' conclusions are summarized below. The groups presented a fairly long "wish list" (see additional data needs section below) but expressed mixed opinions regarding the extent of additional data needed to support a harmonized approach and develop a low-dose model for Compound B.

- *Toxic Effects*: Toxic effects associated with Compound B include cancer, reproductive effects, and other noncancer effects such as eye irritation, nausea, headache, and memory loss.

- *Differences Between Animals and Humans*: Cancer and reproductive effects are observed in humans, rats, and mice, but human data are limited. Irritant, respiratory, and neurological effects have been reported in humans. Developmental effects have been reported in rats and mice.

- *Consistency Across Species/Exposure Routes*: Groups noted that relatively consistent data exist for rodent species, although sex differences were difficult to discern from the case study data set.

- *Administered/Exposure Dose at Which Effect Is Observed*: There is a need for further evaluation of different dose metrics for cancer versus developmental effects. Short exposures may elicit reproductive/developmental effects. Exposure duration issues need to be explored more fully.

- *Mode of Action for Different Toxicities*: Effects are due to genetic damage by alkylating agents; there is a common mechanism, but a variety of targets. A brief discussion was held on the possibility of reaction with proteins/enzymes versus DNA; the consensus was that the focus should be on the overall mechanism (also considering repair mechanisms), not on the action on a single enzyme.

- *Influence of Dose on Mode of Action*: The groups could not fully evaluate dose issues based on the available data set.

- *Common Critical Event for All Toxicities*: Evidence suggests a common mode of action, but additional information is needed to support this hypothesis (see below).

- *Influence of Mode of Action on Risk Assessment Dose Model*: The groups concurred that a low-dose linear model is appropriate for cancer endpoints (parent compound at target site), although some participants were reluctant to assume linearity at low doses. For reproductive endpoints, options include using a low-dose linear model or superlinear model with MOE (if Compound B alkylates "everything," there may be more than one type of damage, and, as cell damage increases, a break and increased slope in the dose-response curve may occur). One group suggested adjusting dose metric for target tissue, considering saturation, cell death, and cell proliferation issues; in addition, high dose excursions may need to be considered. For developmental endpoints, more information is needed (the group hopes to explore this issue at the next colloquium).

- *Additional Data Needs*: The breakout groups differed in opinion regarding the amount of additional information needed to support risk assessment approach decisions. Unanswered questions include: 1) Where are we on the exposure curve (additional low-dose data needed)? 2) Is the parent compound the only bad actor; what about the metabolites? 3) Does glutathione contribute to toxicity? 4) Is detoxification route-specific? 5) At what point is the detoxification mechanism saturated? 6) What is the capacity for repair/cell loss for carcinogenicity versus reproductive capacity versus developmental effects? 7) If Compound B is endogenous, what are sensitive subpopulations (e.g., nutritional aspects)? 8) Are all toxic endpoints covered (has neurotoxicity been explored fully enough)? 9) Were other mechanisms studied (e.g., cell proliferation)? and 10) Is stem cell information available?

  Additional data needs identified by the group include transgenerational/heritable genetic effects data (e.g., low-dose mutation test needed, pre-conceptional exposure data), subchronic exposure data, and exposure duration data for different endpoints.

General Discussions Related to Case Study B

- Discussions focussed on how much weight of evidence and data are needed to support new approaches. The group agreed that extensive research efforts would not be needed for Compound B but that additional low-dose data are needed. The group suggested developing a low-dose model using genetic data as a biomarker of effects; collecting

additional data should be easier than conducting chronic animal studies. The group agreed that it is acceptable to move forward even with data gaps, but uncertainties must be recognized and clearly stated. These issues will be explored more closely in the second colloquium.

- While overall consensus was reached that mode of action considerations are appropriate for Compound B, several participants noted that, in the interest of protecting public health and in light of remaining uncertainties, risk managers may want to opt for the most conservative approach (even if that means by-passing mode of action considerations). Linear extrapolation may not be the most conservative approach, and the MOE approach should, therefore, be explored. More data are needed—especially if superlinearity is explored.

- One participant questioned what extra information a risk manager would gain from MOE data. Response: The MOE could tell the risk manager how far away one is from a given exposure scenario, if the risk manager were uncomfortable dealing with anything below the range of observation.

- Another participant questioned how the risk of reproductive effects would be expressed if a low-dose linear model were used (e.g., 10^{-4} to 10^{-6} risk). None of the groups explored this, however.

- Risk assessors/risk managers need to define the desired risk assessment product and consider the following questions: 1) What endpoint will be used to determine "safe" dose? 2) Are all effects adequately characterized? and 3) What effects should be communicated to an exposed public (risk communication)?

  In addition, program needs should be considered; different models may be appropriate for different exposure scenarios.

Case Study C

Group 1 reviewed and evaluated Compound C, a volatile halogenated hydrocarbon with low water solubility. As described in the case study, Compound C, used as an industrial solvent and anesthetic, is a common groundwater contaminant. The case study focussed on kidney and liver toxicity; neurological effects; and carcinogenicity.

The group concluded that toxic effects associated with Compound C (neurological, kidney, and liver toxicity) vary across species and that different modes of action exist for the three endpoints. Metabolism is a critical event in observed toxic responses. Compound C has multiple metabolic pathways, with unmetabolized Compound C excreted via inhalation in a dose-related pattern. Metabolic pathways are qualitatively similar across species (humans, rats, and mice) but quantitatively different, particularly in rats, with mice being the most rapid metabolizers. Both mutagenic and nonmutagenic metabolites are produced, making conclusions regarding mode of action difficult.

Metabolism of Compound C, as summarized below, involves formation of an aldehyde that is reduced to an alcohol, which is either conjugated and eliminated or oxidized to form an acid. A minor pathway involves the formation of a cysteine derivative.

"C" ⇒ CAL ⇒ COH ⇒ CCOOH
⇓
CCYS (minor pathway)

Group 1 treated Compound C as a collection of metabolites and made a matrix that included the different metabolites and their effects, as well as mode of action information. Three major endpoints for humans, rats, and mice are described in the case study: neurotoxicity, liver toxicity, and kidney toxicity, with cross-species concordance seen for liver and neurotoxic effects.

Mode of Action Considerations

Based on its review of available mechanistic data, Group 1 concluded that different modes of action are implicated for the different target sites because different metabolites are involved. In addition, the group concluded that no common mechanism for cancer and noncancer effects appears to exist. The group noted that looking at different dose metrics may be informative. Too many holes exist, however, regarding the metabolites and independent toxicity to form any definitive mode of action conclusions. Because no real site concordance exists, it was suggested that one might ultimately want to develop different risk estimates for kidney and liver effects.

Kidney: The CCYS metabolite may be mutagenic. Evidence of p53 mutation in humans further suggests a genetic mechanism in the kidney.

Liver: The parent compound and the aldehyde and acid are toxic in rodents. The acid is known to be a peroxisome proliferator mediated by a receptor. The aldehyde has been shown to be clastogenic and produce aneuploidy.

Neuro: Neurotoxic response is observed with exposure to the parent, aldehyde, and alcohol. The acid appears to be nontoxic (indicative of binding activity or increased removal). Additional information is needed regarding mode of action.

Dose-Response Implications

The case study provided insufficient quantitative information to allow the group to report on the linearity in the observable range. Based on available data, Group 1 decided that the default approaches are most appropriate—specifically, the linear default for liver and kidney cancer and the RfC/RfD approach for noncancer effects in the kidney. Justification for the linear default for kidney tumors is based on the CCYS mutagenicity and human TS gene mutation. For liver tumors, the group had lower confidence because of limited data on all

metabolites and the fact that tumors were seen in mice only. Too many holes in the available data set exist to settle on a nonlinear approach for noncancer effects in the liver. Because mode of action is unclear for neurotoxicity, the group felt it necessary to go with the noncancer default approach.

One participant questioned whether both linear and nonlinear approaches might be considered in light of different actions of the different metabolites.

Data Needs

The group concluded that a closer look at both kidney and liver effects is needed to see whether effects are associated with different metabolites and possibly different modes of action. The group identified the following data needs to enable the full evaluation of common mode of action and low-dose extrapolation:

- Data documenting sex and strain of animals for the studies presented.
- Additional data on mode of action of individual metabolites.
- Additional dose-response data to better define linearity within the test range.
- Additional information to evaluate if noncancer effects are associated with cancer effects.
- Additional data on the quality and extent of epidemiological data.

Case Study D

Compound D is a water soluble gas absorbed by inhalation and oral routes. It is a major industrial chemical intermediate and a bacterial breakdown product of related compounds in the environment. Nonneoplastic, preneoplastic, and neoplastic changes in the liver were the focus of the case study.

Metabolism is via cytochrome P450, forming an epoxide which is further rearranged to form an aldehyde; both metabolites have electrophilic character. Metabolites are detoxified mainly through GSH conjugation. The case study does not include metabolic data for humans. Effects observed in humans (as reported in several retrospective and prospective cohort studies) include liver cancer and angiosarcomas. Other liver effects include impaired function and morphological transformations (e.g., hypertrophy/hyperplasia). Liver toxicity is observed in rats, mice, and hamsters exposed to Compound D by oral and inhalation routes. Hepatocellular carcinomas and angiosarcomas were reported in rat dietary studies. Study and dose information for rat studies are detailed in the case study.

Based on the available data, Group 2 concluded that liver toxicity was consistent across species and routes of exposure. The mechanism of action is via mutation of the p53 tumor

suppressor gene and *ras* and *myc* oncogenes. The two critical events appear to be metabolism and interaction with DNA, but additional metabolic data in humans is needed to provide a conclusive link. Because a genetic basis of action is assumed, the group recommended the default linear dose-response model. The case study was relatively straightforward, but the group would like assurances that other (non-liver) effects were studied and not found. In addition, insufficient data were available to evaluate whether similar mechanisms were responsible for toxic effects to the liver (structural changes) and liver cancers.

In followup discussions to the case study presentation, several comments were made regarding preneoplastic and nonneoplastic effects. The significance of reported foci in Compound D exposed rats was questioned. It was noted that the foci are of predictive value from a qualitative perspective, but insufficient quantitative data are available to make a full call; foci could be a "jumping-off point" when looking at hepatocellular carcinomas, but not angiosarcomas. Participants questioned whether the linear default should be used for all liver endpoints (assuming that foci are precursors to the cancers) and not consider nonneoplastic effects (i.e., not develop an RfD). The group did not examine this issue, but recommended exploring it at the next colloquium.

One participant noted that time should not be wasted on re-examining mode of action of a known human carcinogen such as Compound D. Another participant noted, however, that further exploration of mode of action issues for a chemical like Compound D may enable a fuller understanding of how tumors originate.

Case Study E

Compound E is described in the case study as a common contaminant found in drinking water, existing in a variety of oxidation states, complexes, and methylated forms. A range of external (skin) and internal toxicities have been shown to be associated with Compound E.

Human data, some *in vitro* data, and little animal data are available for Compound E. Metabolic reduction leads to toxicity, and metabolic pathways are similar in animals and humans. Methylation leads to detoxification, but Compound E may compete with methylation enzymes, affecting DNA methylation at high doses. Noncancer effects include cardiovascular, skin, blood, and liver effects, and pulmonary effects at high oral doses. Critical doses are within the same range for the observed effects, with skin effects seen at the lowest doses. Cancer effects have been observed in humans in skin, bladder, kidney, lung, and possibly other sites and occur largely via the oral route. Compound E is not an initiator but may serve as a promoter in animals. Most animal tests indicate no noncancer effects.

Compound E is a weak or inactive mutagen, but it does have chromosomal effects (causes breaks in chromosomes); it may be a weak co-mutagen. Group 3 listed the points that led them to their conclusions: 1) liver dysfunction leads to increased skin cancer; 2) Compound E *in vitro* hypermethylates p53; 3) methylated Compound E was a promoter in some initiation/promoter studies in animals; 4) no noncancer effects occur in animals; 5) Compound E

can interact with proteins, including effects on energy; and 5) Compound E appears to affect DNA repair and causes oxidative damage.

Group 3 presented the following "highly speculative" hypothesis on the mode of action of Compound E, emphasizing that pieces of information are missing: Compound E acts via methylation of the p53 gene; the methylated form initiates a promotion effect and interacts with the protein, impairing DNA repair, which leads to oxidative damage. Additional data are needed on the hypermethylation of DNA and possible sensitive subpopulations. In addition, Group 3 could not develop a complete linked model concerning the mode of action of Compound E; while interference with DNA repair could be a common mode of action, multiple mechanisms cannot be excluded.

Dose effects are uncertain and the group could not reach consensus on a low-dose model based on available information. The group discussed options, including the default linear model, but also noted that, because of the chromosomal effects caused by Compound E, a linear dose model may not be appropriate. (One nongroup member did comment, however, that the mechanism seen here actually calls for a low-dose linear model.) Sensitive subpopulations must also be considered when developing an appropriate model. *The group commented that this example points to the need for policy, guidance, or a description of data quality objectives in order to move away from defaults.*

The discussions that followed the group presentation addressed the shape of the dose-response curve, common mode of action, and population sensitivity/genetic predisposition issues related to Compound E. One participant commented that the dose-response may be linear at low dose, but, because of complex interactions, may be various shapes at higher doses; learning how to convey this type of scenario to the risk manager is important and challenging.

