Human & Experimental Toxicology (2005) **24:** 487–527
www hetjournal com

Review

Report of an independent peer review of an acrylonitrile risk assessment

LI Haber and J Patterson*

2300 Montana Avenue, Suite 409, Cincinnati, OH 45211, USA

A peer review panel made up of experts in toxicology, epidemiology, cancer mode of action (MOA), cancer mechanisms, carcinogenicity, genotoxicity, dose–response, US Environmental Protection Agency (EPA) cancer and noncancer methods, pharmacokinetic modeling and acrylonitrile, met on 22–23 September 2003 in Cincinnati, OH. The purpose of the meeting was to provide an independent review of a risk assessment of acrylonitrile that had been prepared by the Acrylonitrile Group (AN Group). Toxicology Excellence for Risk Assessment (TERA) organized the peer review and selected the panel. The panel discussed the toxicity and epidemiology literature of acrylonitrile and MOA information, and reached conclusions regarding its MOA, weight of evidence (WOE) for carcinogenicity, preferred approach for dose–response assessment and risk values. This paper summarizes the discussion and conclusions of the panel regarding the acrylonitrile assessment. Subsequent to the peer review, the authors of the acrylonitrile assessment revised their report and the panel reviewed the revised report. A manuscript of the revised assessment is being published in Regulatory Toxicology and Pharmacology. *Human & Experimental Toxicology* (2005) **24,** *487–527*

Key words: acrylonitrile; carcinogenicity; mode of action; PBPK modeling; risk assessment

Hazard characterization

A peer review panel[a] agreed that the acrylonitrile database contains unusually extensive epidemiology data. No increased cancer risk has been consistently observed in several different large, well-conducted epidemiology studies using several different occupational cohorts in several different countries. These epidemiology studies evaluated tumors of the lung, brain, prostate, and a variety of other organs. The epidemiology data are strong with narrow confidence intervals for most tumor types, and include large numbers of individuals ($n > 50\,000$ across all cohorts, with one cohort including $> 15\,000$ men and > 5000 women). Several cohorts included good exposure data and a long and complete follow-up. Overall, the epidemiological data have three striking features: (1) the size and completeness of the database; (2) the lack of consistently positive findings across studies; and (3) the lack of a clear dose–response relationship for human cancer. The highest human exposures approached or exceeded the lowest exposures found to produce tumors in rats, using some dose measures. Overall, the panel concluded that the epidemiology data do not support an association between acrylonitrile and increased cancer risk in humans, but that such an association could not be ruled out completely. The panel also concluded that, due to the lack of an association between acrylonitrile exposure and increased cancer mortality in humans, it is not valid to estimate a unit risk from the epidemiology data.

In contrast, the panel noted that acrylonitrile is clearly carcinogenic in rats and mice, based on the finding of increased tumors at multiple tissue sites in multiple oral (rats and mice) and inhalation (rats)

[a]The peer review panel included 13 experts: Dr. John P. Christopher, California Environmental Protection Agency; Dr. Michael L. Dourson, Toxicology Excellence for Risk Assessment, Chair; Dr. Linda S. Erdreich, Exponent Inc.; Dr. Susan P. Felter, Procter and Gamble Company; Dr. Timothy R. Fennell, RTI International; Dr. Jeffrey Fisher, University of Georgia; Dr. David W. Gaylor, Gaylor and Associates, LLC; Dr. Kannan Krishnan, University of Montreal; Dr. R. Jeffrey Lewis, ExxonMobil Biomedical Sciences, Inc.; Dr. Alan R. Parrish, Texas A&M University; Dr. Jerry M. Rice, World Health Organization, retired; Dr. James E. Trosko, Michigan State University; and Dr. Vernon E. Walker, Lovelace Respiratory Research Institute. Employers listed for affiliation purposes only. The opinions expressed were those of the individual experts. Panel members did not represent their employers or others with whom they are affiliated. A number of appendices are noted in this text. The appendices (as well as the revised assessment and other materials from the peer review) are available at http://www.tera.org/peer/AN/ANWelcome.htm

*Correspondence: Jacqueline Patterson, 2300 Montana Avenue,

Received 23 May 2005; revised 30 June 2005; accepted 6 July 2005

10.1191/0960327105ht552oa

studies. The reason for the apparent difference between humans and rodents is not known. Therefore, discussion of the animal data focussed on considerations of mode of action (MOA) and how to integrate the apparently disparate animal and human data.

