ITER Peer Review on Acrolein, Acrylamide, & Acrylonitrile Meeting Summary

November 16 and 17, 1998
University of Cincinnati, College of Medicine
Cincinnati, Ohio
USA

An independent panel of expert scientists and risk assessors met on November 16 and 17 to review risk assessment documents on acrolein, acrylamide, and acrylonitrile. This meeting was conducted by Toxicology Excellence for Risk Assessment (*TERA*); a non-profit organization dedicated to the best use of toxicity data in risk assessment. Expert peer reviewers donated their time and talents to provide an independent review of the assessments. A comprehensive overall review of the materials was provided by the combined experience of all the reviewers.

TERA developed the acrylamide document that was reviewed at this meeting. In addition, *TERA* has written an inhalation cancer assessment on acrylonitrile, which was approved by an *ITER* panel and is now available on the *ITER* database. Therefore, to avoid conflict of interest, *TERA* staff did not select the reviewers for this meeting. Ms. Jennifer Orme-Zavaleta, a *TERA* Trustee, selected reviewers to provide appropriate expertise and a balance of organizational affiliation. Ms. Orme-Zavaleta chaired the acrylonitrile session and Ms. Bette Meek of Health Canada chaired the acrylamide session.

At the beginning of each chemical's discussion, the panel considered conflict of interest. Prior to the meeting, each reviewer either identified the potential for conflicts, or certified that he or she did not have a real or apparent conflict of interest associated with the chemical under review or the sponsor. *ITER* staff discussed possible conflicts with the reviewers and Ms. Orme-Zavaleta to determine if measures were needed to manage a potential conflict or appearance of conflict. Options include excluding the reviewer from a particular discussion and polling for consensus, or allowing the reviewer to participate in the discussion, but not to be polled for consensus. The peer review panel discussed and agreed upon how to manage any potential conflicts. This is summarized for each chemical below and documented in Attachment A.

These review meetings follow a standard format beginning with a close examination of the supporting documentation and important references several weeks prior to the meeting. At the meeting, after the conflict of interest discussion, the authors of the assessment or document briefly present their work. For chemical assessments, the panel then systematically discusses the assessment, starting with a discussion of the qualitative weight-of-evidence and a determination of whether adequate data exist on which to base a risk value. This is followed by a discussion of the appropriate critical endpoints and studies. Next, the quantitative aspects of the assessment are discussed, including proposed cancer risk estimates and non-cancer tolerable doses or concentrations. Specific

questions to focus the panel's review are provided to the reviewers with their charge and are considered and discussed at the meeting.

Full discussion and participation are encouraged and agreement is reached by consensus. Consensus, for the purpose of these meetings, is defined as "an opinion held by all or most, or general agreement." The meeting was open to the public and observers from the World Health Organization, Solutia, Cytec Industries Inc., the Acrylamide Monomer Producers Association, Consultox, Elkem Metals Company, Environmental Network and BP Chemicals were in attendance.

Assessment for Acrolein

Sponsor: Health Canada

Presenters: Ms. Bette Meek, Health Canada

Chair: Dr. Michael Dourson, *TERA*

Peer Reviewers:

- Dr. Marilyn J. Aardema, The Procter & Gamble Company
- Dr. John P. Christopher, California Environmental Protection Agency, Department of Toxic Substances Control
- Dr. Marvin Friedman, private consultant
- Dr. Michael L. Gargas, ChemRisk Division of McLaren/Hart
- Dr. George Leikauf, University of Cincinnati, Department of Environmental Health
- Dr. Martha Moore, U. S. Environmental Protection Agency, National Health and Environmental Effects Laboratory
- Dr. Robert G. Tardiff, The Sapphire Group, Inc.
- Dr. Vanessa T. Vu, U.S. Environmental Protection Agency, National Center for Environmental Assessment
- Dr. Vernon Walker, New York State Department of Health
- *Dr. Henry d'A. Heck of CIIT provided written comments which the panel considered and discussed.

The acrolein session opened with a discussion of the potential conflict of interest for the peer reviewers, referring to Appendix A - *Managing Potential Conflicts of Interest*. Several reviewers reemphasized that their comments would be their personal scientific opinions and not that of their employer and Dr. John Christopher asked that additional language to this effect be added to his entry in Appendix A. Dr. Robert Tardiff noted that he had done some work on acrolein for the National Academy of Sciences. The panel agreed that this would not present a conflict and that all panel members may participate

fully in discussions and polling for consensus. Dr. Michael Dourson, the chair, then gave a brief overview of the meeting process.

Ms. Bette Meek from Health Canada presented information on the acrolein assessment and their objectives of the peer review. The presentation was followed by a short period during which reviewers asked clarifying questions. The reviewers then discussed the assessment document, evaluating the hazard characterization for both oral and inhalation routes first, followed by the dose response assessment for each route. One of the observers, Dr. Richard Parent of Consultox, provided a few comments.

PRESENTATION AND CLARIFYING QUESTIONS:

Bette Meek of Health Canada made a brief presentation on the acrolein assessment document. Hazard characterization and dose-response analyses for acrolein are based almost exclusively on studies in animals because of the inadequacy of human data. In animals, acrolein is highly acutely toxic after oral administration, and it is also irritating to the respiratory tract and eyes following inhalation, and to the skin following dermal exposure. Available data are inadequate to serve as a basis for assessment of the carcinogenicity of acrolein following inhalation exposure. The non-cancer assessment for oral exposure was based on a No Observed Effect Level (NOEL) of 0.15 mg/ml in a 13-week study in female rats (NTP 1998). Critical effects were hyperplasia, necrosis, inflammation, and hemorrhage in the forestomach. An uncertainty factor of 100 was applied (10 for interspecies variation and 10 for intraspecies variation), resulting in a Tolerable Concentration (TC) of 0.0015 mg/ml. Since the data from the NTP study were only preliminary, the derived TC is considered provisional. This value is expressed as a concentration, rather than a tolerable dose, since effects at the site of contact are more likely related to administered concentration than dose.

For inhalation exposure, a Tolerable Concentration of 0.11 ug/cu.m was proposed, based on results from a 3-day study in male Wistar rats (Cassee et al. 1996). The critical effect was identified as disarrangement, necrosis, thickening, desquamation, and hyperplasia in the nasal respiratory epithelium. Benchmark concentration modeling was performed with the lower 95% confidence limit (BMCL05) equal to 0.06 mg/cu.m. This was adjusted to a continuous exposure and an uncertainty factor of 100 (10 for interspecies variation and 10 for intraspecies variation) was applied. An uncertainty factor for use of a short-term study was not included because there is no indication that severity of the critical effect increases with duration of exposure.

The panel had no clarifying questions on the presentation.

DISCUSSION

Hazard Identification

The review panel discussed the quality of the genotoxicity information. One reviewer indicated that the database for acrolein genotoxicity is not very rich and includes mixed results. There was discussion on whether data on adduct formation or DNA-protein crosslinks were from isolated DNA or tissue homogenates, or from whole cells. One reviewer noted that there are several publications showing DNA-protein crosslinks in a human airway epithelial cell line after acrolein exposure. Reviewers suggested that the discussion on DNA binding, DNA adducts, and crosslinks might clarify which systems resulted in the specific endpoints.

The panel discussed the presentation of the genotoxicity data in the document. It was suggested that the text should evaluate study quality, for example, noting weak responses and the lack of sensitivity of the hypoxanthine-guanine phosphoribosyltransferase (HPRT) assay. Information on the degree of cytotoxicity should be provided, since negative results from studies that did not achieve sufficient cytotoxicity should be considered "no tests," rather than true negatives.

One reviewer suggested that the risk of heritable damage should be discussed in the section on genotoxicity (Mutagenicity and Related End-Points), while cell transformation and DNA-protein cross-links should be discussed in the Mechanism section. Other reviewers preferred discussing all genotoxicity data in one section, in light of the potential relationship between mutagenesis and carcinogenesis. Health Canada noted that they are currently working on the best format for the genotoxicity and mode of action sections in their documents.

Health Canada specifically asked the panel whether the document had adequately considered data on other aldehydes, or if there are other relevant data that might contribute to a greater understanding. A reviewer stated that although many people consider formaldehyde DNA adducts to be related to mechanism and toxicity, the data only support the use of DNA adducts as an index of exposure. Health Canada agreed to consider data submitted by reviewers to determine whether the comparison with other aldehydes could be strengthened. Health Canada agreed to strengthen the comparison with other aldehydes. However, Health Canada noted that in terms of irritation, the critical effect, acrolein is the most potent irritant of all the aldehydes they have assessed.

Several reviewers questioned the basis for the statement in the document that "Resultingly, it is possible that Tolerable Concentrations developed to protect against non-neoplastic effects may also be protective for potential carcinogenicity." Health Canada clarified that the concern for carcinogenicity was based on data from other aldehydes, however, insufficient data were presented in the document. The panel noted that the data are insufficient to determine *whether* acrolein is carcinogenic following inhalation, and so protection from potential carcinogenicity cannot be evaluated. The review panel recommended that the statement in question should be deleted.