Unanswered questions stemming from these discussions include:

- What do you do when there are multiple chemicals or environmental justice issues?
- Would we change the model for just one chemical?
- Is it possible to come up with a policy that is protective of all sensitive subpopulations? Also, how do we define the subpopulation (wide amount of variability across the population), especially in light of some sensitivities being induced by multiple chemical exposure?
- If the outcome we are looking at has various causes, how do we deal with the added "noise" of the chemical we may be studying? How do we show that Compound "X" adds to the load (e.g., cardiovascular risk, cancer risk)? How do we factor in genetic susceptibility?

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SECTION FIVE

CLOSING PLENARY SESSION

The final session of the colloquium provided participants an opportunity to revisit mode of action and harmonization issues discussed during the previous day and a half and to discuss expectations concerning the second colloquium.

To initiate and guide the closing discussions, the facilitator posed the questions listed below and encouraged participants to look at mode of action and harmonization issues in a broad way.

- Given what is known about the mode of action of various compounds, is there a scientific basis for routinely assuming a different mode of action leading to carcinogenesis and other toxicological effects?

- Mode of action information has been used to influence the approach for low-dose extrapolation. Are there other areas where mode of action information should play a role in risk assessment?

- How do you see mode of action considerations influencing quantitative aspects of risk assessment (e.g., uncertainty factors, dosimetric adjustments, etc.)?

The deliberations that followed covered a variety of related topics, as highlighted in the following sections.

Refining the Definition of Mode of Action

Participants reflected throughout the colloquium on the best way to define mode of action, not only for the purposes of this colloquium but also in the context of risk assessment in general. In the final sessions, the group revisited the definition provided at the opening of the workshop. While this definition did not appear to limit colloquium discussions, some participants challenged the terms "key" biological effects (makes it too narrow), "toxic" responses (how do we define an adverse effect?), and "linked."

The group offered a number of thoughts on defining mode of action and its role in risk assessment; these are listed below. The overall consensus was that having a working definition at this point in the process is not essential, and that a better definition would likely evolve from discussions such as these. In general, the group decided, mode of action is simply the tool that enables scientists to incorporate more biology into risk assessment and do a better job predicting risks.

- The definition is important, though it is more important at this point to to think about the issues, focussing on metabolism/mechanisms and looking for a simplifying step when sorting through available data.

- Some participants noted that it is important to clearly distinguish between "mode of action" and "mechanism."

- Available empirical data, although not mechanistic, might have some relevance and therefore should be considered when discussing mode of action.

- More useful than the definition is thinking about how Compound X brings about toxic effects and how we describe these events to demonstrate that we understand what is happening at the cellular level. Understanding what "it" is doing to the cell is important, after first defining what "it" is.

- Key biological events are dictated by the data being reviewed. It is important to look at available information on biological events and decide what is key, how well characterized and accurate the events are, and how well links have been developed.

- Key biological events may not be independent but rather a series of events leading to an effect; the concept of sequence is important. This statement was qualified by one participant who noted that a set of conditions may exist which is not necessarily a sequence. Even with the existence of a known sequence, the biological point of departure from a common pathway might not constitute the critical rate-limiting step for a particular observed endpoint. The distinction between the critical event and the many biological conditions that contribute to that event is important.

- Conceptually, we are trying to learn more about certain toxic effects, how effects come about, and if/how knowledge of the mechanism helps us to better predict risk in humans, particularly at low exposure doses. Mode of action is the event or series of events that tells what form the risk model should take.

- The following "framework," developed several years ago when first looking at biologically based models, was offered by one participant as a possible way of thinking about mode of action:

  initial exposure ⇒ delivery to target site ⇒ response ⇒ pathogenesis ⇒ outcome

  Additional steps or pathways may exist, but this provides an overall framework.

- Agreeing on a clear definition of mode of action is important. If we say mode of action is "everything" that happens, it is no longer a meaningful term.

Harmonization Issues

The following bullets summarize a group discussion on the need for more emphasis on the commonality across endpoints.

- The concept of threshold is no longer a useful one in the nonneoplastic arena. As with cancer, the approach for noncancer effects is a function of the type of data historically available. The focus now is on what level is adverse and what is causing it. Framing noncancer effects in terms of threshold is not useful, given the level of detail in contemporary bioassays.

- One participant emphasized that the group needs to "push the envelope" more with respect to looking at mode of action across endpoints. The participant noted that the case studies did not allow the group to do that fully (e.g., if a strong mutagen is being evaluated, let's look closely at the noncancer effects and try to establish a possible common mode of action).

Is There a Scientific Basis for Routinely Assuming a Different Mode of Action Leading to Cancer and Other Toxic Effects?

- No. Consistent with discussions throughout the 2-day colloquium, participants agreed that similar modes of action could be responsible for cancer and noncancer endpoints, but that examples certainly exist where different mechanisms may be responsible for the two endpoints. Also, we cannot assume that all cancers are caused by a single mechanism or that all noncancer effects are caused by one mechansim.

Are There Areas Where Mode of Action Information Should Play a Role in Risk Assessment (Other Than in Influencing Low-Dose Extrapolation Methods)?

Expanding upon the examples provided in earlier plenary sessions (see Section Three), the group provided examples where mode of action should be used in risk assessment to accomplish the following:

- Explore the toxicology of a chemical of interest and evaluate what happens to the cell, at what level adversity is observed, and whether it is a predictive indicator of observed effects.

- Evaluate whether high-dose effects also occur at low doses and if there is route extrapolation.

- Evaluate whether the same effects occur and the same mechanisms are observed in animals and humans.

- Encourage risk assessors to look across a range of endpoints and routes, examining the whole toxicological database. Such an approach allows the risk assessor to strengthen his/her position.

- Enable risk assessors to better determine additive effects for chemicals with common modes of action.

- Evaluate multiple routes of exposure within an animal or human to the same chemical (aggregate risk).

- Define a common surrogate for dose and response. The tobacco-specific mutagen NNK is a good example of this; NNK produces cancer in animals and the dose-response curves for NNK and its associated adducts can be overlapped. As a result, the dose-response curve can be extended to doses below which tumors can be measured because the surrogate (i.e., the adducts) is a more sensitive measure of dose. Caution needs to be taken, however, if the chosen surrogate is not the "critical" or "limiting" factor; some measure of the efficiency of the endpoint would therefore be needed, which comes back to the issue of linking the precursor to the adverse effect. In many cases, depending on the questions being asked, surrogates for dose and response may be different: a good surrogate for the dose might identify the biological point of departure, while a good surrogate for the response may reflect the critical event linked to that effect. Ideally, a series of dose-response curves for the endpoints and for the surrogate should be developed.

- Improve the inferences made from structural activity relationships for untested chemicals.

- Evaluate "residual risk" (i.e., what does an exceedance of a "safe" dose really mean?). For example, in a case where an RfD is exceeded by 10 times, looking at mode of action of the chemical enables a further evaluation of public health significance.

- Develop new test methodologies.

In summary, use of mode of action should improve the risk assessment process, enabling us to develop new approaches based on the new science. Developing a useful approach to evaluating mode of action issues when multichemical/multimedia exposures exist is necessary to meet regional risk assessor needs.

How Do Mode of Action Considerations Influence Quantitative Aspects of Risk Assessment?

The group agreed that the examples listed in the preceding section have quantitative relevance. Studying the best way(s) to incorporate these concepts into the quantitative risk assessment is the next step in the process.

Looking Ahead to Colloquium #2

The group expressed interest in and enthusiasm about the upcoming second colloquium, where they will evaluate the more quantitative aspects of mode of action, testing some of the hypotheses discussed during the first colloquium. Participants offered suggestions on the best way to approach the second colloquium. Of particular interest was the prospect of performing a full quantitative exercise using Case Study B (e.g., dose response on adducts). Participants noted that it may be worthwhile to look more closely at real-life examples where mode of action considerations made a large difference in the risk assessment (e.g., alpha-2μ globulin, thyroid tumors, bladder tumors).

Participants agreed to further contemplate approaches for the next colloquium and provide their suggestions to the planning committee.

APPENDIX A

WHITE PAPER

Human Health Risk Assessment:

Current Approaches & Future Directions

September 1997

Risk Assessment Forum
U.S. Environmental Protection Agency

Technical Panel

Co-Chairs

Gary Kimmel, Office of Research and Development
Vanessa Vu, Office of Prevention, Pesticides and Toxic Substances

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Richard Hill, Office of Prevention, Pesticides and Toxic Substances
Edward Ohanian, Office of Water

Contents

Contents *(Continued)*

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 (Katoh 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 mechansisms 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 pint 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^1). 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, 12995; 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	
Interspecies	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%
Intraspecies	Calabrese, 1985			80-95%
	Hattis et al., 1987 p'kinetic factors			100%
	Naumann, 1995 elimination $t_{1/2}$			100%
	therapeutic dose			88%
Subchronic to chronic	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%
LOAEL to NOAEL	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 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.

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APPENDIX B

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Framework for Human Health Risk Assessment Colloquia Series

Colloquium #1

Holiday Inn Arlington at Ballston
Arlington, VA
September 29-30, 1997

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APPENDIX C

CASE STUDIES
(A, B, C, D, E)

Framework for Human Health Risk Assessment Colloquia Series

Colloquium #1

CASE STUDY "A"

1.0 INTRODUCTION

Compound A is a common low molecular weight halogenated compound. It is found in water as a common byproduct of chlorination and occasionally due to contamination from its use as a solvent. Its presence in water can lead to significant oral and dermal exposures. Inhalation is also a significant exposure concern as a result of volatilization from water or pure solvent.

As pure compound, Compound A is a volatile liquid that is denser than water and sparingly soluble. It is relatively slowly reactive (i.e., relatively stable), requiring enzymatic catalysis in the body or exposure to heat, light, and oxygen for reactivity in the environment or in industrial use. Compound A vapor is classified by EPA as a Category 3 gas (low water solubility and low reactivity).

Compound A causes central nervous system, renal, and liver noncancer toxicities in humans and laboratory animals following acute and chronic exposures. It causes nasal toxicity in rodents. In animals, it causes tumors of the liver and kidney. This case study focuses on the toxic and carcinogenic actions on the nasal passage, kidney, and liver from chronic inhalation and oral exposure of rodents to Compound A.

2.0 TOXICOKINETICS

Compound A, like many low molecular weight chlorinated compounds, is readily absorbed by inhalation and oral exposures. Significant kinetic differences in absorption from aqueous versus oil solutions have been reported. It is subject to saturable metabolism primarily by cytochrome P450 2E1. Due to the saturable metabolism, the parent compound is exhaled at high doses regardless of the route of exposure. The major metabolite eliminated by the body is carbon dioxide.

Cytochrome P450 2E1 is present in the liver, kidney cortex, and respiratory tract tissues (tracheal, bronchial, olfactory, and respiratory nasal mucosa; and esophageal, laryngeal, tongue, gingival, cheek, nasopharyngeal, pharyngeal, and soft palate mucosa) of rats. Autoradiography studies in rats demonstrate a good correlation between tissue adducts of Compound A and metabolic capability. Though more limited information is available for mice and humans, similar distributions of 2E1 are observed. Quantitative studies, however, show that human nasal tissue has approximately 10% of the metabolic capacity of rodents.

Metabolism by the oxidative pathway forms an alcohol that spontaneously dehalogenates to form

a highly unstable ketohalogen. This compound reacts with water to form carbon dioxide and acid (HX). Alternatively, Compound A reacts with any available cellular nucleophile, resulting predominantly in glutathione, lipid, and protein adducts. Glutathione depletion can occur at high doses, leading to greater cellular damage. Due to factors such as glutathione depletion and saturation of metabolic pathways, quantitative differences among species or different dose routes, vehicles, and exposure regimens need to be evaluated to provide a consistent understanding of observed toxicities. By accounting for kinetic differences, the role of alterations in the toxicity process (i.e., pharmacodynamics) from factors such as corn oil versus aqueous solution can be evaluated.

Reductive metabolism of Compound A has been demonstrated *in vitro* using anaerobic incubations. Under normal oxygen tension, free radical formation by isolated hepatocytes was reduced but not eliminated. It has been shown that maximal lipid peroxidation from reductive metabolism of Compound A occurs at 10 mm Hg oxygen tension because of opposing requirements for oxygen; low oxygen increases reductive metabolism, but oxygen is required to propagate the lipid peroxidation reaction sequence.

3.0 EFFECTS IN HUMANS

Reports from intentional human exposures demonstrate acute responses similar to those observed in animals. Central nervous system depression and cardiac arrhythmias occur following inhalation of high concentrations. Liver toxicity has been reported following an oral poisoning episode and inhalation of anesthetic concentrations. Renal tubular necrosis and renal disfunction have also been reported following inhalation of anesthetic concentrations.