Several MOAs for the observed animal carcinogenicity have been proposed, including acting as a carcinogenic 'initiator' or a 'promoter' of carcinogenesis. As is the case for many chemicals, no animal experiments have been performed to test acrylonitrile in an initiation/promotion model.

While most panel members agreed that cyanoethylene oxide (CEO) has demonstrated some genotoxic potential, it is not clear whether this is relevant to the mechanism for tumor induction. No consensus was reached on the ability of acrylonitrile or its metabolites to form covalent adducts with DNA as a mechanism of carcinogenesis. The panel agreed that carcinogenicity of acrylonitrile in rodents could be due in part to the parent compound, but is likely due more to its reactive metabolite, CEO, or one of its metabolites. The panel agreed that it is likely that acrylonitrile's carcinogenicity in rodents involves more than one MOA, and different MOAs could predominate at different doses. Varying degrees of data support the different proposed MOAs. For brain tumors, the evidence is most compelling for oxidative stress, either through interactions of reactive oxygen species with DNA or through epigenetic effects. These epigenetic effects include changes in signal transduction and inhibition of gap junction intercellular communication (GJIC), endpoints that might also result from effects besides oxidative stress. The evidence is less compelling for direct DNA damage caused by the acrylonitrile metabolite CEO, or even by acrylonitrile itself, although there are gaps in the available data on the DNA reactivity of acrylonitrile and its metabolites, particularly *in vivo*. However, the data at present do not allow unequivocal determination of acrylonitrile's MOA(s) as an animal carcinogen. The data are insufficient to rule out a direct DNA-reactive MOA for brain tumors, or to definitively identify a specific key event or MOA for brain tumors. Furthermore, there is no information on the MOA for the tumors produced in other tissues in the rat and mouse studies. All of the MOAs proposed for the observed animal carcinogenicity involve general processes (e.g., oxidative stress, GJIC, DNA damage) that are known to occur in humans.

The panel recommended that the assessment document more fully characterize the data regarding the MOA(s) for acrylonitrile carcinogenicity, noting that (1) the proposed MOAs are not mutually exclusive; (2) absence of evidence of an effect should be more carefully distinguished from evidence for the absence of an effect; and (3) some additional published studies supporting DNA reactivity should be included in the document.

In summary, the panels' conclusion for the hazard characterization portion of the assessment document was:

> "Epidemiology data do not support an increased cancer risk from acrylonitrile exposure in exposed workers. In contrast, the experimental animal data clearly support the conclusion that acrylonitrile is carcinogenic in rodents. The proposed cancer MOAs in rodents involve general processes (e.g., oxidative stress, GJIC, DNA damage) that are known to occur in humans, and so the data are presumed to support the use of the rodent data in establishing a quantitative cancer risk value. Although the data are insufficient to rule out any contribution due to direct DNA reactivity, an overall weight of evidence (WOE) evaluation does not support this as a predominant contributor to rodent carcinogenesis. Furthermore, linear extrapolation from the animal data is not supported by the available epidemiology data. Based on this information, the overall weight of the evidence suggests that acrylonitrile may be carcinogenic to humans at high doses based on extrapolation from rat studies, but the cancer risk associated with the low levels to which humans have been exposed in occupational settings is negligible."

Overall, the panel considered that the hazard characterization portion of the Toxicological Review of Acrylonitrile presented a thorough and detailed evaluation of the extensive literature on acrylonitrile. The panel identified several additional published studies and sources of information that should be considered and incorporated into the assessment document. These included studies on acrylonitrile toxicity, and studies on acrylonitrile and on related compounds that provided additional information relevant to evaluating the MOA, particularly the identification of DNA adducts.

Dose–response assessment

The panel concurred with the authors' use of the physiologically-based pharmacokinetic (PBPK) model and choice of peak CEO as a dosimeter. The panel recommended, however, that the assessment document provide a better description of the inability of the area under the curve (AUC) of CEO to provide a consistent dose–response relationship following oral and inhalation exposures in the rat. The panel also requested that the authors clarify in the text the way in which differences in exposure duration were addressed in the calculation of AUC.