The panel agreed the database is sufficient to develop noncancer values.

Inhalation - Choice of Critical Effect and Study

Health Canada proposed use of the study by Cassee et al. (1996) as the most sensitive of the inhalation studies in which the incidences of histopathological changes in the respiratory tract of experimental species have been reported. Degenerative changes were observed in the nasal epithelium of rats exposed by the nose only at the lowest levels (0.25 ppm or 0.57 mg/cu.m) of acrolein vapor for 6 hours per day for 3 days.

One reviewer pointed out that the availability of histopathology in the upper and lower airways provides strong support for use of this study. The panel discussed whether the response was reversible. Health Canada stated that the minimal inhalation data on reversibility suggest no recovery within a 48-hour period. The chair noted that reversible lesions are considered by several groups to be appropriate as critical effects, although a reversible lesion may affect the choice of uncertainty factors.

The panel agreed to the selection of the Cassee et al. (1996) study and the critical effect.

Inhalation - Dose-Response Assessment

Choice of Concentration: Health Canada proposed use of the 95% lower confidence limit of the estimated benchmark concentration (BMCL05) of 0.06 mg/cu.m for effects on nasal respiratory epithelium in male Wistar rats (Cassee et al. 1996).

There was significant discussion concerning the use of benchmark concentration modeling or a LOAEL for calculation of the tolerable concentration. Health Canada acknowledged that this study is not ideal to derive a BMC as there is no no-effect level and only two doses (in addition to the control) were tested. The BMC process was used as an attempt to find the effect level. One reviewer suggested using a higher response level, proposing that the 95% lower confidence limit on concentration corresponding to a 5% response level was beyond the capabilities of the method. It was noted that the small sample size in the study (5 animals/group) led to very wide confidence limits, and thus a conservative estimate. Use of the central tendency would avoid such over-conservatism. Health Canada acknowledged that the choice of a 5% response and the lower bound were arbitrary, but there was a desire to be consistent with other Health Canada assessments, and with the approach used for other aldehydes.

Several reviewers preferred using the LOAEL (0.57 mg/cu.m) with an uncertainty factor to account for the absence of a NOAEL. These reviewers considered the BMC approach less transparent, particularly if the central tendency is used. It was also noted that the lower bound on the BMC (0.06 mg/cu.m) was essentially identical to the LOAEL (0.57 mg/cu.m) divided by 10, although other reviewers objected to such a post-hoc analysis.

Upon initial polling, the review panel preferred the use of the LOAEL approach for this dataset. After additional discussion, however, the group agreed that both approaches (LOAEL and BMC) should be presented. For the BMC, it was suggested to present both the 5% central estimate and the 95% lower confidence estimate.

Health Canada clarified that a duration adjustment of 5 days/7 days is not appropriate and that the only adjustment should be for 6 hours/24 hours. Health Canada noted that there are no data which directly address the need for the adjustment for intermittent to continuous exposure - i.e., there are no directly comparable data where effects were examined in animals exposed continuously versus intermittently. One reviewer noted that effects were observed at the low concentration that were not considered adverse. Therefore, the low concentration is not below a threshold for all effects and severity would increase with continuous exposure. One reviewer proposed either keeping both 6/24 and 5/7 factors or dropping both. Another reviewer suggested removing both factors and adding an uncertainty factor to account for intermittent exposure to continuous exposure because the difference between intermittent exposure and continuous exposure is similar to pharmacodynamic uncertainty in human response. The panel reached consensus that Health Canada should drop the factor of 5/7, but keep the factor of 6/24, and explain more fully the rationale for using 6/24 in the document (as above).

Uncertainty Factors for the BMC Approach: Health Canada proposed an uncertainty factor of 100 (10 for interspecies variation and 10 for intraspecies variation). In view of the fact that there appears to be no indication that severity of the critical effects increase with duration of exposure, an additional uncertainty factor to address the use of a short-term study as the basis for the Tolerable Concentration was not considered appropriate by Health Canada. No additional quantitative element was included to address limitations of the database such as the lack of an adequate carcinogenesis bioassay via the inhalation route. Health Canada also noted that while further studies of the potential relative roles of cytotoxicity, cell proliferation and DNA-protein cross-links observed *in vitro*, are desirable, chronic studies via ingestion are available.

In discussion of the uncertainty factor for interspecies differences, one reviewer stated that data from formaldehyde indicate that monkeys would be less sensitive than rodents. Another reviewer disagreed, and noted that rodents are seven times less sensitive than dogs and monkeys to acrolein in terms of pulmonary lesions and therefore, the default of 10 should be used. Health Canada noted that the data on sensitivity of monkeys to effects of inhaled acrolein are conflicting. The panel reached consensus that an uncertainty factor of 10 should be used for differences between animals and humans.

For intraspecies differences, the panel agreed on the default value of 10 because available data are inadequate to suggest using a different value. One reviewer, while agreeing with the use of a 10-fold factor, noted that there are large human genetic differences in acrolein metabolism enzymes, such as alcohol dehydrogenase, aldehyde dehydrogenase, and GSH dehydrogenase. Another reviewer indicated that the dose-response curves for formaldehyde between asthmatics and healthy people are similar, although another reviewer noted that this is not the case for acetaldehyde dose response.

The review panel also discussed whether an uncertainty factor for database is needed. This factor can include LOAEL to NOAEL extrapolation, and subchronic-to-chronic extrapolation under the Health Canada approach. Several reviewers agreed that progression with increased exposure duration is not an issue. Because the endpoint is

irritation, exposure below the irritation threshold will not result in an effect no matter how long the duration. Another reviewer noted that eye irritation increases at increasing exposure duration, although this effect is sensory irritation, rather than direct irritation. One reviewer indicated that a short-term acrolein exposure study in his lab in which animals were exposed for 6 hours/day for 14 days showed a cumulative effect on airway inflammation and mucus secretion. There was general consensus that cumulative effects are observed in the toxic range, although panel members disagreed about whether cumulative effects are observed below a short-term toxicity threshold. One reviewer thought that an additional uncertainty factor is needed, as there are not enough data on acrolein by the inhalation route. Health Canada indicated that use of a factor of one is based on information from other aldehydes and this should be included in the uncertainty factor discussion. One reviewer noted that the document should provide a stronger rationale (based on knowledge of other aldehydes) for why no uncertainty factor is needed for the short duration of the critical study. The panel reached consensus on a factor of one for database.

The panel agreed that the inhalation TC based on the BMC should present both the central estimate and the 95% lower confidence limit on the concentration associated with a 5% response and include the adjustment for intermittent exposure of 6/24. The total uncertainty factor is 100.

Uncertainty Factors for LOAEL approach: The reviewers agreed that to the 100 uncertainty factor for inter- and intraspecies should be applied. There was also discussion on an additional uncertainty factor for database considerations. One reviewer suggested a factor of 10 to address the difference between a LOAEL and NOEL. Another reviewer supported the additional factor of 10, but indicated that this factor should also account for difference between intermittent and continuous exposure. Another reviewer disagreed and stated that there should be consistency between the BMC and LOAEL approaches with regard to making the adjustment from intermittent to continuous exposure. The consensus position was to use an extra 10-fold factor for both the use of the LOAEL and for intermittent to continuous exposure. This results in a LOAEL-based inhalation TC of 0.57 ug/cu.m, or 0.00057 mg/cu.m (i.e., 0.57 ÷ 1000 = 0.00057).

Oral -- Choice of Critical Effect and Study

Health Canada proposed use of the NTP (1998) study for the derivation of an oral Tolerable Concentration (TC). In this subchronic study, rats and mice were administered acrolein by gavage in solutions of methylcellulose. Lesions in the forestomach were observed at doses as low as 0.25 mg/ml in rats and 0.125 mg/ml in mice. Since the effects at the site of contact are more likely related to administered concentration than dose, a provisional TC based on concentration was derived. Health Canada indicated that they label this TC as provisional because they have only preliminary data from the NTP study and will reevaluate when the full NTP report is available.

The panel discussed whether the irritation of the forestomach in rats reported by NTP (1998) is relevant to humans, and whether it is an appropriate critical effect. Rats have

both a forestomach and a glandular stomach, while humans have only a glandular stomach. The glandular stomach is more resistant than the forestomach to pH changes and irritation. Alternatives to the forestomach lesions that were discussed included use of a NOAEL of 2.0 mg/kg bw/day for stomach lesions in a dog study (Parent et al., 1992a), or the use of lesions in the rat glandular stomach.

In considering whether to use the glandular stomach lesions as the critical effect, the panel noted that this target organ is more relevant to humans than the forestomach, and that both a NOEL and a LOAEL are available for the glandular stomach in the NTP (1998) study. However, several panel members noted that the residence time in the forestomach (approximately 2 hours) is sufficiently long compared to the reaction time in the airway (microseconds in inhalation studies) so that the dose to the glandular stomach may have been much lower than that to the forestomach; in fact, the dose to the glandular stomach is not known. These site of contact effects are a function of the reactivity of acrolein, whether seen in the forestomach or glandular stomach.

t the panel considered use of the dog study of Parent et al. (1992a). Like humans, dogs have only a glandular stomach and high tissue GSH levels. Although the panel noted that histopathological effects were not observed at any dose tested, the use of this highest dose as a NOAEL may be acceptable in light of the observation of an effect level in shorter-term dog studies.