Epidemiological studies of occupationally exposed workers have reported limited evidence of liver toxicity generally described as toxic hepatitis. Studies of chlorinated drinking water consumption and cancer have provided limited associations with urinary bladder and colon cancer and low birth weight. However, because of the presence in chlorinated drinking water of a substantial number of chlorination by-products, the association between Compound A itself and reproductive and/or cancer toxicity is unclear.

4.0 EFFECTS IN ANIMALS

4.1 Nasal Toxicity

Nasal passage toxicity has been observed in rodents following both oral and inhalation exposure, suggesting a systemic response to bloodborne Compound A. Nasal toxicity increases in a dose dependent manner; it occurs at lower doses or concentrations than any other target organ toxicity. In contrast to the other two target organs, no tumors were observed in the nose in any of the chronic assays with rats or mice.

Inhalation exposures of F344 rats produced nasal toxicity, the type and severity of which were dependent upon the exposure concentration (0, 2, 10, 30, 90, 300 ppm) and duration (4 days, 3, 6, and 13 weeks). The lesions, like those observed following oral exposure, were in specific regions of the nasal ethmoid turbinates of both males and females. Following 4 days of

exposure, observations included edema, loss of deep Bowman's glands, periosteal hypercellularity, and new bone growth in portions of the ethmoid turbinates. Focal atrophy of the olfactory epithelium was noted in rats exposed to 90 and 300 ppm. The most prevalent lesion in rats exposed to at least 10 ppm for 3 weeks was loss of deep Bowman's glands and edema in the lamina propria. Following exposures of 6 and 13 weeks, atrophy of the ethmoid turbinates was noted, minimally at 2 ppm and increasing in severity with dose. Labeling index studies found large increases at 10 ppm and higher concentrations following 4 days of exposure. By 3 weeks, labeling had dropped significantly and continued to drop to 13 weeks, although control levels were never attained.

Following oral exposures of female F344 rats, two treatment-related responses were observed in specific regions of the nasal passages, referred to as peripheral and central. Peripheral toxicity included new bone formation, periosteal hypercellularity, and increased cell replication. Following a 3-week exposure, the severity was dose dependent, with minimal changes at 34 mg/kg/day increasing to moderate severity at 400 mg/kg/day (all effects were statistically significant). Central toxicity following 4 days of exposure included degeneration of the olfactory epithelium and superficial Bowman's glands at the highest dose (400 mg/kg/day) and only individual cell loss at the lower doses (34, 100, 200 mg/kg/day). Following 3 weeks of exposure, there was substantial regeneration of the olfactory epithelium; no lesions remained at 34 mg/kg/day. Cell proliferation in the nasal turbinates increased with dose following both 4-day and 3-week exposures at 24 and 100 mg/kg/day, respectively, but little further increase in proliferation occurred at higher doses.

Although some lesions observed in mice were similar to those in rats, they were not identical. Early proliferative lesions were transient, and a late atrophic response was not apparent in the mouse.

4.2 Kidney Toxicity

Increased kidney tumors have been observed in mice and rats exposed chronically.

Male ICI mice exposed orally to 0, 17, and 60 mg/kg of Compound A for 104 weeks had increased adenomas and carcinomas (0/72, 0/37, and 8/38) only at the highest dose, and no increase in females was observed. Inhalation exposure also resulted in tumors in male but not female BDF1 mice. In males, combined adenomas and carcinomas increased at the top two concentrations (0/50, 1/50, 7/50, and 12/48 for 0, 5, 30, and 90 ppm exposures, respectively).

In two studies with OM rats exposed by corn oil gavage and drinking water, an increase in kidney tumors in males was observed. One study also exposed females and a single tumor was seen in the high-dose group. In an oral study with male and female Sprague-Dawley rats (0, 15, 75, and 165 mg/kg) and the inhalation study with F344 rats (0, 10, 30, 90 ppm) no tumors were observed.

Renal tubule injury, cell proliferation, and other cellular and tissue responses to injury were observed in both mice and rats following exposure to Compound A. These effects were observed at the doses used in the cancer bioassays and are observable in tissues that also have neoplasms. Histopathological evaluation of kidneys from a positive rat bioassay, for instance, found evidence of proximal tubule cytotoxicity. Cell injury involved vacuolation, necrosis, and

nuclear enlargement affecting the proximal convoluted tubule of the cortex. Injury was observed in males, but not females, of several mouse strains. Less complete information is available for rats, and much of it is in strains for which there are no cancer data. Kidney damage has been observed in rats, and sex differences appear less pronounced than in mice.

4.3 Liver Toxicity

Compound A has been evaluated for noncancer and cancer effects in rats and mice exposed by the oral and inhalation routes. Under specific exposure conditions, it causes liver and kidney tumors as described in this and the previous sections. Noncancer effects were observed in the liver and kidney in both species, as well as the previously described nasal toxicity.

Noncancer Effects: Hepatotoxicity in various animal species exposed by inhalation has been reported in several studies. Serum sorbitol dehydrogenase (SDH) activity was increased in rats exposed to 153 ppm and above for 4 hours in one study, and SGPT levels were increased in mice exposed to 100 ppm, 7 hour/day for 8 days during various stages of pregnancy in another study. These increased enzyme levels in serum indicate hepatocellular necrosis. Fatty changes were observed microscopically in male and female mice after acute exposure to Compound A concentrations of 100 ppm. Liver necrosis was observed in female rats exposed to 4,885 ppm Compound A for 4 hours and in male mice that died after acute exposure to 692 to 1,106 ppm Compound A, but not in those that survived and were terminated after a 12-month recovery period, indicating that the liver damage was reversible. Centrilobular granular degeneration was observed in rats, rabbits, and guinea pigs exposed to 25 ppm Compound A for 6 months, but not in dogs exposed to 25 ppm for the same time period; however, these pathological findings were not observed in the 50 ppm exposure group of rabbits and guinea pigs or the 85 ppm exposure group of guinea pigs. Although the liver effects in rabbits and guinea pigs were not dose-related, the small number of surviving animals in the higher exposure group may have biased the results of the study and may not fully describe the pathological effects of Compound A at the higher dose.

The liver is also a target organ for Compound A oral toxicity in animals. In acute studies, increased serum levels of transaminases, indicative of liver necrosis, were observed in mice treated with a single gavage dose of 273 mg/kg in oil or 250 mg/kg/day in oil for 14 days. Centrilobular necrosis of the liver with massive fatty changes was also observed in mice after a single dose of 350 mg/kg Compound A in oil. At a dose of 35 mg/kg, minimal lesions consisting of midzonal fatty changes were observed in mice.

Liver effects in animals have been reported in numerous oral studies of intermediate duration. Female mice were exposed to 3, 10, 34, 90, 238, and 477 mg/kg/day of Compound A in corn oil via gavage for 5 days per week for 3 weeks. Compound A treatment resulted in significant increases in liver weights of mice at 90, 238, and 477 mg/kg/day and 34 mg/kg/day resulted in pale cytoplasmic eosinophilia of the centrilobular hepatocytes and mild vacuolation of the centrilobular and midzonal hepatocytes relative to the periportal hepatocytes and livers from control mice. At the 238 mg/kg/day dose, the livers were characterized by a severe centrilobular hepatocyte necrosis. At 477 mg/kg/day, the central zone of the liver was populated by degenerate vacuolated hepatocytes and regenerating hepatocytes with markedly basophilic cytoplasm and small round nuclei with clumped chromatin and prominent nucleoli. Significant dose- dependent increases in ALT and SDH were observed at doses of 34 mg/kg/day and greater.

Cell proliferation was markedly increased in the liver at the 238 and 477 mg/kg/day doses. Mice dosed with 16, 43, 82, 184, or 329 mg/kg/day of Compound A in the drinking water for 7 days a week for 3 weeks showed in no histological changes in livers at all doses studied. Liver weights were significantly increased at 82, 184, and 329 mg/kg/day.

Another study examined the dose response relationships for the induction of cytolethality and regenerative cell proliferation in the livers of male Fischer 344 rats given Compound A by gavage. Groups of 12 rats were administered oral doses of 0, 3, 10, 34, 90, and 180 mg/kg/day Compound A in corn oil by gavage for 5 days per week for 3 weeks. BrdU was administered via an implanted osmotic pump to label cells in S-phase. Cells having incorporated BrdU were visualized in tissue sections immunohistochemically and the LI evaluated as the percentage of S-phase cells. Necropsies and histopathological examinations were performed at death. The relative liver weights were increased at doses of 90 mg/kg/day and greater at 3 weeks. After 3 weeks of exposure, livers of rats in the 34 or 90 mg/kg/day dose groups did not differ from controls. In the 180 mg/kg/day dose group, effects were similar to those seen at 4 days after exposure. Dose-dependent increases in both ALT and SDH were observed after 3 weeks in the 180 mg/kg/day dose group only.

The toxicological effects of Compound A administered in the drinking water in rats were studied. Groups of 12 rats were administered Compound A in drinking water at concentrations of 0, 60, 200, 400, 900, and 1,800 ppm for 7 days/week for 3 weeks. BrdU was administered via an implanted osmotic pump to label cells in S-phase. Cells having incorporated BrdU were visualized in tissue sections immunohistochemically and the LI evaluated as the percentage of S-phase cells. Necropsies and histopathological examinations were performed at death. Average daily doses of Compound A ingested from drinking water were: 0, 6.0, 17.4, 32.0, 62.3, and 106 mg/kg/day for 3 weeks of exposure for 0, 60, 200, 400, 900, and 1,800 ppm concentration levels, respectively. Only mild hepatocyte vacuolation was observed in rats given 900 or 1,800 ppm in water for 3 weeks. No increase in the hepatic LI was observed at any time.

Fatty changes, necrosis, increased liver weight, and hyperplasia have been observed in rats exposed to 150 mg/kg/day Compound A via gavage for 90 days. Fatty and hydropic changes, necrosis, and cirrhosis were observed in mice treated by gavage with 50 mg/kg/day Compound A in oil for 90 days or 86 mg/kg/day in drinking water for 1 year. In contrast, centrilobular fatty changes observed in mice at 64 mg/kg/day Compound A in drinking water for 90 days appeared to be reversible, and no liver effects were found in mice treated with 50 mg/kg/day Compound A in aqueous vehicles.

In chronic exposure studies, liver effects have been observed in rats, mice, and dogs after oral exposure to Compound A. Necrosis was observed in female rats treated by gavage with 200 mg/kg/day Compound A in oil for 78 weeks. Nodular hyperplasia occurred in all groups of male and female mice similarly treated at 138 mg/kg/day. Fibrosis of the liver was observed in both sexes of rats exposed to 200 mg/kg/day Compound A in the drinking water for less than 180 weeks. Increased SGPT was observed in dogs given Compound A in toothpaste capsules for 7.5 years. The lowest oral dose administered to animals in chronic studies was 15 mg/kg/day, which increased SGPT in dogs.

Cancer Effects*:* A chronic study of B6C3F1 mice exposed to Compound A in corn oil gave the largest increases in liver neoplasms using high doses (time-weighted averages of 138 and 277

mg/kg for males and 238 and 477 mg/kg/day for females). Observed incidences of hepatocellular carcinomas were 1/18, 18/50, 44/45 and 0/20, 36/45, and 39/41 for control, low-dose, and high-dose males and females, respectively. By contrast, a drinking water study with female B6C3F1 mice exposed to time-weighted average doses of 0, 34, 65, 130, and 263 mg/kg found no increased tumor incidence despite using a dose equal to a positive dose in the corn oil gavage assay. A study using orally exposed (0, 17, 60 mg/kg) ICI mice found no effect in males or females, though a second group of males exposed using a different vehicle showed an increase. An inhalation study using BDF1 mice exposed to 0, 5, 30, or 90 ppm Compound A found no statistically significant increases in adenomas, carcinomas, or combined tumors though a trend analysis was positive for the combined neoplasm rates.

There are five chronic studies using four strains of rats exposed by corn oil gavage, drinking water, and inhalation. These studies were negative or showed marginal increases in hepatocellular neoplasia that were not statistically significant, even when doses were similar to those used in mice. One drinking water study appears positive with 0/18 adenomas in control females and 10/40 in treated female Wistar rats. However, this study is difficult to interpret for several reasons: i) exposed females lived about 185 weeks versus only 145 weeks for controls, and ii) the number of control animals is small, making the incidence more uncertain.

The positive results in mice with corn oil gavage and the negative findings in mice exposed through drinking water raises questions about the appropriate dose metric for dose-response assessment. Neither the daily dose nor the cumulative dose of Compound A are predictive of the tumor outcome. Results from several studies suggest that the greater toxicity with corn oil gavage is due to some combination of pharmacokinetic and pharmacodynamic factors.