The panel discussed at length the issue of using a linear versus a nonlinear approach for low-dose extrapolation of the carcinogenic effects of acrylonitrile. Although the panel recognized that the data are insufficient to rule out a role for genotoxicity, the consensus was that a nonlinear approach was preferred. The majority of the panel believed that using a nonlinear approach from the animal data, with the epidemiology data providing perspective on reasonable risks (an informal sort of bounding), seemed to be the only approach that incorporated the two most important features of the acrylonitrile data set – the strongly positive response in rats and several large, well-conducted occupational epidemiology studies that do not support an association between acrylonitrile exposure and cancer in humans. These panel members stated that, based on the WOE, linear extrapolation is inappropriately conservative. Panel members also supported a nonlinear extrapolation based on mechanistic data, with different panel members placing varying degrees of weight on the mechanistic data. However, the panel recommended by a simple majority that both linear and nonlinear approaches should be shown, with the linear approach described, perhaps, in an appendix. This recommendation to present both approaches is consistent with what the authors proposed in their assessment document.

For the quantitative cancer assessment, the consensus of the panel was:

- The authors should use their best judgment regarding whether the point of departure (POD) should be based on the pooled data or individual studies. This was based on the panel's recognition of the overall high quality of the authors' assessment document.
- A POD of 5% increased tumor incidence should be used as the basis of the assessment. Two members preferred a 1% POD as the basis of the assessment, but one of these panelists would then use a reduced uncertainty factor. No members preferred a 10% POD as the basis of the assessment.
- The authors should clarify that the uncertainty factor of 1.8 for human toxicokinetic variability was obtained by averaging the oral and inhalation values, which were very close.
- The composite uncertainty factor for the cancer assessment should be 180, based on a default uncertainty factor of 3.2 for differences between animal and human toxicodynamics, a default uncertainty factor of 3.2 for human toxicodynamic variability, a factor of 1.8 for human variability in toxicokinetics, and a factor of 10 for severity of response with the POD of 5%. An uncertainty factor for differences between animal and human toxicokinetics is not needed because a PBPK model is used.
- The rationale for the choice of POD and uncertainty factors in extrapolating from the animal data should use the epidemiology data to bound the quantitative cancer estimate, such that the resulting risk value (based on rat data) should not be inconsistent with the epidemiologic data.
- No additional factor is needed for protection of children.

The panel recommended that the overall quantitative assessment be based on the cancer endpoint, as the endpoint of concern, but also saw value in estimating reference concentrations (RfCs) and a Reference Dose (RfD), based on noncancer effects, in order to ensure that the cancer value was protective of noncancer effects.

Thus, the panel reached consensus on the following approach for deriving an RfC for noncarcinogenic effects based on human data:

- Derive the RfC based on the NOAEL of 10 ppm (22 mg/m^3) in the study of Sakurai *et al.*,[1] but characterize the NOAEL better.
- Consider eliminating the dosimetric adjustment (e.g., not adjusting for intermittent exposure and the occupational minute volume), but justify the decision toxicologically based on the fact that local effects, such as nasal and eye irritation, are determined more by concentration than by the product of concentration and time.
- Use an uncertainty factor of 3.2 for human variability (instead of a factor of 10), based on a toxicokinetic subfactor of 1 for irritant effects, and provide the rationale.
- Use an uncertainty factor of 1 for addressing extrapolation from subchronic to lifetime exposure (instead of 10).
- Use a database uncertainty factor of 1, but further justify this with the three-generation study. In light of the neurotoxicity seen with acrylonitrile given orally, and the neurotoxicity of the acrylonitrile metabolite cyanide, the panel also felt that it is important to note that the three-generation study included some consideration of neurodevelopmental toxicity.

For an RfC based on animal data, the panel reached consensus on using the lower bound on the concentration causing a 10% response (LEC10), or the lower bound on the benchmark concentration corresponding to a 10% response (BMCL10) of 0.38 mg/m^3 based on nasal lesions in rats in the

study by Quast *et al*.[2] The panel recommended an uncertainty factor of 1 for extrapolating from a LOAEL (based on the minimal severity of the lesion and use of the BMCL). A value of 1 was also recommended for the uncertainty factor for subchronic to chronic extrapolation, and for the database uncertainty factor, based on the same rationale as used for the RfC based on human data. Overall, the recommended composite uncertainty factor was 30, based on 10 for the uncertainty factor for human variability and 3 for the uncertainty factor for interspecies extrapolation, to account for toxicodynamic differences remaining after interspecies extrapolation using RfC dosimetry.