Health Canada pointed out, however, that acrolein toxicity is more closely related to concentration of administration than dose. Since dosing by Parent et al. (1992a) was by gelatin capsule, no concentration can be calculated for effects seen in dogs. One reviewer suggested that if acrolein toxicity is concentration-related, the glandular stomach and forestomach should have the same response. Another reviewer suggested that, in light of concerns about the use of the forestomach and the concentration-related nature of the toxicity, there might not be sufficient data to derive a TC. One reviewer noted that eye irritation by acrolein is related to cumulative exposure, while adaptation to nasal irritation is observed, and questioned whether there are enough data to determine whether stomach irritation is cumulative or adaptive. Because concentration data are not available for dosing of dogs, the panel preferred the NTP (1998) study in rats.

The review panel agreed that the NTP (1998) rat study should be used, with forestomach lesions as the critical effect. Health Canada recognized the conservativeness in using the forestomach lesions, and agreed to expand the discussion addressing the uncertainty of different responses between the forestomach and glandular stomachs, along with noting that humans do not have a forestomach.

Oral - Dose Response Assessment

Choice of Concentration: Health Canada proposed use of the NOEL of 0.15 mg/ml for effects in the gastrointestinal tract seen in female rats (NTP, 1998). The panel reached consensus on use of the forestomach effects in rats and the selection of 0.15 mg/ml as the

critical concentration. Health Canada agreed to note the differences between the glandular stomach and the forestomach as discussed above.

Uncertainty Factors: Health Canada proposed an uncertainty factor of 100 (10 for interspecies variation and 10 for intraspecies variation). Because there was no indication that severity of the critical effect increases with duration of exposure, Health Canada did not consider an additional uncertainty factor to address the use of a subchronic study necessary. The panel agreed with Health Canada and reached consensus on an uncertainty factor of 100.

Health Canada proposed a provisional TC of 0.0015 mg/ml. Health Canada thought it most appropriate to present this oral TC as a concentration because the effects at the site of contact are more likely related to administered concentration than dose. Because of the preliminary and limited reporting of data from the NTP (1998) study, Health Canada considers this TC as provisional. The panel agreed that the provisional oral TC of 0.0015 mg/ml was appropriate.

RECOMMENDATIONS

1. A number of suggestions were offered to improve the presentation of the genotoxicity discussion. These included:

better delineation of effects on DNA binding, adducts, and crosslinks

more information on the study quality of the genotoxicity data,

information on the degree of cytotoxicity and experimental details of acrolein dosing in mutagenicity studies,

deleting the statement "Resultingly, it is possible that Tolerable Concentrations developed to protect against non-neoplastic effects may also be protective for potential carcinogenicity."

2. The review panel recommended expanding the discussion addressing the uncertainty of different responses between the forestomach and glandular stomachs, and note that humans do not have a forestomach.

3. The review panel suggested that derivations of Tolerable Concentration based on both LOAEL and BMC approaches should be presented. For the BMC approach, it was suggested to present both the 5% central estimate and the 95% lower confidence estimate.

4. It was suggested that the document could include a stronger rationale, based on knowledge of other aldehydes, for no extra uncertainty factor adjustment for the short duration studies used.

REFERENCES

Cassee, F., J. Groten and V. Feron. 1996. Changes in the nasal epithelium of rats exposed by inhalation to mixtures of formaldehyde, acetaldehyde, and acrolein. Fund. Appl. Toxicol. 29:208-218.

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Integrated Biologically-Based, Multiple Endpoint Risk Assessment for Acrylamide – Phase 1: Cancer and Modes of Action

Sponsor: Acrylamide Monomer Producers Association

Presenters:

- Dr. Marvin A. Friedman, Private Consultant
- Dr. Michael Dourson, *TERA*
- Dr. Lynne Haber, *TERA*
- Mr. Andrew Maier, *TERA*
- Dr. Kenneth Poirier, *TERA*

Chair: Ms. Bette Meek, Health Canada

Peer Reviewers:

- Dr. Marilyn J. Aardema, The Procter & Gamble Company
- Dr. John P. Christopher, California Environmental Protection Agency, Department of Toxic Substances Control
- Dr. Michael L. Gargas, ChemRisk Division of McLaren/Hart
- Dr. George Leikauf, University of Cincinnati, Department of Environmental Health
- Ms. Bette Meek, Health Canada

- Dr. Martha Moore, U. S. Environmental Protection Agency, National Health and Environmental Effects Laboratory
- Dr. Robert G. Tardiff, The Sapphire Group, Inc.
- Dr. Vanessa T. Vu, U.S. Environmental Protection Agency, National Center for Environmental Assessment
- Dr. Vernon Walker, New York State Department of Health

Staff of Toxicology Excellence for Risk Assessment (*TERA*) and Dr. Marvin Friedman prepared a document entitled *Integrated Biologically-Based, Multiple Endpoint Risk Assessment for Acrylamide. Phase 1: Cancer & Modes of Action*. The assessment document was prepared for the Acrylamide Monomer Producers Association, Inc. (AMPA) which includes four member companies: SNF S.A.; Cytec Industries Inc.; Nalco Chemical Company; and Allied Colloids.

Because of *TERA's* involvement in the development of this document, *TERA* staff did not chair the meeting or select peer reviewers. Ms. Bette Meek of Health Canada chaired the acrylamide session and Ms. Jennifer Orme-Zavaleta, a *TERA* Trustee, selected reviewers. The panel discussed conflict of interest and agreed to full participation of all panel members as reflected in Appendix A.

Drs. Marvin Friedman and Michael Dourson presented background and information on the acrylamide document. The presentation was followed by a short period during which reviewers asked clarifying questions. The document authors and sponsors requested peer input on their documentation to provide them with comments and suggestions for improvements and how to proceed. To focus the discussion, they provided the panel with a list of questions and issues.

PRESENTATION AND CLARIFYING QUESTIONS

Dr. Michael Dourson of *TERA* began the presentation with a brief introduction. The purpose of this review was to provide the authors with input on cancer mode of action (MOA) as the first stage of a longer-term effort toward an integrated assessment. The authors requested that the panel review the draft document in order to identify areas needing more work, as well as the strengths and weaknesses in the arguments.

Acrylamide monomer is used in the production of polyacrylamide, which is used as a flocculent in the treatment of sewage, wastewater, and drinking water, as well as in pulp and paper production. Other uses of polyacrylamide include as grouting agents and in molecular biology laboratories. A very small amount of unpolymerized acrylamide is contained in polyacrylamide. Human exposure is primarily occupational via dermal contact and inhalation, although exposure to the general public can result from the leaching of the acrylamide monomer from polyacrylamide flocculents used in water treatment. The net result is that large populations may be exposed to very low

concentrations, emphasizing the significance of using linear or non-linear extrapolation for risk assessment.

The primary toxic effect seen in humans from elevated levels of acrylamide is neurotoxicity. Other effects are seen in experimental animals at higher levels, including male reproductive toxicity, male germ cell mutations, and tumor formation. The document under review focused on the data surrounding tumor endpoints.

Dr. Marvin Friedman then provided a brief overview of the project plans and issues on which the document team wished to obtain panel input. The team is currently in Phase 1 of the overall project that is to develop an integrated biologically-based multiple endpoint risk assessment for acrylamide. The four major toxicological endpoints under consideration are carcinogenicity, neurotoxicity, mutagenicity, and male reproductive toxicity. The project objective is to use all of the toxicology data to develop an integrated risk characterization that includes all the toxicological endpoints, including cancer. This Phase 1 document and the questions for the panel focus on modes of action and how they relate to cancer risk assessment. The team is seeking information, advice, and counsel for the next steps. With resolution of the identified issues, the team's next step will be to develop a comprehensive risk assessment and characterization which will then undergo peer review.

Dr. Friedman presented the metabolic pathway for acrylamide. To show that acrylamide acts via multiple modes of action, even for a single endpoint and dose range, Dr. Friedman then presented data on the induction of dominant lethal mutations by acrylamide under various conditions. Acrylamide alone induces dominant lethal mutations. Much of this effect can be attributed to the acrylamide metabolite glycidamide, since dominant lethal mutations are markedly reduced by 1-aminobenzotriazole (ABT), an inhibitor of P4502E1 production of glycidamide. However, some dominant lethals are produced even in the presence of ABT, indicating there may be a second mode of action for their generation. By contrast, inhibition of mating behavior by acrylamide occurs at the same doses and is essentially unaffected by ABT, suggesting that the mode of action for mating inhibition differs from those of dominant lethal induction.

In the two primary animal carcinogenesis studies (Johnson et al., 1986 and Friedman, et al., 1995), mammary gland fibromas and adenocarcinomas (in the Johnson study only) in females, thyroid follicular adenomas in males and females, and testes mesothelioma - tunica vaginalis in males were seen. The Johnson study identified several additional target organs where tumors developed. These studies were conducted with the same species and strain of rats, duration and route. Friedman et al. (1995) repeated the Johnson study protocol because of uncertainty in results due to a virus that affected the Johnson rats and to provide adequate data for risk assessment. Friedman used an unbalanced design intended to detect a 5% increase in cancer incidence over a 1.5% estimated background level.