5.0 ADDITIONAL DATA RELEVANT TO MODE OF ACTION

5.1 Genotoxicity

More than 40 studies using *in vitro* and *in vivo* assays for a large number of endpoints indicative of various kinds of DNA damage have been undertaken with Compound A. Studies have looked at a variety of endpoints, including those associated with direct or secondary DNA damage. Direct DNA damage endpoints included mutation (i.e., point mutations, small insertions, or deletions), clastogenicity, recombination, sister chromatid exchange, DNA breakage, and DNA adduct formation. Secondary damage endpoints included DNA repair, cell transformation, and anueploidy. The results for mutagenicity assays and sister chromatid exchange are briefly summarized here as representative of the kind of results obtained for most of the endpoints.

Mutagenicity studies for Compound A have been conducted primarily in bacteria (*S. typhimurium*, *E. coli*, *Photobacterium*) with additional studies in yeast, Aspergillus, and cultured chinese hampster V79 cells. Clear positive results were obtained in the studies with *Photobacterium* and yeast. One study used bacteria bioengineered to express glutathione transferase theta which has been implicated in the genotoxicity of related compounds, including methylene chloride and bromochloromethanes. This study was clearly negative with Compound A. In vivo studies included two *Drosophila* sex-linked recessive lethal assays, which were both negative, and two host-mediated assays with bacteria, of which one was positive. Although yeast appear susceptible to Compound A, these results overall appear strongly negative across a range of species.

Sister chromatid exchange is a very sensitive indicator of chemical effects on DNA, although its relationship to carcinogenesis remains unclear. Of the seven *in vitro* studies, four were positive, including one using plant cells. Studies using mammalian cells and chinese hamster ovary (CHO) and human lymphocyte cultures gave equal numbers of positive and negative responses. The one *in vivo* study of male mice exposed to 200 mg/kg Compound A for 4 days reported a statistically significant increase in SCEs in bone marrow cells. Notably, none of these assays use cells from the two organs where tumors are reported. A range of factors can contribute to the mixed results. Compound A is volatile, so *in vitro* assays done in closed containers are preferable to those in open systems. Formation of genotoxic compounds may arise due to the use of stabilizers, even in highly purified preparations of the compound.

5.2 Metabolism and Cell Proliferation in Kidney Tissue

Metabolism is one major factor leading to the variations between sexes. Cytochrome P450 2E1 is present in kidneys of mice and rats, with the highest levels in the proximal convoluted tubules, the site of toxicity. Several studies demonstrate a correlation between levels of covalently bound radiolabel derived from Compound A (an indicator of metabolic activity) and kidney tissue damage. Order of magnitude differences in bound radiolabel have been demonstrated between males and females; two-fold differences were shown between strains with differing susceptibility to neoplasms. The sex differences are under hormonal control, as demonstrated by reduced radiolabel binding and nephrotoxicity in castrated males and increased binding and renal injury in testosterone-treated females. There also appear to be differences in tissue sensitivity; a neoplasm-susceptible strain of mice had greater radiolabel accumulation in kidney compared to a nonsusceptible strain, even after correcting for the higher metabolism in the susceptible strain.

Quantitative studies of cell proliferation in the kidney have been carried out in mice and rats. In the mouse strain used for the inhalation bioassay, for instance, 7- to 10-fold increases in labeling index were observed in males but not females following 4-day exposure to 30 and 90 ppm; no change was observed at 5 ppm. These results correlate with the observation of tumors in the chronically exposed high-dose males. Other studies have shown cell proliferation to vary over dose, exposure duration (decreasing in low-dose groups and continuing in high-dose groups), and exposure route (e.g., no increase with drinking water, but increased with corn oil gavage). These studies are in a variety of species that are untested for cancer or nonsusceptible to kidney tumors, so the results provide a general perspective but are not directly applicable to the cancer studies.

5.3 Cellular Damage and Repair in the Liver

A highly reactive metabolite of Compound A is formed by enzymes of the endoplasmic reticulum and reacts with water, soluble nucleophiles on small molecules (e.g., glutathione) and macromolecules (e.g. proteins), and macromolecular constituents of nearby organelles (e.g., lipids, proteins). Over time, this damage can lead to other damage (e.g., to DNA or organelles dependent upon normal cell function) and becomes histologically observable as necrosis and atrophy. The response to cellular damage includes repair processes in cells, cell proliferation by other cells in the tissue, and tissue repair (e.g. immune cell clearance of damaged tissues). Studies *in vivo* have found cell proliferation to be dependent upon a range of pharmacokinetic and pharmacodynamic factors, including dosing vehicle (corn oil versus aqueous gavage), exposure regimen, strain, and species.

As described for kidney toxicity, there are studies strongly supporting the correlation of metabolism with liver toxicity. CYP2E1 levels are highest in centrilobular regions of rats and humans, the region of greatest damage from ethanol (a 2E1 inducer) and halogenated alkanes. Further, GSH levels are lower in centrilobular regions, likely contributing to observations of GSH depletion following high oral doses.

Histological observations in exposed livers vary with dose, exposure duration, and strain/species. Effects include fatty infiltration, glycogen depletion, cytotoxicity, and necrosis. Induction of cytotoxicity and regenerative cell proliferation following high-dose bolus administration of Compound A in corn oil correlates with the development of hepatic neoplasms in mice exposed to Compound A administered in corn oil. Release of liver enzymes into serum, enhanced labeling indices in hepatocytes, and clear signs of cytotoxicity have been observed in mice exposed by corn oil gavage above 60 mg/kg. In initiation-promotion studies, Compound A showed no initiating or co-carcinogenic activity, although when dosed in corn oil gavage, it appeared to promote liver tumor development in some assays.

Framework for Human Health Risk Assessment Colloquia Series

Colloquium #1

CASE STUDY "B"

1.0 INTRODUCTION

Compound B is produced and widely used as an intermediate in chemical synthesis and in other specialty uses. It dissolves easily in water and is a gas under ambient conditions. In occupational settings, Compound B is sometimes used as an aqueous solution to which workers may be exposed. Inhalation is considered to be the most important route of exposure for Compound B. The compound is a reactive electrophilic species that adducts cellular nucleophiles including DNA and proteins. It also is a metabolite formed in the body from chemicals derived from endogenous and exogenous sources.

Compound B causes a range of effects in humans, including irritation of the eye, skin, and mucous membranes and neurotoxicity. Animal studies have demonstrated cancers of several sites, reproductive and developmental toxicities, lymphocytic necrosis, and kidney toxicity.

This case study focuses on cancer and reproductive/developmental effects associated with Compound B, primarily for inhalation exposures.

2.0 TOXICOKINETICS

Compound B is well absorbed from the respiratory tract, but its reactivity may limit distribution from some exposure sites. Once in the blood, the compound can distribute throughout the body with little apparent selectivity for any tissue (i.e., partitions into all tissues about equally).

The reactive parent is removed by reaction with cellular nucleophiles, metabolism, or exhalation. Alkylation products (adducts) of the reaction of Compound B with blood proteins, including hemoglobin, can be readily followed in humans and animals, providing an internal measure of exposure from both endogenous production and exogenous sources. A number of DNA adducts have been identified and can be measured in DNA from readily collected white blood cells or from internal tissues. Formation of DNA adducts in rat tissues is linear over the range 1 to 30 ppm for 6 hours. The metabolic pathways reduce the chemical's reactivity by hydrolysis or by conjugation with glutathione. Inhalation exposures lead to dose dependent depletion of glutathione at sufficiently high concentrations (e.g. 20% depletion at 100 ppm for 4 hr and 60 to 70% depletion at 600 ppm for 4 hours). Urinary metabolites are derived from the oxidative and glutathione conjugation processes; the spectra of metabolites observed varies quantitatively across species.

3.0 EFFECTS IN HUMANS

Effects associated with humans exposed to Compound B are generally qualitatively consistent with those observed in animals, although available studies are generally limited and inconclusive for reasons including small cohort size and uncertainties about exposure levels.

3.1 Cancer Effects

Some epidemiological studies of workers exposed to Compound B have indicated elevated leukemia, stomach and pancreatic cancers, and Hodgkin's disease, although exposure levels are uncertain. Other studies revealed no excess in these cancers.

3.2 Reproductive/Developmental Effects

Studies of occupationally exposed women have reported mixed results for increased incidences of spontaneous abortion. Estimated, not measured, exposure levels associated with adverse outcomes in one study ranged from 0.1 to 0.5 ppm, with peaks up to 250 ppm.

3.3 Other Noncancer Effects

Exposure to high concentrations of Compound B gas is irritating to the eyes, while exposure to aqueous solutions can produce injury to the eyes and skin. Reports of respiratory effects (e.g. bronchitis) in workers with different exposures are mixed. Central nervous systems effects are frequently reported, including heachache and nausea. Other studies have reported peripheral neuropathy, impaired hand-eye coordination, and memory loss.

4.0 EFFECTS IN ANIMALS

4.1 Cancer Effects

Chronic studies have reported increases in cancer in rats and mice exposed by the inhalation, oral, and injection routes. Oral (7.5 and 30 mg/kg/day) and injection exposure produced dose-dependent increased tumor incidences at local exposure sites but not internal tissues, perhaps indicating that the compound reacted with cellular constituents at the exposure sites and that little systemic distribution occurred. Inhalation exposures produced dose-dependent (33, 100 ppm) increases in mononuclear cell leukemia in females, brain tumors in both sexes, and peritoneal mesotheliomas in male rats that did not survive to study termination. An inhalation study in mice found increases in benign and/or malignant alveolar/bronchiolar and harderian gland tumors in males exposed to 50 and 100 ppm. Females had increases at those two sites and three others, lymphomas, uterine, and mammary tumors.

4.2 Reproductive/Developmental Effects

The reproductive and developmental toxicities of Compound B have been the subject of a number of studies in mice, rats, and rabbits.

Inhalation studies in which both male and females rats were exposed to three concentrations (0,

10, 33, 100 ppm), starting 12 weeks prior to fertilization and continuing through 21 days following parturition, demonstrated effects in the groups with the highest exposure. The gestation period was longer for more females in this group. There were decreases in the number of implantation sites, pups born, and the ratio of pups born to implantations. No effects were observed on parental body weights or organs. A study of Sprague-Dailey rats exposed to 0 or 150 ppm found decreases in maternal body weight, increases in resorptions per litter, and increase in resorptions per implantation site in a group exposed for 3 weeks prior to mating and on days 1 to 16 of gestation. Decreased fetal weights and lengths and reduced ossification of the sternebrae and skull were observed in this group. Another inhalation study with Sprague-Dailey rats on days 6 to 15 of gestation looked at the effects of single or repeated short (1 x 0.5 hour, 3 x 0.5 hour) exposures to high concentrations. Decreased fetal weight was observed following repeated exposure to 800 and 1,200 ppm. Because these studies included exposures during gamete development, fertilization, and fetal development, they do not identify periods of sensitivity to Compound B-induced effects.

Studies of effects on sperm: A variety of studies have shown that sperm abnormalities and genetic changes, including dominant lethal mutations and heritable translocations, occurred in post-meiotic stages of sperm development. No effects were apparent in stem cells from which sperm develop. Studies in mice exposed to 200 or 400 ppm for 5 days by inhalation found increased frequencies of sperm abnormalities.

Dominant lethal mutation is determined by exposing males, mating them with unexposed females, and determining if fetal survival is affected. Inhalation studies in mice exposed to 0, 300, 400, or 500 ppm for 4 days found dose-dependent increase in dominant lethality, though 300 ppm was considered a slight effect. Another inhalation study in mice using 0, 300, 600, and 1,200 ppm for varying times (maintaining a total 1800 ppm-hour exposure) showed a dose rate effect; i.e., increased incidence with short exposure to a high dose rather than equal incidence for all groups. An upward curved dose-dependent increase in dominant lethal effects and heritable translocations has been observed in mice exposed for an extended period (8.5 weeks) to 165, 204, 250, 300 ppm. A dominant lethal effect was observed in offspring of male rats exposed to 1,000 ppm for 4 hours. The mechanism for these effects is not resolved, as stage-specific alkylation of specific proteins have been demonstrated, as well as alkylation of DNA. Although the literature tends to describe these as mutually exclusive options, this may not be the case.

Studies of effects on ova: Limited studies with Compound B indicate that exposure of females can result in altered pregnancy outcomes, likely due to genetic changes in oocytes. Studies with a related chemical have shown that transfer of oocytes to an unexposed mother does not alter the increased incidence of fetal deaths or externally abnormal fetuses.

Studies of fertilized egg (zygote) effects: Studies using inhalation (1,200 ppm) and ip injection (125 mg/kg) have demonstrated increases in fetal death and abnormalities among surviving fetuses when pregnant females are exposed shortly after conception. These effects are highly specific for particular developmental stages (e.g., inhalation exposures at 1 and 6 hours produced effects, while marginal changes were seen with exposures at 9 and 25 hours post-fertilization). The malformations observed were varied. Hydrop and eye defects were the major anomalies observed on day 17 of gestation among offspring of mothers exposed 1 and 6 hour post-fertilization. Other defects were small fetal size, cleft palate, and cardiac, abdominal wall, or extremity and/or tail defects. Deaths occurred from near the time of implantation until day 17 of

gestation when exposure occurred near fertilization. Injection (ip 125 mg/kg) 3 hour post-mating caused fetal deaths and cleft sternum. Skeletal defects due to zygotic exposure differ in kind from those following exposure during organogenesis. The mechanism for these effects is not clear because cytogenetic studies failed to show either structural or numerical chromosome aberrations.