For the RfD, the panel reached consensus on the following points:

- As proposed by the authors, an RfD should be derived from the human equivalent dose calculated as a benchmark dose of 32 mg/kg per day, based on neurotoxicity in the Gagnaire *et al* study[3]
- The panel agreed with the use of an uncertainty factor of 1.8 for human variability in toxicokinetics, based on the ratio of the 95th percentile to the mean for acrylonitrile in blood;[4] a factor of 3.2 for human variability in toxicodynamics; a factor of 3.2 for toxicodynamic differences between rats and humans; and a factor of 10 for subchronic to chronic extrapolation.
- As for the RfC, the panel concurred with the use of a factor of 1 for the database uncertainty factor, but recommended that the authors support this factor better, using the three-generation study to show that young rats were tested adequately. Thus, the composite uncertainty factor is 180 ($1.8 \times 3.2 \times 3.2 \times 10$).
- The resulting RfD would be 0.2 mg/kg per day (32 mg/kg per day $\div$ 180).
- The authors should note in the text that the issue of whether to include a factor for use of the BMDL (which represents a defined *effect* level) is actively being discussed by the Environmental Protection Agency (EPA).

Final conclusion

In general, the panel agreed that the conclusions in the report could be supported based on the available data, with the specific recommendations noted in the meeting report. Many panel members commended the authors on the thoroughness of their review of the literature, and on compiling an extensive database into a well-written, well-analysed, unified assessment document. Panel members agreed that the report should focus on presenting the strongest case, with alternative approaches presented in an appendix. The database to support a cancer risk assessment for acrylonitrile is unique in that it includes robust epidemiological data, bioassay data from two rodent species by two routes of exposure, a PBPK model, and extensive mechanistic data. The panel agreed that it is important that all of these sources of data be integrated into the risk assessment for acrylonitrile. Several panel members stated that the MOA is the major outstanding issue, and noted the importance of considering multiple MOAs.

The panel requested the opportunity to review the revised assessment document, including a summary table showing how the risk values were modified. This review will be completed prior to loading the assessment on the International Toxicity Estimates for Risk (ITER).

Background

An independent panel of expert scientists met in Cincinnati to peer review a toxicological review and assessment of acrylonitrile (CAS No. 107-13-1). The Sapphire Group, Inc. on behalf of the Acrylonitrile Group (AN Group) has prepared the assessment document. The AN Group is composed of the following member companies: Bayer Corporation; BP Chemicals Inc.; Cytec Industries Inc.; The Dow Chemical Company; DuPont Company; GE Plastics; Solutia, Inc; and Sterling Chemicals, Inc. Expert peer reviewers donated their time and talents to provide an independent review of the assessment. The objective of the meeting was a comprehensive overall review of the materials as provided by the combined experience of all the reviewers. This meeting report summarizes the major discussions and conclusions of the panel as a whole.

This peer review meeting was organized and conducted by Toxicology Excellence for Risk Assessment (TERA), a nonprofit organization dedicated to the best use of toxicity data in risk assessment. TERA independently selected the panel of experts, which was made up of scientists from industry, government, consulting, and academia. TERA strives to create a balanced panel, while carefully identifying and managing potential conflict of interest (COI) and bias issues. The peer review meeting followed a standard TERA process, beginning with a close examination of the supporting documentation and important references by the panel prior to the meeting. At the meeting, the authors of the assessment and other scientists (on behalf of the AN Group) briefly presented the assessment and issues. The panel then systematically discussed the assessment,

starting with a discussion of the qualitative WOE for the key toxicity endpoints, followed by a discussion of the quantitative aspects of the assessment.

TERA developed the Charge to Peer Reviewers to guide the panel's discussions. The Charge is based upon a standard format TERA uses for its peer reviews of assessments that derive risk values. General questions regarding literature completeness, and interpretation of data and key decisions for hazard assessment and dose–response are included, as well as more specific questions relevant to acrylonitrile. To ensure the Charge was complete, TERA requested input from EPA regarding important issues that should be covered. EPA suggested including (1) whether oxidative stress can be accepted as the mechanism for brain cancer in rats and its relevance to humans; (2) whether the proposed WOE is supportable under the EPA cancer guidelines and the power of the epidemiology studies; (3) whether acrylonitrile can be considered non-genotoxic, and therefore a nonlinear carcinogen, under the EPA cancer guidelines; and (4) whether one can assess acrylonitrile as a nonlinear carcinogen, and if so, is the MOE approach the best way to do it? These EPA suggestions were fully incorporated into the Charge. TERA asked the sponsors to review the draft charge to make sure that they thought it complete and clear, and that it would meet their needs. The charge questions are listed at the beginning of the discussion for each major issue, and Appendix C includes the entire list of charge questions.