To explain the tumorigenicity of acrylamide, the authors identified four possible modes of action, and evaluated the evidence supporting and contradicting these MOAs. The authors hypothesize that more than one MOA is at work. The four possible MOAs are:

MOA 1: Gene mutations arising from glycidamide-DNA adducts may result in acrylamide-induced tumorigenesis.

MOA 2: Aneuploidy and chromosomal defects may result from interactions of acrylamide with protein (possibly kinesin-related proteins or tubulin). Mutations in germ cells may result from interaction of acrylamide with protamines in sperm.

MOA 3: Stimulation of hormones by acrylamide may result in unregulated tissue stimulation.

MOA 4: Acrylamide up-regulates pro-growth signals in target tissues through activation of transcription factors, leading to loss of normal cell growth regulation.

Clarifying Questions on the Presentation

After the presentation, the panel asked a number of clarifying questions. One reviewer asked how long ABT remains in the animal before it is metabolized or excreted. Dr. Friedman responded that the residence time is long enough to ensure that no glycidamide is produced from the single dose of acrylamide.

Several panel members asked what is meant by an "integrated risk assessment." Dr. Friedman responded that an integrated risk assessment would cover multiple endpoints. For example, it might tie together aneuploidy and neurotoxicity as they each use the same protein mechanisms, resulting from effects on motor proteins. With the same MOA, one could quantitatively assess the risks of each endpoint together. Another reviewer indicated that EPA is also developing integrated assessments, but they are looking at all the different endpoints and the linkages between them. Reviewers asked why the document focused on cancer. Dr. Friedman responded that approaching all endpoints at once is too big an undertaking at the start. Rather, there are some cancer issues that the presenters wish to resolve that will help with that aspect of the assessment, before going to the next level and integrating across endpoints. All of these endpoints are presented separately in the document; they will be assimilated or integrated to come up with a working hypothesis. The Chair clarified that the panel is providing input at this time, but not seeking consensus on a cancer assessment.

Questions Posed to the Peer Panel

The panel was provided with a list of eight questions to consider as they reviewed the assessment document. Just prior to the meeting, these questions were revised and reordered for a smoother discussion and to reflect the priorities of the authors. The authors identified an over-riding issue: for each of the proposed modes of action, are the available data sufficient to support their relevance to acrylamide carcinogenesis?

Discussion of the first question covered MOAs 1 and 2, while MOAs 3 and 4 were specifically discussed at a later point in the meeting.

Specific Questions (In the order they were discussed)

1. Are genotoxic lesions, which appear only at the chromosome level, as significant as point mutations? What is the impact of genotoxicity, which may not be the result of DNA reactivity, but rather protein reactivity? Are the data sufficient to conclude whether acrylamide causes gene mutations in somatic cells? Is clastogenesis (effects on chromosome structure) as significant as gene mutations? Is the genotoxicity in somatic cells due to DNA reactivity or protein reactivity? Is this an important distinction? How can DNA reactivity and protein reactivity be distinguished?

2. What criteria are needed to make a choice between linear or non-linear low-dose extrapolation when more than one MOA is occurring? How do we allocate the contribution of several MOAs to the assessment of risk?

3. Are non-reproducible findings between the two cancer bioassays relevant to hazard and risk assessment? Of the two studies, which is a better one for extrapolating to humans? Should the cancer data be combined between these studies as has been done in the draft analysis? Is there a more appropriate way to combine the data?

4. What extrapolation methodology is used for a material that appears to decrease latency period or increased the rate of appearance of background tumors?

5. Is a biologically based model possible with the existing acrylamide data? If not, what data are needed? If so, what should the general structure of the model be?

6. Is there a better correlation between tumors and acrylamide and glycidamide hemoglobin adducts (as the basis of tissue dose) or to acrylamide administered dose? Is it appropriate to address the issue of linearity vs. nonlinearity by considering dose metrics (e.g., hemoglobin adducts) that reflect total systemic dose, rather than measured dose to the target tissues? If so, which dose metric (administered dose, acrylamide Hb adducts, or glycidamide Hb adducts) correlates best with tumors?

7. Are benign tumors only significant as precursor lesions to malignant tumors? If so, how should the factors for MOE (margin of exposure) be addressed with a non-linear extrapolation?

8. Does the use of a MOE (margin of exposure) for within human variability and slope of dose-response curve overlap the underlying technical data?

DISCUSSION

1. Are genotoxic lesions, which appear only at the chromosome level, as significant as point mutations? What is the impact of genotoxicity, which may not be the result of DNA reactivity, but rather protein reactivity? Are the data sufficient to conclude whether acrylamide causes gene mutations in somatic cells? Is clastogenesis (effects on chromosome structure) as significant as gene mutations? Is the genotoxicity in somatic cells due to DNA reactivity or protein reactivity? Is this an important distinction? How can DNA reactivity and protein reactivity be distinguished?

In response to this series of questions, reviewers agreed that the data are sufficient to conclude that acrylamide causes mutations in somatic cells, but that one must distinguish between point mutations and chromosome mutations.

Reviewers commented that the chromosome mutations are as significant as gene mutations. An increased risk of cancer is associated with chromosomal mutations. Thus, chromosomal mutations are a marker of effect, while 10 years ago they were considered only a marker of exposure. The reviewers noted that acrylamide itself probably does not react with DNA, but its metabolite, the epoxide glycidamide, does, and that this direct interaction is of potential importance. The predominance of chromosomal effects *in vivo* and *in vitro* and that these effects could result from protein reactivity were noted. A number of reviewers agreed that most chemicals that are mutagenic produce some of both point mutations and chromosome aberrations. There are also many other chemicals that produce more chromosome aberrations than point mutations. A reviewer noted that transgenic systems would not detect these kinds of lesions. One needs to focus on the basic biology and what sorts of events acrylamide induces. In the final analysis the relative importance of each type is tied to health outcomes.

A reviewer suggested that the types of tumors produced by acrylamide be compared with those produced by ethylene oxide (which is an epoxide, like the acrylamide metabolite glycidamide). Ethylene oxide also produces chromosomal mutations, although not to the same extent as acrylamide. Most of the deletions produced by ethylene oxide are due to strand breaks.

There was considerable discussion about the difficulty of separating direct DNA reactivity from indirect DNA effects resulting from interaction with proteins. Chemicals that bind to DNA also bind to protein, although the relative affinities may differ. Thus, acrylamide binds to DNA, but at extremely low affinity, while binding to protein is at much higher affinity. By contrast, binding of the epoxide (glycidamide) to DNA may be quantitatively more significant. One reviewer noted that the state of the science does not, in general, allow the determination of the tumorigenic mechanism for every individual tumor type. It was also noted that acrylamide could interact with proteins in other ways than those hypothesized by the authors, such as by interacting with DNA repair enzymes. One reviewer expressed concern that repair mechanisms may be saturated. If portions of DNA are damaged and repair enzymes are working on those, the result may be other DNA damage not getting repaired.

A reviewer noted the importance of the observation that glycidamide is mutagenic in the Ames assay. Since acrylamide is negative in the Ames assay, even in the presence of S9 activation (which would be expected to produce glycidamide), a key question is whether glycidamide is, indeed, produced by S9 *in vitro*. Acrylamide should not be considered exclusively a clastogen, since both point mutations and chromosome aberrations have been observed *in vivo.*

The reviewers had a number of additional suggestions and comments. Consistency between other chemicals and acrylamide was noted in that, like acrylamide, chemicals that cause aneuploidy often are neurotoxic. One reviewer agreed with the authors that the tumors seen with acrylamide are in tissues with a relatively high incidence of spontaneous tumors. It was suggested that acrylamide decreases tumor latency, and increases the number of tumors by DNA damage modulating effects occurring via other pathways.

A number of reviewers suggested that looking at structure activity relationships (SAR) with other compounds with the same tumor profile might be useful and asked if there are other reproductive tumor producing agents like acrylamide. If an identical tumor spectrum cannot be identified, a reviewer suggested looking at endocrine-related tumors in rats. Another reviewer commented that the narrow spectrum of tumors observed with acrylamide is typical of epoxides, with species- and strain-specific differences in tumors related to species- and strain-specific metabolism and species-specific cancer genes.

Another reviewer noted that carcinogens typically produce DNA adducts in all tissues, even tissues that do not develop cancers. Due to this inconsistency, adducts are used as measure of exposure and not effect. A reviewer noted that there are many chemicals that cause mutations in transgenic animal models but that do not produce liver tumors. Thus, the acrylamide data are consistent with results for other related chemicals, although it is not known why the other chemicals do not produce liver tumors. One reviewer noted that, in contrast to the Syrian hamster embryo (SHE) cell transformation data discussed in the context of the hormonal MOA (MOA #3), acrylamide has produced cell transformation by itself in other studies and cell systems.