Studies of organogenesis effects: Several studies have demonstrated that exposure during organogenesis can cause fetotoxicity and malformations. In a study in which F344 rats were exposed to 0, 10, 33, and 100 ppm on days 6 to 15 of gestation, no gross external abnormalities were observed. However, the high dose group had reduced fetal body weights and variations in ossification of vertebrae. Two groups of Sprague-Dailey rats exposed to 0 or 150 ppm on days 7 to16 of gestation and days 1 to 16 of gestation had reductions in fetal weights and lengths and decreased ossifications of the sternebrae and skull. Maternal toxicity was observed in the highest dose groups in CD1 mice dosed intravenously with 0, 75, and 150 mg/kg for three days beginning on day 4, 6, 8, or 10 of gestation. Mean fetal weights were reduced 20% in the high-dose groups, as were increased incidences of skeletal malformations. Studies in rabbits dosed intravenously during gestation found dose-related trends for decreased numbers of live fetuses per litter and increased resorptions when dosed days 6 to 14 of gestation.

5.0 ADDITIONAL DATA RELEVANT TO MODE OF ACTION

5.1 Genotoxicity

Much data exist in the literature on the genotoxicity of Compound B using *in vitro* and *in vivo* systems, representing a wide range of prokaryotic and eukaryotic species. Resulting genetic damage includes formation of mutations, specific DNA adducts, increased micronuclei formation in mice and humans, and increased sister chromatid exchanges in peripheral lymphocytes of rats, rabbits, monkeys, and humans. Compound B is clearly a potent mutagenic, alkylating agent whether formed *in vivo* or from exogenous exposure.

5.2 Other Alkylation Targets

Compound B readily alkylates proteins, lipids, RNA, glutathione, and other small molecules present intracellularly or in bodily fluids (e.g. albumin and hemoglobin in blood). The relative importance of adduction of these other cellular molecules as compared to DNA remains an unresolved question. For instance, Compound B alkylates specific proteins in sperm responsible for maintaining DNA integrity. This protein alkylation occurs at those germ-cell stages that are sensitive to Compound B-induced toxicity.

Framework for Human Health Risk Assessment Colloquia Series

Colloquium #1

CASE STUDY "C"

1.0 INTRODUCTION

Compound C is a volatile halogenated hydrocarbon liquid classified by EPA as a Category 3 gas (relatively stable and low water solubility). It has been widely used as an industrial solvent and anesthetic and is a common ground water contaminant.

Compound C was used as an anesthetic due to its ability to depress central nervous system functions; sporadic reports of liver toxicity in humans were associated with this use. Acute nervous system toxicities are observed in animals. Results of epidemiological studies have been controversial with some studies suggesting increased cancer incidences while others do not; noncancer endpoints have not been well studied. The major findings reported in chronic animal studies include kidney and liver toxicity and carcinogenicity, and neurological effects. These effects will be the focus of this case study.

2.0 TOXICOKINETICS

Compound C is rapidly absorbed by the inhalation and oral pathways. Exhalation of unmetabolized Compound C is a major dose-dependent excretory pathway for both oral and inhalation exposures. Compound C is metabolized by a major oxidation pathway catalyzed by cytochrome P450 2E1 and a minor glutathione conjugation pathway, both primarily in liver. The product of the oxidative pathway is an aldehyde which spontaneously adds water, CAL. CAL is reduced to an alcohol (COH) which is conjugated and eliminated in urine. This conjugate is subject to varying amounts of enterohepatic recirculation in different species. CAL can also be oxidized, forming CCOOH. The haloacid is excreted in urine along with other minor metabolites. The glutathione conjugation pathway involves several steps leading to formation of a cysteine derivative, CCYS. CCYS can then be conjugated and excreted in urine or metabolized to a reactive species.

Qualitatively, the pathways are similar across humans, rats, and mice, but quantitatively there are substantial differences. Mice metabolize Compound C very rapidly (more than predicted by body weight scaling), while humans clear CCOOH relatively slowly. Significant interindividual metabolic variations have been observed in humans given a single dose of Compound C or CAL as indicated by urinary excretion of CCOOH ranging from 5 to 50% of the oral dose. Induction of 2E1 by ethanol is observed, although at low concentrations Compound C metabolism is perfusion limited and the increased metabolic capacity will not increase the amount of Compound C metabolized. In addition, there are differences in the extent of CCYS formation,

and the subsequent split between conjugation and formation of reactive species. A polymorphism of the glutathione transferase is known to exist in humans; about 10% of the population is lacking this particular isoform.

3.0 EFFECTS IN HUMANS

3.1 Liver Toxicity and Carcinogenicity

Liver toxicity (noncancer) has been sporadically reported following anesthesia, occupational use, or accidental/intentional ingestion in medical case reports, but it is unclear if other factors were primarily responsible (e.g. preexisting disease). Several epidemiological studies reported no statistically significant increased liver cancer risks in workers exposed to Compound C. A review panel, however, judged that available data in aggregate indicates a slight increase in biliary/liver tumors.

3.2 Kidney Toxicity and Carcinogenicity

Kidney toxicity (noncancer) has been reported sporadically in humans. Studies at one factory where workers were frequently exposed to high concentrations have found tubular degeneration and increases in kidney carcinomas. Concentrations were not measured, so estimates of possible concentrations have been based upon reports of neurological effects such as dizziness. Several other well-conducted epidemiological studies of workers exposed to lower concentrations have found no increase in deaths due to kidney cancer.

3.3 Neurotoxicity

Neurological effects are associated with exposures to a wide range of concentrations of Compound C in air. Anesthesia required approximately 2,000 ppm. Controlled studies with volunteers exposed for short times (hours) found neurological effects including sleepiness, reductions in motors skills, and altered rates of breathing and heart beat. One study (200 ppm for 7 hours for 5 days) reported mild fatigue and sleepiness. Another study (27 and 81 ppm for 4 hours) reported a slight trend toward slower pulse rate. A third study (200 ppm for 2.5 hours) found no effect on heart beat or breathing rates. A fourth study (110 ppm for 8 hours) found decreased performance on skills tests. Controlled studies with exposure to the metabolites, CAL and COH, report similar effects.

4.0 EFFECTS IN ANIMALS

4.1 Liver toxicity

Liver toxicities observed in acute and subchronic studies in mice and rats included increased liver weight to body weight ratio, hypertrophy, small increases in serum levels of liver enzymes, and limited necrosis. These effects were dose dependent both for severity and incidence over dose ranges of approximately 50 to 2,000 mg/kg/day (by oral gavage) and 25 to 600 ppm (by inhalation). Chronic studies in multiple rat strains report no significant pathology in liver . Increased hepatocellular adenomas and carcinomas were found in mice chronically exposed to

approximately 1,000 and 2,000 mg/kg/day or 300 and 600 ppm. Incidences were much higher following corn oil gavage dosing than inhalation exposure. Tumors were observed only in mice following dosing with CAL and CCOOH. A 37 week study with CCYS exposed mice did not find an increase in liver tumors. Acute liver toxicity is increased by several compounds such as ethanol and phenobarbital, reflecting some combination of increased Compound C metabolism at high doses and alterations in the development of liver toxicity.

4.2 Kidney Toxicity

Kidney toxicity, described as degenerative changes in tubules, has been observed in mice and rats of both sexes following chronic oral or inhalation exposure (mice: 1,000 [LOAEL] and 2,000 mg/kg/day; rats: 50 [NOAEL], 250, 500 and 1,000 mg/kg/day or 100 [NOAEL], 300, 600 ppm). This effect is truly chronic; reexamination of tissues from 90 day exposures found only slight indications of kidney toxicity at doses higher than used in the chronic studies. Incidences were particularly high following high dose corn oil gavage exposure of rats. This kidney toxicity is believed responsible for increases in mortality in these chronic studies. Low (<10%, generally 1 or 2 animals per group of 50) incidences of kidney tumors were reported in five strains of male rats in several studies, with statistical significance achieved only in one. CCYS dosing of mice produced kidney toxicity, but not tumors in a 37 week exposure; no lifetime studies are available in rats or mice.

4.3 Neurotoxicity

Chronic studies reported altered behavioral effects in high dose animals (e.g., mice at 1,000 and 2,000 mg/kg/day; rats at 500 and 1,000 mg/kg/day) exposed orally; no data were reported for inhalation studies. In a 42 day study with rats exposed to 50, 100, and 300 ppm increases in brain waves indicative of sleep occurred with dose as did decreases in heart rate. Similar effects have been reported in animals exposed in acute and subchronic exposures to CAL and COH; no neurological effects are observed following dosing with CCOH.

5.0 OTHER DATA RELEVANT TO MODE OF ACTION

5.1 Genotoxicity

Genotoxicity has been the subject of numerous studies with Compound C, CAL, CCOOH, CCYS, and some other minor metabolites of Compound C. No effects are associated with the parent compound. *In vitro* studies with Compound C including metabolic capability have largely been negative, but some positives or equivocal positives have been reported. Some of these latter studies reflect a mutagenic stabilizer, while others used pure material. Studies of CAL have found it to cause clastogenesis and aneuploidy. Studies with CCOOH have been negative. Finally, CCYS is mutagenic in several *in vitro* assays.

5.2 Liver

Liver effects have been observed following exposures to Compound C, CAL, and CCOOH; liver tumors (hepatocellular adenomas and carcinomas) were observed in mice, but not in rats. These species differences in response reflect quantitative pharmacokinetic differences and differences in pharmacodynamics. The acid (CCOOH) is known to cause a range of effects in liver via the peroxisome proliferator-activated receptor (PPAR). Activation of PPAR by a wide range of compounds leads to pleiotrophic responses in the liver. Early liver effects of Compound C exposure include hypertrophy due in large part to proliferation of the subcellular organelles - peroxisomes, induction of specific cytochromes P450 involved in lipid and xenobiotic metabolism, and a brief period of cell proliferation. These responses occur to a much greater extent in mice than rats. Metabolism of Compound C to the acid is also significantly greater in mice than in rats.

Increases in liver to body weight ratio (due to the cell proliferation and enlargement) follow both inhalation and oral exposures to Compound C. A maximum liver weight is reached with increasing dose or for a single concentration, with increasing time up to about 30 days. Studies with other compounds have demonstrated that peptide factors are produced in response to this growth stimulus and stop the cell proliferation and liver enlargement. A selective environment is created due to the continued presence of the original mitogenic stimulus and the antimitogenic signal. Under these conditions, a subsequent genetic change allowing a cell to escape the antiproliferative signal will permit it to proliferate in response to mitogenic stimulus. A number of commonly used human pharmaceuticals activate PPAR, but the pleiotrophic responses observed in rodents with CCOOH do not appear to occur in humans exposed to these PPAR inducers. Structural characteristics of PPAR differ between mice, rats, and humans and PPAR expression is lower in humans.

5.2 Kidney

An extensive database with related compounds and metabolites, including CCYS, has demonstrated that metabolites of CCYS can lead to kidney toxicity, such as tubular degeneration.

Several aspects of kidney disease in exposed factory workers have been studied. Among those workers with kidney cancer, all had varying degrees of tubular damage. Comparable kidney cancer patients without high exposures to Compound C showed tubular damage in about a half of the cases. Alterations in a kidney-specific tumor suppressor gene were observed in 100% of the Compound C exposed workers while these alterations were observed in 33 to 55% of those with kidney cancer but not exposed to the chemical.

5.3 Neurotoxicity

Studies with the metabolites CAL and COH, as mentioned previously, have demonstrated acute or subchronic effects in humans and animals; animal studies reported no effects following CCOOH dosing. An analysis of studies using Compound C or COH was carried out to evaluate potential internal dose metrics in relationship to observed effects. The 42 day animal study showed a nonlinear (curved) dose response curve when altered brain waves or heart beat were plotted versus Compound C exposure dose or estimated peak blood concentrations of Compound C. Versus peak blood concentrations of COH, the response gave a straight line, one indicator of a direct dose response relationship. Analysis of the controlled human studies found peak COH concentrations similar to or greater than those in the 42 day animal study.

Framework for Human Health Risk Assessment Colloquia Series

Colloquium #1

CASE STUDY "D"

1.0 INTRODUCTION

Compound D is a gas at ambient temperatures and is soluble in water. It is a major industrial chemical intermediate for synthetic purposes and arises from bacterial breakdown of related compounds in the environment.

Compound D causes nonneoplastic, preneoplastic, and neoplastic changes in liver which are the focus of this case study; effects in other target organs occur at higher doses.