TERA determined that in order to provide a complete and thorough review of the assessment document, it was important to locate scientists with experience in the following key subject areas: toxicology, epidemiology, cancer MOA, cancer mechanisms, carcinogenicity, genotoxicity, dose–response, EPA cancer and noncancer methods, pharmacokinetic modeling, and acrylonitrile. TERA, as the independent group convening the peer review, was solely responsible for selection of panel members. TERA solicited suggestions on types of expertise and specific individuals from the EPA and the sponsors. In selecting peer reviewers, TERA explored potential conflict of interest (COI) and bias issues (including employment, financial, professional affiliations, public positions, publications and other sources of bias) with each prospective peer reviewer, and excluded those with conflicts from further consideration. Information on employment, professional affiliations, and other panelist information relevant to COI and bias were disclosed publicly at the beginning of the peer review meeting and are part of this meeting report.

Full discussion and participation was encouraged by the Chair and panel agreement was reached by consensus. Consensus for the purpose of these meetings is defined as 'an opinion held by all or most, or general agreement'. This meeting report is structured to reflect both the full discussion of the issues by different members of the panel and the consensus of the panel as a whole. The report will indicate when consensus was unanimous. The discussion is included to inform readers who were not present at the meeting of the reasoning of the panel in arriving at conclusions. Individual peer reviewer's comments are not identified by name as it is only the panel consensus statements that represent the final outcome of the peer review.

The meeting was open to the public. Individuals from member companies of the AN Group, as well as scientists from the US EPA were in the audience. Meeting observers were offered the opportunity to provide written and/or oral comments.

Conflict of interest disclosures

After a brief welcome by TERA, each peer reviewer introduced him or herself and noted whether they had additions or changes in their disclosure statements. (Copies of panel members' biosketches and COI and bias disclosure statements were provided to all attendees; see Appendix A). Two panel members had additional information. Dr. Lewis clarified that his employer, ExxonMobil, does not make acrylonitrile. Dr. Walker noted that his more recent work (e.g., with Dr. B. Ghanayem) was paid for by Health Canada, out-of-pocket, and by other research organizations. The Chair then raised the issue of whether there are any COI or bias concerns due to the fact that several peer reviewers received funding for acrylonitrile work from the AN Group or its member companies in the past. He specifically asked those peer reviewers to whom this did not apply to comment. Individual panel members to whom this did not apply remarked that the expertise that results from such previous work is needed for the peer review and it is helpful to have individuals who are not coming in cold to this large volume of information. All exempt panel members agreed that it was not a concern to include individuals with previous support from the sponsors on the panel.

TERA staff passed out additional written comments from Dr. Samuel M. Cohen of the University of Nebraska Medical Center, along with a biosketch and a COI disclosure statement. Dr. Cohen had been invited onto the panel, but could not attend the meeting due to scheduling conflicts. See Appendix B for his comments.

Introduction

Dr. Dourson as Chair of the panel presented ground rules for the conduct of the meeting and noted that there are four possible outcomes to the review:

- assessment is endorsed as written, assessment can be loaded onto the IIER data base;
- assessment is endorsed after some items are revised, assessment can be loaded onto IIER;
- assessment has major problems that preclude consensus; or
- assessment is rejected

The panel discussed both the human and animal data on acrylonitrile and the physiologically-based pharmacokinetic model for acrylonitrile. They discussed MOA and a WOE statement for potential carcinogenicity to humans. Finally, they discussed quantitative estimates of risk for acrylonitrile. For each major issue, the general format of the meeting consisted of a presentation by the sponsor's representatives, clarifying questions from the panel, a public comment session, and open discussion among panel members. This report is intended to summarize the highlights of the discussions that took place during the meeting and report on the conclusions, recommendations and suggestions of the panel.

The charge questions are listed in italics at the beginning of the discussion for each major issue. Please note that the discussion ranged broadly over all of the charge questions and associated issues related to each major topic, rather than following the charge questions one by one. Appendix C presents the entire list of charge questions, with cross-references to the relevant section and pages of the meeting summary.

Cancer hazard assessment

Sponsor presentations

The meeting began with a series of short presentations by several individuals on behalf of the AN Group. Copies of the slides used in each presentation are found in Appendix D.