A reviewer noted that the statement on page 13 that "the ability of N7-DNA adducts to generate mutations has not been adequately tested" is misleading. The majority of DNA monoadducts will be in alkyl adducts which do not produce mutations directly, but do lead to apurinic sites, which can lead to mutations. The question on whether the apurinic sites lead to mutations relates to whether the rate is above background. Studies of the persistence of these sites would be useful, including a comparison of the half-life of these sites with acrylamide and ethylene oxide.

One reviewer recommended that a tabular summary of the genetic toxicity data would be useful to provide an overall picture. Another reviewer noted that the Dearfield et al. (1995) review has many tables that may be useful.

The panel agreed that acrylamide is clearly clastogenic *in vivo* and *in vitro*. Whether acrylamide tumorigenesis is also caused by induction of point mutations is not clear. The mutagenic epoxide should be taken into account, although it is not know how to assimilate this quantitatively.

2. What criteria are needed to make a choice between linear or non-linear low-dose extrapolation when more than one MOA is occurring? How do we allocate the contribution of several MOAs to the assessment of risk?

Reviewers suggested that the document first needs to develop MOA support for each tumor type (mammary, thyroid, etc.). Both linear and non-linear extrapolation could be done and then one could characterize where the two meet. If one were to use a Margin of Exposure (MOE) approach, one would look at how rapidly the risk is reduced. One reviewer indicated that a graph displaying the risk at one in one million and where that coincides with a MOE might be helpful.

The authors indicated that the document can present both approaches and select what best matches the data, but asked which approach would ultimately be used. It is unclear as to what level of certainty is necessary to choose a non-linear approach. A reviewer indicated that the information should be laid out in a transparent fashion and then measured against clearly identified criteria for causality, as one would for an epidemiology study. Another reviewer indicated that EPA is working with the World Health Organization (WHO) to determine how much evidence is needed to choose a non-linear approach. One reviewer stated that there are not enough data on acrylamide to support the hypothalamus-pituitary model for thyroid tumors. Regarding allocation of contribution, reviewers thought that one could group tumors with the same or perhaps similar MOAs, but the weight of evidence should be clearly evaluated for each MOA. If there were insufficient information on the MOA for a tumor type, then defaulting to a linear approach would be more appropriate.

3. Are non-reproducible findings between the two cancer bioassays relevant to hazard and risk assessment? Of the two studies which is a better one for extrapolating to humans? Should the cancer data be combined between these studies as has been done in the draft analysis? Is there a more appropriate way to combine the data?

The reviewers indicated that the recent study (Friedman et al., 1995) using the same dose, strain and source of rats as the Johnson et al. (1986) study, is a better bioassay, but both studies should be presented and considered in the hazard identification discussion. The control and experimental rats in the Johnson study were infected with a sialodacryoadenitis virus, which may have confounded the results of that study.

The panel did not consider the results "non-reproducible" as some of the same tumors were seen, and those that were not seen were tumors with smaller incidences. One reviewer pointed out, and the authors agreed, that the glial cell tumors seen in female rats in Johnson et al. (1986) are a concern that cannot be dismissed. Dr. Friedman noted that

thyroid follicular cell adenomas and adenocarcinomas were seen in females in the Johnson study, but only adenomas were seen in the Friedman study. Reviewers noted that because different pathologists may interpret the same slides slightly differently (and thus one pathologist could identify a lesion as an adenoma, while another might call it an adenocarcinoma), it might be useful to have the slides from both studies read by the same pathologist in a blind reading. Dr. Friedman indicated that while he is not a pathologist, he did look at all the slides and thought that the Johnson animals looked like they had tumors that were more virulent.

The panel agreed that both studies are relevant for the hazard characterization. Since there is greater confidence in the Friedman study results, it would be a better study for extrapolating to humans for the dose-response assessment, however, the concerns about glial cell and thyroid tumors need to be addressed.

New Issue – Discussion on weight of evidence for the hormonal and signal transduction MOAs (MOA 3 and MOA 4).

At the request of the authors, the panel deviated from the specific questions to address the evidence to support modes of action (MOAs) numbers 3 and 4.

MOA #3: Stimulation of hormones by acrylamide may result in unregulated tissue stimulation.

One reviewer noted that the document presented a good evaluation of MOAs #3 and #4 and noted that the data are stronger for mesothelioma than for the thyroid. This reviewer considered MOA #3 to be a plausible hypothesis, but that more data are needed on the sequence of events. Although there are more data gaps for mesothelioma, there are no major inconsistencies. A reviewer noted that ethylene oxide also produces mesotheliomas and there is a lot of work on that chemical which may be relevant to acrylamide.

For the thyroid, there is no consistent pattern of effects on hormone levels, weakening the hypothesis. It was suggested that there might be a connection to changes in serotonin levels. Another reviewer expected to see increases in gonadotropic hormones. Dr. Friedman indicated that one could not show effects on circulating hormone levels because of the weak effect of acrylamide, as indicated by the relatively low incidence of acrylamide-induced thyroid tumors. A reviewer asked whether the scrotal tumors are acrylamide related or metabolite-mediated. Dr. Friedman indicated that this is not known and would be a very time-consuming and expensive study.

Another reviewer suggested that how to bridge the gap between sex hormones and their trophic hormones has to be determined, as well as what is going on in the nearby cells. Dr. Friedman suggested that a rapidly growing population of cells in this area might be the target. Cells grow in one area and move out to coat the scrotum. Cell proliferation studies are currently being conducted, but without much success. The document authors agreed that the document needs to deal with each tumor independently.

Several reviewers noted that the section on hormonal effects and the related MOA evidence is a good model for weight of evidence (WOE) and causality discussions with the inconsistencies and research gaps well presented. Dr. Haber asked for the reviewers' opinions on what the SHE cell transformation data mean and how to interpret these data in light of the other cell transformation data. A reviewer responded that it would be surprising if acrylamide did not get positive results in SHE cells in light of its clastogenicity in other test systems. It was suggested that chromosome aberrations and HGPRT gene mutations be evaluated in the SHE system to compare results in that system with the other *in vitro* test systems. If genotoxicity is not observed, this supports the endocrine MOA, while the observation of genotoxicity in the SHE cells (and the absence of transformation when only acrylamide is present) would cloud the issue. It was noted that a key aspect of sorting out the potential role of endocrine modulation in SHE cell transformation (and in evaluating all of the *in vitro* data) is having an *in vitro* assay in which it is known that glycidamide is formed.

The panel concluded that a key aspect of evaluating the weight of evidence regarding MOA #3 is to consider the data supporting the hypothesis for each tumor type separately. As presented in the document, there are generally consistent data (with significant gaps) for the mesotheliomas, and inconsistent data regarding the thyroid tumors.

MOA #4 Acrylamide up-regulates pro-growth signals in target tissues through activation of transcription factors, leading to loss of normal cell growth regulation.

One reviewer commented that this MOA is far less developed than the others and thought that the document is several steps away from showing that the observed changes in protein and RNA levels are part of a mode of action for tumor development. Another reviewer indicated that the authors should be cautious, that the data support an association, but not a causal relationship at this point. It was also noted that the magnitude of the observed changes, and the concentrations at which changes were seen, were not particularly striking for this test system. In response to a reviewer question, Dr. Friedman noted that AP-1 was measured because an involvement of this protein was indicated by the observation that dexamethasone inhibits the effects of acrylamide on neurofilament protein synthesis (Lin et al., 1996). There is ongoing research on AP-1 and NFκ B. Studies on gap junctions, oxidative stress, or apoptosis have not indicated significant changes. A reviewer also noted that much of the signal transduction data come from PC12 (pheochromocytoma) cells, but that acrylamide has not been observed to induce pheochromocytomas.

Key questions noted by the reviewers were whether the observed changes occur in the systems of interest (i.e., the target tissues for tumors), and identification of the pathway from acrylamide to protein kinase C (PKC) activation. It was suggested that MAP kinase and PKC activities be measured. Reviewers also asked whether the observed activation is due to glycidamide or acrylamide, and how robust the response is. A reviewer suggested that the response be compared to that observed with TPA, a phorbol ester that is known to act through the PKC pathway proposed for acrylamide. It was also suggested that

researchers determine whether the observed protein increases are specific to the pathway measured, or reflect changes in protein levels in general.

Overall, the reviewers considered MOA #4 to be a hypothesis worth pursuing, but needing substantial additional data before a link to tumorigenicity is established.

4. What extrapolation methodology is used for a material that appears to decrease latency period or increase the rate of appearance of background tumors?

Before choosing an extrapolation method, a reviewer pointed out that one must first assume sufficient weight of evidence of carcinogenicity to extrapolate. It was also noted that in most bioassays one sees an increase in the rate of spontaneous tumors (i.e., tumors with a high background rate), however the reviewer could not suggest how to handle this, nor (as one reviewer noted) were there ideas on addressing this when it was discussed at NTP (National Toxicology Program) meetings. Another reviewer noted that the observation of increased incidence of spontaneous tumors is consistent with a tumor promoter. It was stated that there appears to be sufficient evidence that one or more MOAs are nonlinear, but that the authors will have to show that acrylamide is a classical promoter. One reviewer noted that environmental chemicals rarely cause unique tumors and that an increase in the background rate of tumors might be more relevant to environmental concerns. These chemicals may be acting by reducing the latency period.