2.0 TOXICOKINETICS

Human and animal data indicate that Compound D is rapidly and efficiently absorbed via the inhalation and oral routes, rapidly converted to water-soluble metabolites, and rapidly excreted.

Compound D is metabolized mainly by the liver and, at low concentrations, metabolites are excreted primarily in urine. At high exposure concentrations, unchanged Compound D is also eliminated in exhaled air. Overall, the data indicate that neither Compound D nor its metabolites are likely to accumulate in the body.

The primary route of metabolism of Compound D is by the action of the cytochrome P450 2E1 on Compound D to form an epoxide (DO). DO is a highly reactive, short-lived epoxide that rapidly rearranges to form an aldehyde (DALD), also a reactive compound. Metabolite DO is also a substrate for epoxide hydrolase. These two metabolites are detoxified mainly via glutathione (GSH) conjugation.

3.0 EFFECTS IN HUMANS

3.1 Cancer effects

Several independent retrospective and prospective cohort studies demonstrate a statistically significant elevated risk of liver cancer, specifically angiosarcomas, from exposure to Compound D. Liver angiosarcomas are an extremely rare tumor, with only 20 to 30 cases per year reported in the U.S. Since the introduction of the Compound D manufacturing, a significant percentage of reported angiosarcoma cases have been associated with Compound D exposure.

3.2 Histopathological Liver Changes

Occupational studies have also associated Compound D exposure with impaired liver function and/or biochemical or histological evidence of liver damage. Such damage includes hypertrophy and hyperplasia of hepatocytes, activation and hyperplasia of sinusoidal lining cells, fibrosis of the portal tracts and the septa and intralobular perisinusoidal regions, sinusoidal dilation, and focal areas of hepatocellular degeneration.

4.0 EFFECTS IN ANIMALS

Studies in rats, mice, and hamsters administered Compound D via both the oral and inhalation routes indicate liver toxicity. These studies have all reported increased incidences of liver angiosarcomas. Hepatocellular carcinomas have been reported only in rats exposed orally. As described below, altered hepatic foci are observed at low doses, but it is unclear whether to consider these a noncancer effect or simply a very early effects in the cancer process. Other studies have reported increased liver weight and necrosis at relatively high doses compared to the lowest giving rise to cancer.

4.1 Critical Studies in Rats

Wistar rats were administered diets containing 10% polyCompound D with varying proportions of Compound D monomer. Diets were available to experimental animals for 4 hours per day and food consumption and Compound D concentrations were measured at several times during the feeding period in order to account for loss of Compound D from the diet due to volatilization. This information was used to calculate the ingested dose. Evaporative loss averaged 20% over 4 hours. The ingested dose was adjusted downward by the amount of Compound D measured in the feces to arrive at the bioavailable doses of 0, 1.7, 5.0, or 14.1 mg Compound D/kg/day which were fed to Wistar rats (n = 80, 60, 60, and 80, respectively) for a lifetime. An additional group of 80/sex were administered 300 mg/kg bw/day, by gavage in oil for 5 days/week for 83 weeks. Rats were weighed at 4 week intervals throughout the study. Hematological values were obtained at 13, 26, 52, 78, and 94 weeks, and blood chemistry was performed at 13, 26, 52, and 106 weeks (n=10). Urinalysis was performed on 10 animals per group at 13, 25, 52, 78, and 94 weeks. All surviving animals were necropsied at week 135 (males) or week 144 (females). Interim sacrifices of 10 animals at 26 and 52 weeks included animals from the control and high dose group.

There was no difference in body weights in the Compound D treated animals, although all groups (including the control) weighed significantly less than the controls fed ad lib (treated animals had access to food for only 4 hours/day). Significant clinical signs of toxicity in the 5.0 and 14.1 mg/kg/day groups included lethargy, humpbacked posture, and emaciation. Significantly increased mortality was seen consistently in males at 14.1 mg/kg/day and in

females at 5.0 and 14.1 mg/kg/day. No treatment-related effects on hematology, blood chemistry, or urinalysis parameters were observed. Relative liver weight was significantly increased at 14.1 mg/kg/day, but was not reported for the other dose groups.

A variety of liver lesions were observed histologically to be dose-related and statistically significant in male and female rats. These included clear cell foci, basophilic foci, eosinophilic foci, neoplastic nodules, hepatocellular carcinoma, angiosarcoma, necrosis, cysts, and liver cell polymorphism. Several of these endpoints were significantly increased in the group exposed to 1.7 mg/kg/day. Of the above lesions, all except the angiosarcoma derive from hepatocytes; angiosarcoma is derived from sinusoidal cells. The neoplastic nodules, cysts, and altered hepatocellular foci are proliferative lesions indicative of changes in the cells from which hepatocellular carcinomas are derived. However, an ambiguity in the designation of neoplastic nodules should be noted. This study designated the lesions as neoplastic nodules according to the criteria of Squire and Levitt (1975). More recent diagnostic nomenclature adopted by the National Toxicology Program (NTP) uses the terms "hepatocellular adenoma" and "hepatocellular hyperplasia" for the lesions previously diagnosed as "neoplastic nodules". The NTP classification reserves the term hyperplasia for "proliferative lesions that are perceived to be a secondary, nonneoplastic response to degenerative changes in the liver." By contrast, the report states, "foci of cellular alteration, hepatocellular adenoma, and hepatocellular carcinoma are believed to represent a spectrum of changes that comprise the natural history of neoplasia."

Thus, the "neoplastic nodules" observed in this study include both neoplastic and nonneoplastic lesions, and the altered hepatocellular foci are preneoplastic lesions. Consistent with this designation, the foci occur at lower doses and higher incidences than the hepatocellular carcinomas. These lesions occur at doses one to two orders of magnitude lower than other liver lesions. The incidence of necrosis was increased in a dose-related manner that was statistically significant in males at 14.1 mg/kg/day and in females at 5.0 mg/kg/day. Proliferation of sinusoidal cells showed a dose-related increase in males, but did not achieve statistical significance.

This study defines a NOAEL of 1.7 mg/kg/day and a LOAEL of 5.0 mg/kg/day for liver effects that are not thought to be preneoplastic. Increased tumor incidence was noted in all treated groups. Almost exclusively angiosarcomas were observed in males and females administered 300 mg/kg/day by gavage, while a mixture of angiosarcomas and hepatocellular carcinomas was observed at the mid- and high dietary doses. Only hepatocellular carcinomas were reported at the low dose.

The lifetime dietary study was performed in order to study a range of oral doses below that delivered in the previous study, since tumors were observed at all doses in the previous study. The oral doses were delivered in the same way except that the diets contained a final concentration of 1% polyCompound D, rather than 10%. Wistar rats (100/sex/dose) were administered doses of 0, 0.014, 0.13 or 1.3 mg Compound D/kg/day for 149 weeks. Mortality differences were not remarkable for males, but were slightly increased for females receiving 1.3 mg/kg/day. Relative organ weights were not evaluated. Angiosarcomas were observed in one high-dose male and two high-dose females. Other significant increases in tumors were limited to neoplastic nodules in females and hepatocellular carcinomas in males. An increased incidence of basophilic foci was observed in both sexes at 1.3 mg/kg/day and only in females in the two lower dosage groups. Rats exposed to 1.3 mg/kg/day also had a significantly increased incidence of liver cell polymorphism, hepatic cysts, neoplastic nodules, and hepatocellular carcinoma. No increases in nonneoplastic endpoints were observed.

5.0 ADDITIONAL DATA RELEVANT TO MODE OF ACTION

5.1 Genotoxicity

In vitro genotoxicity assays indicate that Compound D is mutagenic, causing point mutations in the presence of exogenous metabolic activation. Similar assays show that the major Compound D metabolite, Compound DO, is positive without metabolism in genotoxicity tests. *In vivo* genotoxicity tests with Compound D also provide evidence of genotoxicity. DNA adducts formed from Compound D metabolites have been identified following both *in vivo* and *in vitro* exposures. These include a major, but short lived metabolite and several minor, but more persistent adducts.

5.2 Role of Metabolites

Compound D must be metabolized to cause toxicity or carcinogenicity. The reactive, short-lived metabolites, Compound DALD and Compound DO, are responsible for the toxic and carcinogenic effects of Compound D. Both Compound DALD and Compound DO can react with tissue nucleophiles, but Compound DALD appears to be the most important source of tissue protein adducts. Compound DO is the reactive metabolite responsible for DNA adducts. In part this difference may result from the ability of the more lipophilic metabolite Compound DO to reach the nucleus, as opposed to Compound DALD which, although it is produced in greater quantities, is too water soluble to cross the nuclear membrane.

5.3 Liver tumorogenesis

Mutations in the p53 tumor suppressor gene are the most common gene alteration identified in human cancers and have been associated with human hepatocellular carcinomas and angiosacrcomas, including those due to exposure to Compound D. *Ras* oncogene mutations have also been found in human liver cancers; Compound D-induced human angiosarcoma is also associated with mutations of *ras* oncogenes. Rodent liver tumor response is more variable in nature. While liver angiosarcoma is a rare tumor in all species, hepatocellular carcinoma has a high spontaneous incidence in some rodent strains. Knockout of the p53 tumor suppressor gene in mice results in the spontaneous development of angiosarcomas, along with malignant lymphomas, but not hepatocellular carcinoma. In contrast, accelerated development of hepatocellular carcinomas in rodents is associated with overexpression of the *myc* and *ras* oncogenes, but not with mutational loss of p53 function. Rat angiosacrcomas due to Compound D exposure show mutations of p53.

Framework for Human Health Risk Assessment Colloquia Series

Colloquium #1

CASE STUDY "E"

1.0 INTRODUCTION

Compound E is a common contaminant found in drinking water. It is an element that exists in a variety of oxidation states, complexes (e.g., oxides), and organic derivatives (e.g., methylated forms). These various forms occur naturally, synthetically, or as byproducts of industrial processes.

A range of external (skin) and internal toxicities have been observed. Oral exposures have resulted in nonneoplastic and neoplastic skin diseases, cardiovascular effects, irritation of the gastrointestinal tract, anemia, and cancers of the lung, bladder, kidney, and perhaps other internal organs. Inhalation exposures have been associated with nonneoplastic and neoplastic changes in the respiratory tract (e.g., lung).

This case study describes chronic toxicities associated with Compound E exposures.

2.0 TOXICOKINETICS

Following exposure, Compound E is well absorbed by the oral and inhalation routes; dermal data is lacking. The two major inorganic oxidation states of Compound E are interconverted in the body. The other major metabolic fate of Compound E is methylation in the liver; methylated forms are the major urinary metabolites. The reduced form interacts with sulfhydryls, particularly neighboring sulfhydryls that result in a 5-membered ring as the product. This is a major contributor to toxicity, although the oxidized form can substitute for phosphorus in a wide variety of endogenous compounds (e.g., ATP) so it may contribute to toxicity.

Metabolic pathways in humans and rodents are qualitatively similar with no striking quantitative differences beyond those associated with typical interspecies differences of scale. Methylation is essentially linearly related to metabolism with increasing dose in humans, though under controlled experimental conditions saturation of methylation can be demonstrated at sufficiently high acute doses. Similar findings occur in mice.

3.0 EFFECTS IN HUMANS

3.1 Noncancer

Ingestion of Compound E by humans is usually not associated with serious injury to the respiratory system, although pulmonary edema and hemorrhagic bronchitis may occur in moderate to severe cases. Insufficient data exist on the exposure levels in these studies to identify a no-effect level for respiratory tract irritation with confidence, but it appears such effects are minor or absent at exposure levels of about 0.1 to 1 mg/m^3.

A number of studies in humans indicate that Compound E ingestion may lead to serious effects on the cardiovascular system. Long-term low-level exposures may also lead to damage to the vascular system. The disease is characterized by a progressive loss of circulation in the hands and feet, leading ultimately to necrosis and gangrene. Studies indicate that ingestion of 0.6 to 0.8 ppm Compound E in drinking water (corresponding to doses of 0.02 to 0.06 mg/kg/day, depending on age) leads to circulation changes. Workers exposed to Compound E dusts may also have an increased incidence of Raynaud's disease and an increased constriction of blood vessels in response to cold at exposure levels above about 0.05 to 0.5 mg/m^3.

Anemia and leukopenia are common effects of Compound E poisoning in humans, and have been reported following acute and chronic oral exposures. Hematological effects are usually not observed in humans exposed to levels of 0.07 mg/kg/day or less, although intermediate-duration exposure to 0.05 mg/kg/day resulted in mild anemia in one study. Although anemia is often noted in humans exposed to Compound E by the oral route, red blood cell counts are usually normal in workers exposed by inhalation. The reason for this apparent route specificity is not clear, but might simply be related to dose.

A number of studies in humans exposed to inorganic Compound E by the oral route have noted signs or symptoms of hepatic injury. Clinical examination often reveals that the liver is swollen and tender, and analysis of blood sometimes shows elevated levels of hepatic enzymes. These effects are most often observed after chronic exposure to doses of 0.019 to 0.1 mg/kg/day. Histological examination of the livers of persons chronically exposed to similar doses has revealed a consistent finding of portal tract fibrosis leading in some cases to portal hypertension and bleeding from esophageal varices. Hepatic toxicity has not been investigated in humans following inhalation exposure.