It was suggested that the authors improve the time-to-tumor discussion and consider a time-to-tumor model.

5. Is a biologically based model possible with the existing acrylamide data? If not, what data are needed? If so, what should the general structure of the model be?

The panel agreed that there are not enough data for a biologically based dose response (BBDR) model at this time. Missing data include identification of the tumors upon which to base the model and what key mutation is causing the tumors. It was suggested that the document develops a qualitative "story" for each tumor site and identify the data gaps. After sufficient support has been developed for the qualitative story, a BBDR might be developed. The need in BBDR development to be very quantitative at the tissue level, including identification of all relevant rate constants and binding coefficients, was noted by a reviewer. One reviewer suggested that it might be helpful to create a large diagram of all the interactions with the various receptors. By laying out what is known, and what the document authors think is taking place, they can identify the unknowns and formulate hypotheses, which can then be tested.

6. Is there a better correlation between tumors and acrylamide and glycidamide hemoglobin adducts (as the basis of tissue dose) or to acrylamide administered dose? Is it appropriate to address the issue of linearity vs. nonlinearity by considering dose metrics (e.g., hemoglobin adducts) that reflect total systemic dose, rather than measured dose to the target tissues? If so, which dose metric

(administered dose, acrylamide Hb adducts, or glycidamide Hb adducts) correlates best with tumors?

The panel agreed that selection of the appropriate dose metric is dependent on the MOA and which chemical, acrylamide or glycidamide, is responsible for the tumor of interest. One reviewer suggested that the selection of the dose metric is not appropriate until the mode of action is understood for each tissue. While it is generally better to use an internal dose, the identification of the appropriate internal dose is dependent on knowing the MOA. If the MOA is not known it may be better to use administered dose. Alternatively, another reviewer suggested using the MOA to hypothesize the dose metric and then see which fits the data best. A third reviewer noted that it would be even more difficult to choose the dose metric if two mechanisms are operating. Another reviewer stated that since you measure dose in people and not adducts, you have to regulate on the basis of dose, but ultimately one needs to consider both and to link the two.

A reviewer indicated concern about use of the Calleman et al. (1992) paper discussing the relationship between hemoglobin adducts and administered dose, and the use of adduct levels for risk assessment. These concerns included how well the relationship between administered dose and hemoglobin adducts was validated in rats, the questionable nature of any extrapolation to humans, since the Calleman model is not a true PBPK (physiologically-based pharmacokinetic) model, and how good hemoglobin adducts are at predicting tissue dose. Another reviewer indicated that the data are there; he has done some validation in rats, and that doses are the same in tissues with little tissue difference in dose delivered to DNA.

The panel agreed that based on what is known about metabolism and adduct distribution, having hemoglobin adduct data is sufficient without tissue specific data for use in modeling. However, more information is needed on the quantitative relationship between hemoglobin adducts and tumor formation, including intermediate endpoints related to the MOA for each tissue. As noted earlier, studies of the persistence of apurinic sites would be useful, including a comparison of the half-life of these sites with acrylamide and ethylene oxide.

In response to an author's question, one reviewer indicated that with human data one could develop a PBPK model, but that there are not enough human data at this time, with many of the kinetic steps unaccounted for. Another reviewer suggested the authors work with Tim Fennel at CIIT.

One reviewer suggested reorganizing the presentation of the four modes of action (page 13 and 14 of the document) to separate supporting evidence, contradictory evidence, and what is unknown about each proposed MOA.

7. Are benign tumors only significant as precursor lesions to malignant tumors? If so, how should the factors for MOE be addressed with a non-linear extrapolation?

The authors clarified that this question refers to the severity factor for weight of evidence in the EPA proposed cancer guidelines (U.S. EPA 1996) and is not specific to acrylamide. One reviewer offered her personal opinion that there is a continuum of responses and benign tumors are part of that. One could use MOE with good qualitative information on the MOA, but without quantitative information. Benign tumors are not malignant but are certainly a frank effect and one needs a factor for this. The point is whether one has data to believe that benign tumors lead to malignant tumors. If so, a factor of 10 should be used; if not, something less than 10 should be used. However, in the absence of knowledge one assumes benign tumors lead to malignancy. The bottom line is that one can only use MOE with a good understanding of the biology, and ideally precursor data (as opposed to tumors) should be used for the extrapolation.

Other reviewers cautioned that the site of tumors is important, as well as cross-species differences, although the difficulty in extrapolating tumor types to another species is recognized. Another reviewer pointed out that an MOE analysis also looks at exposure, that one considers the adequacy of MOE in context of exposure. This is a large area of uncertainty.

8. Does the use of a MOE for within human variability and slope of dose-response curve overlap the underlying technical data?

The authors indicated that it might be premature to discuss this question. However, one reviewer indicated that when the slope of the dose response curve is steep, the response rapidly decreases with dose, and that one does not need a large MOE. A shallower slope would mean one needs a larger MOE. Another reviewer stated that one does have overlap if exactly the same mechanism applies in animals and humans.

RECOMMENDATIONS

The panel agreed that with the authors' proposal that is reasonable to revise the existing document with any additional written comments from the panel and input from this meeting. The team then plans to proceed to the next phase where they will more completely evaluate each potential MOA on a tissue-by-tissue basis, with the ultimate goal of producing an integrated assessment.

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Assessment for Acrylonitrile

Sponsor: Health Canada

Presenters:

- Mr. George Long, Health Canada
- Ms. Bette Meek, Health Canada

Chair: Ms. Jennifer Orme-Zavaleta, Trustee, *TERA*

Peer Reviewers:

- Dr. Marilyn J. Aardema, The Procter & Gamble Company.
- Dr. Michael L. Dourson, Toxicology Excellence for Risk Assessment
- Dr. Susan Felter, The Procter & Gamble Company
- Dr. Marvin A. Friedman, Private Consultant
- Dr. Michael L. Gargas, ChemRisk Division of McLaren/Hart
- Dr. Robert G. Tardiff, The Sapphire Group, Inc.
- Dr. Vanessa T. Vu, U.S. Environmental Protection Agency, National Center for Environmental Assessment
- Dr. Vernon Walker, New York State Department of Health

PRESENTATION AND CLARIFYING QUESTIONS

Representatives from Health Canada presented information on the acrylonitrile assessment and the objectives of the peer review in the context of the Priority Substances Program. The presentation was followed by a short period during which reviewers asked clarifying questions. The reviewers then discussed the assessment document, evaluating the hazard characterization first, followed by the dose response assessment. In addition, two observers, Dr. James Collins of Solutia and Dr. Dale Strother of BP Chemical made short presentations on epidemiological analysis and toxicological aspects of the acrylonitrile database, respectively, prior to discussion of the pertinent part of the document.

Mr. George Long of Health Canada gave an overview of the hazard characterization and dose-response analysis for acrylonitrile. The critical endpoint for the Health Canada assessment was carcinogenicity. This was based primarily on consistent findings of tumors (brain/spinal cord, ear canal, gastrointestinal, and mammary gland) in rats, by both oral and inhalation exposure. In humans, there is no clear evidence of cancer in several epidemiological studies; the primary toxicity observed in exposed workers is acute irritation. In summarizing the information on mode of action, it was noted that there has been no consistent pattern of results in genotoxicity assays. Acrylonitrile appears to be mutagenic *in vitro* in the presence and absence of metabolic activation, but, at most, weakly mutagenic *in vivo*. Acrylonitrile appears to form DNA adducts in the liver, but results have been inconsistent in the brain where the primary tumors of concern are observed. In addition, there is inconclusive evidence that tumors are associated with oxidative DNA damage. In the dose-response assessment, Quast et al., (1980b) was selected as the basis of the inhalation cancer risk estimate. Although there were only two doses, a large number of animals were tested. Human equivalent values for the inhalation risk estimate were derived by using the ratio of animal to human inhalation volume/body weight. Bio/Dynamics (1980) was selected as the basis of the oral cancer estimate because it had five dose levels and tested large numbers of animals.

Ms. Bette Meek of Health Canada gave a short presentation on the objectives of the Health Canada Priority Substances Program and their needs from the peer review. She noted that their goal was to evaluate health risk in the general environment and that a full risk characterization will be done as a final product, but has not been submitted to this panel for review. The Program has a five-year timeframe for completion of assessments on all 25 compounds included on the second Priority Substances list. The cut-off for inclusion of new data in the assessment on acrylonitrile was the second stage of the external peer review (early Autumn, 1998). Health Canada is only able to include fully reported studies in their assessment documents. Since much of the new data on acrylonitrile is only available in abstract form without full documentation, many new studies are mentioned in the document, but do not serve as the basis of any conclusions. The literature review portion of the assessment has already undergone a first stage peer review to ensure that all available data has been included and a preliminary second stage review to address adequacy of coverage and defensibility of conclusions in the draft Hazard Evaluation and Dose-Response analyses. The relevant issues on which Health

Canada requested peer review comments included their conclusions regarding mode of action and the appropriateness of the dose scaling used.

The reviewers had no clarifying questions related to the presentations.

DISCUSSION

Data Adequacy

The panel discussed the adequacy of the oral and inhalation databases separately. Overall, the panel agreed that the oral and inhalation databases were adequate to derive risk values. The panel had few comments on the oral data. It was noted that one developmental study reported developmental effects in the absence of maternal effects based on findings in just one animal. In addition, one study (SRI, 1996) reported conclusions regarding a decrease in sperm motility and the effect on fertility. There was a question regarding whether this conclusion was that of Health Canada or the study authors. Health Canada noted that the conclusion was that of the study authors. It also noted that neither of these studies was critical to the assessment, but that these issues would be clarified.

The focus of the discussion of inhalation data was how the findings of the human and animal data can be reconciled given that animal bioassays clearly show that acrylonitrile is a carcinogen in rats, but the weight-of-the-evidence of the epidemiological studies does not show a definitive association between cancer and acrylonitrile exposure in humans. Thus, the panel attempted to define when it is possible to state that a chemical is a human noncarcinogen in the presence of positive animal data. Several reviewers felt that this is only possible when the data are available to demonstrate that the animal data are not relevant to humans. Such data are not available for acrylonitrile. Several reviewers also noted that the differences in response between animals and humans could be related to a difference in sensitivity/potency of the two species; and, therefore, it is not possible to rule out the potential for carcinogenicity in humans.

Several reviewers suggested that Health Canada establish some bounding estimates for human exposure and relative risk to facilitate a comparison with the rat data in order to answer the question of relative sensitivity. Health Canada noted that it did do crude bounding of the human relative risk based on the lower confidence intervals and that the limitations of a more quantitative approach, in particular bounds on exposure estimates, preclude very useful comparisons. The panel felt that presenting the entire confidence interval for the relative risk would be a more balanced approach. One reviewer noted that it might be possible to use a human PBPK (physiologically based pharmacokinetic) model to help answer this question. By modeling the internal dose of cyanoethylene oxide (CEO), a metabolite of acrylonitrile, one would be able to predict the human doses of acrylonitrile where a response would be observed. However, others countered that there are insufficient data to know what the most appropriate dose metric is for cross-

species extrapolation. Several reviewers suggested that the quantitative differences between humans and rats, which had been characterized in the background sections of the document, be reiterated in relation to the epidemiological data.

Several reviewers suggested that Health Canada more fully describe the relevance to humans of the different tumor types observed in animals. Health Canada asked for suggestions on how to do this. A reviewer noted that the brain tumors are relevant while the Zymbal gland and forestomach tumors are not as relevant. Health Canada noted that while the Zymbal gland does not exist in humans, it is a good marker for genotoxic carcinogens and that no one has ever expected site concordance between animals and humans. Another reviewer noted that the consistent observation of astrocytomas across studies was good, but the reviewer would like to see the document evaluate each tumor type. Subsequent to the meeting, Health Canada noted that there are no specific data on relevance to humans upon which to base exclusion of tumor types (e.g., metabolic variations) and that they selected the tumor type which occurred at highest incidence across studies, for which there is no basis to exclude its relevance.

Mode of Action

Overall, the panel agreed with the characterization of the *in vitro* mutagenicity data by Health Canada.

Based on additional critical review of the mutagenicity studies, however, one of the reviewers felt that firm conclusions could not be drawn on the *in vitro* data due to their inadequacy. Several other reviewers suggested that additional text would help demonstrate that no conclusions could be drawn from the *in vivo* studies due to their inadequacy as well.

Several reviewers also suggested the addition of a structure activity relationship (SAR) analysis if desired by Health Canada, since this would benefit the document by bringing in available information on the entire class of compounds. For example, ethylene oxide, rather than vinyl chloride, was considered as possibly appropriate because the types of DNA adducts formed by vinyl chloride (7-oxoethylguanine and N-2,3-ethenoguanine) are not consistently observed following treatment with acrylonitrile. Ethylene oxide treatment results in the formation of cyanohydroxyethyl DNA adducts; these same types of adducts have been observed *in vitro* (Yates et al., 1993, 1994) following acrylonitrile treatment. Since no studies have been conducted which evaluated the presence of these types of adducts *in vivo*, one reviewer felt that the document's conclusion that mutagenicity may occur by a mechanism other than the formation of ACN-DNA adducts (p 73) was too strong. DNA adducts cannot be ruled out until studies on the presence of cyanohydroxyethyl adducts *in vivo* have been conducted. In addition, a reviewer suggested that a study by Fedtke et al. (1989) be included in the document.

Cancer Dose-Response

The panel discussed the dose response analysis for the oral and inhalation routes of exposure separately. A key issue discussed was the appropriate dose scaling to be used in the generation of the Tumorigenic Concentration 5% (TC05) and Tumorigenic Dose 5% (TD05) values.

Oral Dose-Response

Overall, the panel agreed that the appropriate study (Bio/Dynamics 1980) and the appropriate data set (astrocytomas) were selected for the oral dose-response assessment. Several reviewers suggested that each tumor type could be evaluated separately, noting its significance and relevance to humans. If Health Canada wished to provide additional rationale for the critical study, reviewers suggested including a table that compares the results of both Bio/Dynamics (1980) and Quast et al. (1980a) and shows the range of all astrocytoma values.

Other reviewers questioned the decision to exclude animals that died before 6 months when the tumors were first observed between 7 and 12 months. This reviewer felt that the mortality adjustment should be based on the time when the first tumor of the type selected for the dose-response analysis was observed. Health Canada noted that the data from the study did not clearly specify the exact time point within the above range that animals died. Though tumors of the brain should be presented as reported in the reports of the original studies, several reviewers suggested that it should be noted in a general discussion that ACN-induced brain tumors are actually gliomas rather than astrocytomas.

Health Canada opened a discussion of the dose scaling for the TD05 by stating that it did not believe that dose scaling was appropriate for acrylonitrile. One reviewer commented that Health Canada should be consistent with its internal policy on this issue, but that if it decided to do dose scaling, body weight to the three-quarters power should be used rather than body weight to the two-thirds power. Another reviewer agreed that dose scaling should not be used in this case because the toxicity appeared to be due to a metabolite rather than the parent compound. This reviewer noted that observations of many other chemicals for which PBPK models are available show that when the toxicity of a chemical is due to a metabolite, the two species being compared are, at worst, equal. More often, the "bigger animal" is much less at risk when the toxicity is due to a metabolite because metabolism is not related to surface area. The panel agreed that no scaling factor would be appropriate and it approved the TD05 value of 2.3 mg/kg-day, based on incidence of brain and spinal cord astrocytomas in male and female rats.

Inhalation Dose Response

The panel had no comments on the choice of study (Quast et al., 1980b), data set, and tumor type (astrocytomas) used for the inhalation dose-response assessment. The panel agreed with the modeling as conducted by Health Canada. Several reviewers suggested that the assessment should present a more quantitative discussion of the factors that could contribute to the differences between animals and humans. One reviewer suggested that the bounding estimates from the human studies also be discussed in the dose-response

analysis. One reviewer questioned whether the tumors should more properly be identified as gliomas, as was suggested for the oral dose-response assessment. All agreed however that the assessment should label the tumors as the study authors reported them.

The panel next discussed the dose scaling used by Health Canada to derive human equivalent concentrations. Health Canada had multiplied the TC05s by (0.11 m^3 per day/0.35 kg bw) and (70 kg bw/23 cu.m per day), where 0.11 cu.m per day is the breathing rate of a rat, 0.35 kg is the body weight of a rat, 23 cu.m per day is the breathing rate of a human, and 70 kg is the body weight of a human. The panel did not disagree with this approach. One reviewer questioned whether there should be some extra factor to account for the fact that there is no first pass effect following inhalation exposure, but another reviewer noted that the models take this into account. Health Canada confirmed that the concentrations had been adjusted for duration.

The panel agreed with the final human equivalent values for TC05 of 8.9 mg/cu.m based on brain and spinal cord astrocytomas in male rats and 6 mg/cu.m based on brain and spinal cord astrocytomas in female rats. The reviewers suggested that it might be useful for the document to compare the TD05s and the TC05s. One reviewer noted that the values were, in fact, comparable, but another reviewer noted that there are too many uncertainties in extrapolation across routes to call out the first reviewer's suggested 4-fold difference.

It was noted that there is a slight discrepancy between the Health Canada document and the earlier *TERA* assessment in the incidence of brain and spinal cord tumors to be modeled after excluding animals due to early mortality. Health Canada could not duplicate the *TERA* numbers. Although possible reasons for this discrepancy were discussed, the issue was not resolved at the meeting.

RECOMMENDATIONS

- The panel encouraged that more work be done on developing the human PBPK model in order to address the differences between rats and humans.

- Several reviewers suggested that the characterization section of the document reiterate the quantitative discussion of the factors that contribute to the differences observed between animals and humans, which was presented in background documentation.