One of the most common and characteristic effects of Compound E ingestion is a pattern of skin changes that include generalized hyperkeratosis and formation of hyperkeratotic warts or corns on the palms and soles, along with areas of hyperpigmentation interspersed with small areas of hypopigmentation on the face, neck, and back. These effects have been noted in a large majority of human studies involving intermediate- or chronic-duration oral exposure. Numerous studies in humans have reported dermal effects at chronic dose levels ranging from about 0.01 to 0.1 mg/kg/day. Dermal effects are usually not mentioned in studies of persons exposed primarily by inhalation.

3.2 Cancer

Most epidemiological studies of Compound E carcinogenicity focus on populations drinking Compound E-containing waters or workers exposed occupationally by inhalation of smelter dusts. Other groups studied have included residents living near industrial releases, occupational cohorts, and humans treated medically with Compound E. Chronic oral exposures increased the risk of developing skin cancer and cancers of some internal organs; inhalation exposures increased risk for lung cancer.

The most widely studied location had well water containing Compound E concentrations ranging from 0.01 to 1.82 mg/L. The population in this area largely shared similar socioeconomic status and living conditions, including medical care, so that variations in Compound E levels were the only apparent major environmental difference. The study population was classified into four groups, according to concentrations in the wells: <0.1 ppm (13 towns), 0.1 to 0.29 ppm (8 towns), 0.3 to 0.59 ppm (15 towns), and greater than 0.6 ppm (6 towns). In this area, 10.6 people per 1000 were found to have skin cancer. The male to female ratio was 2.9 to 1 and the prevalence of skin cancer increased with increasing Compound E concentration in drinking water. Using age-adjusted mortality rates of this same population, significant dose-responses have been reported between Compound E levels in well water and mortality from several cancers. Skin, bladder, kidney, and lung cancers were reported most consistently while cancers of the nasal cavity, colon, liver, and prostate have been less frequently identified.

Some uncertainty exists concerning the quantitative comparability of the population of this area with others throughout the world. For instance, Compound E-induced skin cancer prevalence in the residents was increased by other risk factors including liver dysfunction among carriers of hepatitis B surface antigen and dietary factors. The liver cancers observed have been suggested to indicate interactions between hepatitis B, aflatoxin, and Compound E. Other potential risk factors that have been raised, but for which data are not always available include the oxidation state of the inorganic Compound E, the presence of other disease states whether Compound E-induced or not, smoking, and the length of exposure. Thus, studies from other populations exposed orally are of significant interest.

Epidemiological studies of drinking water exposure have been reported in other locations around the world. Findings of skin cancer or internal cancers have been mixed reflecting differences in many factors, including population size studied, drinking water concentrations, length of exposure, and length of time since exposure (latency period).

Two towns in a second location were compared with regard to Compound E levels in drinking water. The well for the exposed population was found to contain 0.41 mg/L, while the well in the control town had an Compound E concentration of 0.007 mg/L. Increased incidences in skin pigmentation were found. Of the exposed individuals found to have pigment alterations, 1.4% had ulcerative zones classified as skin cancer, but a statistically significant excess incidence of skin cancer has not been reported. Recent studies in these populations have measured chromosomal alterations in blood cells and found higher incidences among those with Compound E exposure compared to a control population.

Another cohort, comprised of individuals exposed to well water containing Compound E concentrations greater than 1 mg/L for about 5 years, was reported to have an increased observed

standard mortality ratio for both lung and urinary tract cancer, relative to expected mortality. This study also suggests synergism between oral Compound E intake and smoking for the development of lung cancer.

A study in another location evaluated 20,000 residents who were exposed long-term to drinking water that contained Compound E concentrations estimated at 0.17 to 0.33 mg/L as compared to a similar number of people with very little exposure. No significant differences in peripheral vascular disorders, peripheral neuropathy, or cancer frequency were observed. Studies of populations in a another location exposed to drinking water containing Compound E have been negative for skin cancer and internal cancers. Among residents in one region, no correlation was found between Compound E levels in drinking water and incidence of skin cancer. In this study, only 5% of water samples contained 100 mg/L or more as compared to 48% of the samples in the first studies described above which were positive. Another study evaluated the association between Compound E intake, which ranged from 0.0005 to 0.16 mg/L (mean 0.005 mg/L), and bladder cancer. No relationship was found between bladder cancer and either cumulative Compound E exposure or intake concentration. An ecological study of skin cancer cases did not find an increased incidence in the two counties presumed Compound E-exposed as compared to the control counties. No water concentrations are reported in this study. Several other studies from this country are also available with similar findings.

Although medicinal exposures to Compound E are not identical to drinking water exposures, studies of this population provide other data on cancer following oral intake. Cancers of the skin and internal organs have been reported. A significant excess incidence of fatal bladder cancer and a weak dose-response trend for respiratory cancer have been reported among treated with Compound E for periods ranging from 2 weeks to 12 years. It was also noted in these studies that among a group of patients examined for dermatological signs of Compound E exposure, all cancer deaths occurred among those showing evidence of skin disease.

Inhalation of Compound E dusts represents the other major route of exposure. Studies of several worker populations who were exposed to Compound E via inhalation, reported associations between occupational Compound E exposure and increased lung cancer mortality rates. One study established a dose response for increased respiratory cancer using categorization of low (<100 $\mu g/m^3$), medium (100 to 499 $\mu g/m^3$), high (500 to 4,999 $\mu g/m^3$) and very high (5,000 $\mu g/m^3$). Two studies used the multistage model of carcinogenesis to analyze inhalation data. In both cases, the effects of Compound E were found to likely be at late stages of the cancer process.

4.0 EFFECTS IN ANIMALS

Carcinogenicity of Compound E has been extensively studied in laboratory animals. Cancers did not result except in some studies with methylated forms following dosing with a genotoxic chemical as initiator (i.e. initiation-promotion protocol). Noncancer effects have been less extensively studied in laboratory animals. Histopathological observations of gastrointestinal irritation, blood alterations, dermal effects, and other noncancer effects observed in humans have generally not been seen with chronic exposure of rodents. Little data are available for some potential effects such as reproductive or developmental toxicities.

5.0 ADDITIONAL DATA RELEVANT TO MODE OF ACTION

5.1 Genotoxicity

Results from *in vitro* mutagenicity tests of Compound E with both bacterial or mammalian cells indicate that Compound E alone is either an inactive or extremely weak mutagen. Concentrations of Compound E that were weakly mutagenic were also cytotoxic.

Compound E has been reported to be a comutagen, enhancing the mutagenic response to ultraviolet (UV) light in E.coli, UV and methyl methanesulfonate (MMS) in Chinese hamster ovary (CHO) cells, and with N methyl-N-nitrosourea (MNU) in V79 cells. Clastogenic effects, such as sister chromatid exchanges (SCEs) and chromosomal aberrations, have also been observed following administration of Compound E compounds to mammalian cells in vitro. These aberrations were observed over the same concentration range for which cell transformation was observed, with the reduced form being more active than the oxidized form. SCEs were also observed for both chemicals. These types of clastogenic effects have also been observed in human cells following treatment with Compound E as have DNA-protein crosslinks.

No oncogene or tumor suppressor gene changes have been clearly associated with Compound E-induction of human tumors, though there is one report of an unusual spectra of mutational changes in the p53 gene in bladder tumors from Compound E exposed individuals. Alterations in this tumor suppressor gene are common late in human tumor processes, but are rare in rodent tumors. p53 plays a role in the check function for cell replication at the G1 → S checkpoint preventing replication of cells with DNA damage.

A study examined the frequency of lymphocyte chromosomal aberrations and of micronuclei in exfoliated oral mucosal or urothelial cells from residents of towns with low or high Compound E exposure. A significant increase in the frequency of lymphocyte chromosomal aberrations, consisting of chromatid or isochromatid deletions, was reported in the population with high Compound E compared to that with low exposure. Also, a significant increase in the frequency of micronuclei in oral mucosal epithelial cells or urothelial cells was observed.

5.2 Observations Focused On Proteins

Compound E is highly reactive with peptide and protein sulfhydryl groups. However, it is now known that Compound E can also be selective in this process, reacting with only a small number of closely spaced dithiol groups. One target is lipoic acid, a cofactor for pyruvate dehydrogenase involved in mitochondrial production of acetylCoA. Others include proteins important to DNA repair.

Compound E compounds, therefore, could cause or potentiate chromosomal damage by interfering with DNA repair enzymes. Different mechanisms may be responsible for the induction of chromosomal aberrations and SCEs. Restriction endonucleases that induce only DNA double-strand breaks have been shown to induce chromatid exchanges and deletions, but not SCEs. *In vitro* studies of DNA ligases (which contain closely spaced dithiols) involved in excision repair have shown that Compound E is a selective inhibitor of one of the two ligases present in Chinese hamster V79 cells. When these cells were treated with Compound E, no radiolabeled CTP was incorporated; following MNU treatment, which causes single strand breaks, dCTP was incorporated and then removed indicating DNA repair had occurred. When cells were treated with MNU followed by Compound E, there was no decrease in radiolabeled dCTP indicating repair had been inhibited. Further studies determined that DNA ligase II was involved. Decreases in the activity of alkyltransferase proteins, involved in removing alkylated bases from DNA, have been found with Compound E but were not dose dependent. Compound E is also known to induce expression of so-called heat shock proteins. These proteins play a variety of roles in cellular responses to stress; Compound E-protein complexes may appear "denatured" and induce this stress response.

Gene amplification can be an important process in carcinogenesis that can arise from chromosomal instability and recombination initiated by unrepaired single-strand breaks. Compound E treatment of mouse 3T6 cells results in a dose-dependent increase in colonies made methotrexate-resistant by amplification of the dihydrofolate reductase gene. The difficulty in detecting Compound E carcinogenicity in animals may be related to its ability to cause gene amplification, but not gene mutations. Amplification of an altered or activated oncogene may occur in a late stage of carcinogenicity and induction of this process could increase the incidence of tumors.

5.3 Oxidative Damage

Metabolic formation of free radicals and the production of oxidative stress may contribute to the toxicity of Compound E. In a series of experiments, the effects of Compound E on cultured human skin fibroblasts, CHO cells, and E-resistent cell lines were studied. The CHO cells were 10-fold less sensitive to acute toxicity than the human skin fibroblasts. Treatment with Vitamin E, an antioxidant, was partially protective in fibroblasts, but had no effects on CHO cell survival. Sensitivity to oxidative damage may be a function of cellular antioxidant capabilities which were greater in CHO than fibroblast cells. Management of oxidative stress may explain differential cell toxicity; some resistant cells have higher levels of heme oxygenase which may act by reducing cellular heme pools and thereby reduce oxygen radical formation.

Increases in single DNA strand breaks were observed in lungs of male ICR mice administered 1,500 mg/kg of a metabolite of Compound E. No increases were observed in several other tissues including liver and kidney. Because of the elution pattern, strand breakage was assumed to be caused by a free radical of this metabolite. Furthermore, clumping of heterochromatin in the nuclei of endothelial cells of the alveolar wall capillaries in these mice was attributed to radicals.

5.4 DNA Methylation

Methylation of DNA plays a major role in regulation of gene expression, both in normal tissues

and in preneoplastic and neoplastic tissues. Because Compound E is detoxified via methylation, its metabolism might alter DNA methylation which are dependent upon the same enzymes (methyltransferase) and methyl donor molecules (S-adenosylmethionine). Exposure of human lung adenocarcinoma cells to Compound E with two different oxidation states, but not a methylated metabolite of Compound E, produced significant dose-response hypermethylation in the promoter region of the p53 tumor suppressor gene. This was determined by restriction mapping and sequencing. Limited data also suggest that hypermethylation may exist over the entire genome in response to exposure of these cells to Compound E.

APPENDIX D

CHARGE TO THE PARTICIPANTS

Framework for Human Health Risk Assessment Colloquia Series

Colloquium #1

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 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 first colloquium will focus on the role of mode of action information as the basis of risk assessment approaches.

Charge to the Participants

Prior to the first colloquium, each participant is receiving: a paper entitled *Human Health Risk Assessment: Current Approaches and Future Directions;* a series of five case studies; a list of questions; a working definition of "mode of action"; and a list of the breakout groups.

Human Health Risk Assessment: Current Approaches and Future Directions was developed by a Risk Assessment Forum work group to serve as a perspectives piece. The paper discusses a number of issues regarding the Agency's risk assessment approaches and their scientific basis. It should be useful as a basis for further discussion of the scientific basis for current and future risk assessment approaches. We encourage each participant to read this document in preparation for the colloquium. These first two colloquia will focus primarily on the first issue, "Mode of Action/Dose-Response Considerations." The working definition of "mode of action" is intended to provide a frame of reference for this colloquium that can be employed both in the plenary sessions and the breakout groups.