- Overall, the panel agreed with the conclusions made regarding mode of action. However, several reviewers felt that the document would be strengthened by a more in-depth critical review/evaluation of the *in vivo* mutagenicity studies because additional review would demonstrate that no conclusions could be drawn from the *in vivo* studies due to their inadequacy.

- The panel agreed with the oral and inhalation dose-response modeling using only the brain and spinal cord tumors.

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Attachment A

Managing Potential Conflicts of Interest

ITER Peer Review Meeting

November 16 and 17, 1998

(Approved by panel)

ITER peer reviewers donate their time and talents to this effort. They are selected based upon their expertise and qualifications and are employed by many types of organizations. *TERA* strives to create a balance of expertise and affiliations for each meeting. However, individual peer reviewers are representing their own expertise and views, not those of their employer. The *TERA* Board of Trustees approves *ITER* peer reviewers for inclusion in this program. A complete list of potential reviewers and more information on the *ITER* peer review program are available at http://www/tera/org/peer. Additional, *ad hoc* reviewers are selected to participate for their special expertise that may be needed for a particular chemical or discussion.

TERA had requested that each peer reviewer identify potential conflicts of interest related to the review of the health risk assessment of acrolein, acrylonitrile, and acrylamide, and/or the sponsors of these discussions. Each reviewer signed a statement indicating that he or she does not have a conflict of interest concerning these chemicals, or identified any potential conflicts or appearances of a conflict, with which he or she is aware.

Because of *TERA* involvement in two of the three chemicals for review (*TERA* co-authored the acrylamide issues document and has previously developed an assessment on acrylonitrile), the sponsors agreed that it would not be appropriate for *TERA* staff to select reviewers for this meeting. The sponsors agreed that an impartial Trustee from the *TERA* Board of Trustees should make the selection of reviewers. Trustee Jennifer Orme-Zavaleta, who is employed by the U.S. Environmental Protection Agency, reviewed the larger list of *ITER* peer reviewers, and those identified as potential *ad hoc* reviewers, to make the following selection. *TERA* staff discussed any issues concerning conflicts, or the potential or appearance of a conflict, with the individual reviewers, and with Trustee Orme-Zavaleta.

A summary of identified conflicts for each reviewer was presented to the panel and discussed prior to each chemical's session for panel discussion and approval. Most reviewers served as reviewers for all three chemicals; however, a few participated in less than three reviews as noted below. The following text was agreed to by the peer review panel.

Marilyn Aardema – Dr. Aardema is a Principal Scientist with the Human Safety Department of The Procter & Gamble Company. She has been asked to participate as an *ad hoc ITER* peer reviewer because of her expertise in genetic toxicology. She does not have any conflicts and will participate fully in all discussions and consensus.

John Christopher - Dr. Christopher is a Toxicologist with the Department of Toxic Substances Control of the California Environmental Protection Agency (Cal EPA). Cal

EPA regulates various aspects of production, use, sale or disposal of many chemicals, including those under discussion. However, Dr. Christopher does not have a specific conflict of interest with any of these chemicals and will participate fully in all discussions. Dr. Christopher requested inclusion of the following note: "Dr. John Christopher performs scientific peer review for *TERA* as a private individual. His employer, the California Department of Toxic Substances Control, is not bound in any way by the opinions he expresses or by consensus agreements to which he chooses to be a party."

Michael L. Dourson -- Dr. Dourson is Director of Toxicology Excellence for Risk Assessment (*TERA*). **Acrolein** – Dr. Dourson does not have any conflicts with acrolein and will chair that portion of the meeting. **Acrylamide** - Dr. Dourson is a co-author of the acrylamide document and so will not participate in that panel. **Acrylonitrile** - *TERA* previously developed an assessment on acrylonitrile, which was approved by an *ITER* panel and is now available on the *ITER* database. Dr. Dourson did not contribute as an author to that assessment. Dr. Dourson believes he can provide impartial comments in the discussion, but has requested that he not be polled for consensus to avoid any possible appearance of a conflict of interest. The panel agreed to this request and Dr. Dourson will participate in the discussion but not be polled for consensus.

Susan P. Felter – Dr. Felter is a Toxicologist with The Procter & Gamble Company. Dr. Felter previously worked for *TERA* and was the principal author of *TERA*'s acrylonitrile assessment, which was approved by an *ITER* panel and is now available on the *ITER* database. Dr. Felter no longer works for *TERA* or on acrylonitrile issues and will participate fully in discussions and consensus. Dr. Felter is not participating in the acrolein or acetaldehyde panels.

Marvin A. Friedman – Dr. Friedman is a consulting toxicologist, who recently retired from Cytec Industries, Inc. **Acrolein** – Dr. Friedman has no conflicts and will participate fully in discussion and consensus. **Acrylamide** -- Dr. Freidman is a co-author of the acrylamide document and will not participate in that panel. **Acrylonitrile** -- Cytec is a producer of acrylonitrile and while with Cytec Dr. Friedman was responsible for toxicological research on this chemical. He was associated with the acrylonitrile industry association as both member and chair. He was a reviewer on the early carcinogenicity reports, which came from the Dow Laboratory and served on the steering committee for the CIIT research program on acrylonitrile. Dr. Friedman believes he can provide impartial comments and opinions on the acrylonitrile document because he currently has no interest in the chemical, however he acknowledges that his past may bias his views. It is agreed that Dr. Friedman will participate in the acrylonitrile discussion, but not be polled for consensus.

Michael L. Gargas – Dr. Gargas works for the ChemRisk Division of McLaren/Hart. **Acrolein –** Dr. Gargas has no conflicts and will participate fully in discussions and consensus. **Acrylamide –** Dr. Gargas has no conflicts and will participate fully in discussions and consensus. **Acrylonitrile –** Dr. Gargas has conducted research in the past on acrylonitrile and is currently addressing comments on a manuscript submitted to *Risk*

*Analysis (*on behalf of BP Chemical). Dr. Gargas will be providing comments to a confidential client on the EPA Ambient Water Quality Criteria for acrylonitrile. It is agreed that Dr. Gargas will participate fully in the acrylonitrile discussions and consensus.

Henry d'A. Heck – Dr. Heck works for the Chemical Industry Institute of Toxicology (CIIT). He did not attend the meeting but provided written comments on the acrolein assessment for the panel's consideration. Dr. Heck has done research on acrolein for CIIT, but does not have any conflict of interest.

George Leikauf – Dr. Leikauf is Director of the Toxicology Division of the Department of Environmental Health at the University of Cincinnati, College of Medicine. **Acrolein and Acrylamide** Dr. Leikauf does not have any conflicts and will participate fully in the acrolein and acrylamide discussions and consensus**.** Dr. Leikauf did not participating in the acrylonitrile panel.

M.E. (Bette) Meek – Ms. Meek is Head of the Priority Substances Section of the Bureau of Chemical Hazards of Health Canada. Health Canada is sponsoring the acrolein and acrylonitrile assessments, therefore, Ms. Meek is not on those panels. Ms. Meek has no conflicts with the discussion on acrylamide and will participate fully in that discussion and consensus and serve as chair for acrylamide.

Martha M. Moore – Dr. Moore is Chief of the Genetics and Cellular Toxicology Branch of the National Health and Environmental Effects Research Laboratory of the U.S. EPA. She has been asked to participate as an *ad hoc ITER* peer reviewer because of her expertise in genetic toxicology. Dr. Moore has no conflicts and will participate fully in the acrolein and acrylamide discussions and consensus. She was not able to participate in the acrylonitrile review, but provided written comments that were considered by the panel.

Jennifer Orme-Zavaleta – Ms. Orme-Zavaleta is a Trustee of *TERA* and is employed by the U.S. EPA. *TERA* asked Ms. Orme-Zavaleta to assist with this peer review meeting in the selection of peer reviewers because of *TERA* staff involvement in the acrylamide (authors and presenters) and acrylonitrile (previous published assessment) assessments. *TERA* also asked her to chair the acrylonitrile panel. She has no conflicts and will participate fully as the chair.

Robert G. Tardiff – is the Director of the Sapphire Group. Dr. Tardiff worked on a National Academy of Sciences study including acrolein in the past, but has no conflicts and will participate fully in all discussions and consensus.

Vanessa T. Vu – Dr. Vu is Associate Director for Health at the U.S. EPA's National Center for Environment Assessment (NCEA). She has management oversight of the Agency's IRIS (Integrated Risk Information System) program which develops health assessments of environmental agents. Dr. Vu, like other reviewers represents her own

personal opinions and not necessarily those of the EPA. She has no conflicts and will participate fully in all three chemicals' discussions and consensus.

Vernon E. Walker – Dr. Walker is a Research Scientist/Veterinary Pathologist in the Laboratory of Human Toxicology and Molecular Epidemiology of the New York State Department of Health. He has been asked to participate as an *ad hoc ITER* peer reviewer because of his expertise in molecular biology and chemical DNA interaction. Dr. Walker performed DNA adduct research at CIIT in the past which was partially sponsored by BP Chemicals, Cytec and Monsanto. He also performed mutation studies for the Brain Tumor Society. However these activities do not create a conflict for these reviews and he will participate fully in all discussions and consensus.