The case studies and accompanying questions will guide the discussions throughout the colloquium. The colloquium will begin with more general questions, but participants will spend the bulk of the two days reviewing the individual case studies and the more specific questions. The colloquium's final session will address critical harmonization issues. It is important that each participant review all of the case studies, since each case study will be discussed in plenary session, as well as in detail in at least one breakout group.

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.

APPENDIX E

DISCUSSION POINTS

Framework for Human Health Risk Assessment Colloquia Series

Colloquium #1

DISCUSSION POINTS

Mode of action: For the purposes of this workshop, mode of action is defined as those key biological events that are directly linked to the occurrence of toxic responses. These events include absorption and entry into the body up to the final manifestation of toxicity.

I. General questions for discussion at the beginning of the workshop

A. What are the variety of different purposes for which EPA conducts risk assessments?

B. How has mode of action information been used in risk assessment to date?

C. Are there differences in the importance of mode of action information for conducting risk assessments for different human health endpoints/toxicities?

II. General questions for the case studies

A. What are the toxic effects associated with the compound?

B. How similar are the effects in studies of animals and humans?

C. How consistent are the data across species, routes of exposure?

D. At what administered doses or exposure concentrations are the effects observed?

E. What do we know about mode of action for the different toxicities?

F. Is mode of action influenced by dose (i.e., administered dose or exposure concentration)?

G. Are there commonalities in mode of action for the various toxicities?

H. Do we have enough information to determine a common critical event that leads to all subsequent toxicities for the compound? Is such a common precursor effect expected as a general rule?

I. Qualitatively, how does mode of action information influence decisions about choice of risk assessment models for the dose response analysis?

II. General questions for discussion at the end of the workshop

A. Given what is known about the mode of action of various compounds, is there a scientific basis for routinely assuming a different mode of action leading to carcinogenesis and other toxicological effects?

B. Mode of action information has been used to influence the approach for low dose extrapolation. Are there other areas where mode of action information should play a role in risk assessment?

C. How do you see mode of action considerations influencing quantitative aspects of risk assessment (e.g., uncertainty factors, dosimetric adjustments, etc.)?

APPENDIX F

BREAKOUT GROUP ASSIGNMENTS

Framework for Human Health Risk Assessment Colloquia Series

Colloquium #1

Breakout Group Assignments

Monday - Tuesday, September 29-30, 1997

Breakout Group 1
Case Studies A, B, & C
Chair: Jeanette Wiltse
Rapporteur: Vicki Dellarco

- Jim Cogliano
- Vicki Dellarco
- Gary Foureman
- Terry Harvey
- Michael Ioannou
- Annie Jarabek
- Gary Kimmel
- Aparna Koppikar
- Robert McGaughy
- Jennifer Orme-Zavaleta
- Jennifer Seed
- William Sette
- Paul White
- Yin-Tak Woo
- Wendy Yap

Breakout Group 2
Case Studies A, B, & D
Chair: Don Barnes
Rapporteur: James Rowe

- Charles Abernathy
- Carole Braverma n
- Jane Caldwell
- Chao Chen
- Marion Copley
- Dan Costa
- William Farland
- Oscar Hernandez
- Richard Hertzberg
- Kim Hoang
- Carole Kimmel
- Arnold Kuzmack
- David Lai
- James Rowe
- Hugh Tilson
- Bill Wood

Breakout Group 3
Case Studies A, B, & E
Chair: Dick Hill
Rapporteur: Mark Stanton

- Jerry Blancato
- Eric Clegg
- Kerry Dearfield
- Arnold Den
- Julie Du
- Susan Griffin
- Bob Kavlock
- Elizabeth Margosches
- Bill Pepelko
- Rita Schoeny
- Cheryl Siegel Scott
- R. Woodrow Setzer
- Mark Stanton
- Vanessa Vu
- John Whalan

APPENDIX G

AGENDA

United States Environmental Protection Agency
Office of Research and Development
Risk Assessment Forum

Framework for Human Health Risk Assessment Colloquia Series

Colloquium #1

Holiday Inn Arlington at Ballston
Arlington, VA
September 29-30, 1997

Agenda

M O N D A Y , S E P T E M B E R 2 9 , 1 9 9 7

8:00AM **Registration**

9:00AM **Welcome Remarks** . . . . . *William Wood, U.S. Environmental Protection Agency (EPA), Risk Assessment Forum, Washington, DC*

9:05AM **Goals of the Human Health Risk Assessment Framework** . . . . . *Vanessa Vu, EPA, Office of Pollution Prevention and Toxics (OPPT), Washington, DC*

9:20AM **Evolution of Human Health Risk Assessment: Using Biological Information to Define Modes of Action, Develop Exposure-Response Models, and Refine Default Assumptions** . . . . . *Rory Conolly Chemical Industry Institute of Toxicology, Research Triangle Park, NC*

9:50AM **Introduction to Case Studies and Colloquium Issues, and Charge to Breakout Groups** . . . . . *Mel Andersen ICF Kaiser, Inc., K.S. Crump Division, Research Triangle Park, NC*

10:15AM BREAK (move to Breakout Rooms)

10:20AM **Breakout Groups Convene to Address General Questions**

MONDAY, SEPTEMBER 29, 1997 (continued)

11:30AM **Plenary Session: Breakout Group Reports and Discussion**

12:30PM LUNCH (on your own)

1:30PM **Case Study A**
— Each of the three breakout groups convene to discuss Case Study A

3:00PM BREAK

3:15PM **Plenary Session: Case Study A Breakout Group Reports and Discussion**

4:00PM **Case Study B**
— Each of the three breakout groups convene to discuss Case Study B

5:00PM ADJOURN (Plenary session for breakout group reports and discussion for Case Study B will take place on Tuesday morning)

T U E S D A Y , S E P T E M B E R 3 0 , 1 9 9 7

8:30AM **Review of Day Two Charge**

8:50AM **Plenary Session: Case Study B Breakout Group Reports and Discussion**

9:30AM **Individual Breakout Groups Convene**

— Case Study C
— Case Study D
— Case Study E

11:00AM **Plenary Session: Case Study C Breakout Group Reports and Discussion**

11:40AM LUNCH (on your own)

12:40PM **Plenary Session: Case Study D Breakout Group Reports and Discussion**

1:20PM **Plenary Session: Case Study E Breakout Group Reports and Discussion**

2:00PM BREAK

2:15PM **Discussion of Critical Harmonization Issues**

3:00PM **Review of Progress Made in Colloquium 1 and Preview of Colloquium 2: Quantitative Concepts**

4:00PM ADJOURN

Keynote Speaker

Rory Conolly, a Senior Scientist at CIIT, provided the group with his views on risk assessment approaches, speaking about the relevance of mode of action and dose-response modeling in shaping future risk assessments. The central question he addressed in his presentation, entitled "Evolution of Human Health Risk Assessment: Using Biological Information to Define Modes of Action, Develop Exposure-Response Models, and Refine Default Assumptions," was, How do we move forward and bring the newer science into risk assessment at a reasonable and responsible rate? Highlights of the presentation are provided below; a copy of the speaker handout is presented in Appendix H.

Historical Perspective

In the 1970s, only a limited understanding of mechanism of action existed. Default methods and models were based on state of the science at that time and were therefore appropriate.

In deriving risk assessment methodologies, regulators have strived to minimize uncertainty and derive reasonable risk estimates, balancing the desire not to miss any risks with the desire not to overestimate risk and incur unnecessary compliance costs. Looking to the future, risk assessors should continue to seek to reduce uncertainty in risk assessment, using mechanistic data where possible to improve predictions of risk.

Where Are We Today?

Today we have a larger data base and a better understanding of mode of action in cancer and noncancer response. It is, therefore, appropriate to use the latest science and to update risk assessment practices.

A lag time between availability of new science and acceptance and use in practice is inevitable. Moving forward requires reaching consensus, which involves working out the details and developing methodology to use the new science.

How to Get Science Into Risk Assessments

As understanding improves, risk assessment policies need to be re-evaluated; EPA's new cancer guidelines show how the Agency is starting to do this.

More sophisticated validated models (PBPK and biologically based) for dose-response need to be developed. Ideally, we need models to describe the whole exposure response process. Exposure-response models are available but are not as well developed as PBPK models; they have been used for dioxin, 5-fluorouracil, chloroform, and formaldehyde.

When are models mature enough for widespread use? To be used in risk assessment, a model needs to be validated against animal and human data; receive adequate quality control; and be peer-accepted.

Challenges for Regulators/Where Is Risk Assessment Going?

Regulators face the challenge of incorporating evolving and more sophisticated approaches into risk assessment methodology. Guidelines need to be developed to identify acceptable models for use in risk assessment; criteria can be qualitative (e.g., taking component parts of a model, comparing it to the default approach, and deciding whether uncertainty is increasing or decreasing).

Evaluation of mechanistic data will not be easy. A wide spectrum of interactions/mechanisms exist; some do not result in toxic effects. Therefore, substantive questions exist concerning how one evaluates various biomarkers and relates them to toxicity.

Additional data are needed to fully understand the shape of the dose-response curve at low levels of exposure and to effectively incorporate these data into the quantitative risk assessment. Experimental work could be performed to address this knowledge gap.

Computer models have become cheaper, faster, and increasingly sophisticated. We can now incorporate biology into models (e.g., models showing airflow through the nasal passages of rats and humans)—something that could not be done 10 years ago. The nasal passage models demonstrate that detailed anatomical modeling can make a difference in risk predictions; this is a lesson for any organ with anatomical complexity—we should continue to develop such models and use them in risk assessment.

Defaults will continue to be important in risk assessment, but we need to keep up with the science. Some of yesterday's defaults will not be good enough for tomorrow. Most chemicals will not have rich data sets (may have limited but targeted data collection [e.g., PBPK models, short-term assays, predictive computer model]). Defaults will still need to

be used in the future, but they will be enriched by the newer science and modeling technologies.

Well-articulated risk assessment strategy can motivate research, specifically in terms of how biologically based modeling is incorporated into risk assessment. EPA could take the lead in specifying data needs (e.g., the kind of descriptive data needed, the criteria needed for validation of models, and the role human models should play in model validation). Promulgation of new risk assessment guidance using mechanistic data is needed to encourage industry to pay for research. Industry needs to be sensitive to the fact that some lag time is inevitable, but regulators should not let the lag time get too long.

Introduction to Case Studies and Colloquium Issues and Charge to the Breakout Groups

Melvin Andersen of ICF Kaiser, Inc. facilitated the colloquium. In his introductory remarks he encouraged the group to engage in active discussions on how to use mode of action information wisely. To open discussions, Dr. Andersen reviewed the definition of mode of action developed for the purposes of this colloquium.

> *Mode of action is defined as those key biological events that are directly linked to the occurrence of toxic responses. These events include absorption and entry into the body up to the final manifestation of toxicity.*

Dr. Andersen suggested that the group keep the following questions/issues in mind when thinking about mode of action.

What is the nature of the chemical causing the effect?

What are the initial interactions that a chemical has with macromolecules or cellular components?

All details may not be necessary, but the challenge lies in deciding on how to incorporate available new information.

Information on what is happening at the molecular level will continue to grow. New guidance emphasizes mode of action (e.g., IARC, NRC, EPA). Our choices are either to continue to be proactive or be reactive later.

Dr. Andersen charged the group to begin exchanging ideas and perspectives in the first breakout session, specifically discussing the definition of mode of action and general questions pertaining to mode of action and risk assessment (see Section Three).

Dr. Andersen then provided the group with a brief overview of the case studies, explaining that the case studies were developed to emphasize diverse issues and that the nine general

questions (see Section Four) provided to participants were intended to guide discussions. The first four questions are somewhat generic in nature, while the remaining questions focus more on mode of action and how information can be used to influence decisions on risk assessment approaches.

Questions/Comments

As summarized below, a brief group discussion followed the keynote address and introductory remarks by EPA and the facilitator.

One attendee questioned how feasible it might be to apply work done in the pharmaceutical industry (where a significant amount of human data and mode of action information are available) to developing PBPK models and validating existing animal models for the chemicals of interest to EPA, FDA, etc. Responses indicated that while some of this information is available for the therapeutic effects of anticancer drugs and has been used in the development of fundamental pharmacokinetic models, data are largely unavailable for the toxic effects of those drugs. The goal of most pharmacokinetic studies in the pharmaceutical industry is to get information on the therapeutic dose range, not to learn specifically how the chemical acts. PBPK models for pharmaceutical drugs have not been widely used for the type of extrapolations used by EPA in studying toxic chemicals (e.g., species or high to low dose extrapolations). The industry is beginning to see biologically based models as a good adjunct to human data. Such models may be used by the industry to evaluate developmental/reproductive effects where little human data are available. A few participants noted that acquiring any available data may be difficult because of confidentiality issues and the existence of a great deal of chemical-blind data.

Another participant commented that we may never have low-dose information for the endpoints EPA is currently studying, but emphasized the importance of starting to study mechanistic effects in the low-dose range and linking those events to the observed effect of regulatory interest.