Dr. Randy Deskin of Cytec Industries, Inc. spoke briefly for the AN Group, Inc. Members of the AN Group include the Bayer Corporation; BP Chemicals, Inc.; Cytec Industries, Inc.; The Dow Chemical Company; DuPont Company; GE Plastics; Solutia, Inc.; and Sterling Chemicals, Inc. Dr. Deskin briefly described the mission of the AN Group and uses of acrylonitrile. He noted that the AN Group has sponsored research on acrylonitrile for the past 25 years and their goal for this risk assessment is to develop updated risk values using all relevant information and the best available scientific understanding of the toxicological properties of acrylonitrile. He noted that EPA's existing assessment on the Integrated Risk Information System (IRIS) is based on old data and obsolete methodologies and that many new data have been published in the last 20 years. The AN Group plans to publish the completed risk assessment in the peer-reviewed literature, and share a finalized version of this risk assessment with others who are interested in the health risk assessment of acrylonitrile, including the US EPA's Air Office. Acrylonitrile is listed as a hazardous air pollutant (HAP) and as such is on a list of chemicals that the EPA must consider for regulation to reduce emissions under the Clean Air Act. The AN Group has engaged in an active dialog with the EPA Air Office and shared drafts of the Sapphire Group's assessment document. The Air Office provided some questions that have been included in the charge to peer reviewers. Others who may be interested in an updated health risk assessment of acrylonitrile include The National Sanitation Foundation International, the US Food and Drug Administration, the US Occupational Health and Safety Administration, and the European Union.

Dr. James J. Collins of The Dow Chemical Company presented information on the human epidemiology data on acrylonitrile. Dr. Collins noted that at the time of the current EPA IRIS review (1982) there were limited epidemiology data and that EPA utilized a DuPont study by O'Berg,[5] as the basis for the inhalation cancer risk value. Limitations of this study were recognized at the time, including its small size, short follow-up and 'weak documentation of exposure'. Since then, the O'Berg study has been updated,[5] and there have been three other large, well-conducted studies with detailed exposure assessments and longer follow-up. Up to 26 000 workers are included in the current available studies with detailed exposure estimates. The studies of national cohorts cover the full range of past occupational exposures to acrylonitrile in the respective countries.

While most of the epidemiology studies focussed on lung and brain cancer, other sites have also been evaluated. Dr. Collins observed that the cumulative relative risk (RR) for lung cancer has declined from the O'Berg study,[5] to the more recent studies and the 95% confidence intervals have narrowed. There are four studies in particular,[6–9] (UK cohort; Dutch cohort; DuPont cohort; NCI/NIOSH cohort) that are large and have narrow confidence intervals and RRs

close to 1. In a meta-analysis of 25 published and unpublished epidemiology studies,[10] the confidence limits (CL) were even more narrow and the meta RR for lung cancer was <1. Dr. Collins noted that power calculations for epidemiology studies are for planning purposes, and once a study is complete, the CLs constitute the power of the study, except for the effects of bias and confounding.

Dr. Collins compared animal and human results by comparing *P*-value functions of the projected RR for brain cancers from the animal data and the measured RR from the human studies. The human RR was estimated at 1, with narrow CLs (0.8–1.2), while the animal-derived RR was almost 2.5, with wider CL (due to the smaller sample size), but no overlap with the human data. This analysis assumed the humans and animals had the same exposure levels (explained further in Clarifying questions from the panel). Dr. Collins also noted that bias and confounding are unmeasured factors and so the true CLs in the human studies are probably wider than those used in this analysis.

Dr. Collins concluded by stating that the current IRIS assessment needs to be updated to consider the extensive new high-quality epidemiology data. The new data also allow for assessment of rare cancers and reduce uncertainties around the estimates. He noted that the International Agency for Research on Cancer (IARC) re-reviewed acrylonitrile in 1998 and downgraded the cancer classification from 2A 'probably carcinogenic to humans' to 2B 'possibly carcinogenic to humans'.[11]

Dr. Michael L. Gargas of the Sapphire Group, Inc. was a lead author of the assessment and presented information on the cancer hazard assessment for acrylonitrile. He noted that acrylonitrile is a multi-site and multi-species carcinogen in many bioassays and a number of tissues. Tumor sites in the rat include the central nervous system (CNS), mammary gland, brain, Zymbal gland, forestomach, tongue, and small intestine. Mice have been studied to a much lesser extent, but increases in forestomach and Harderian gland tumors have been observed. Potency is highest for brain tumors in rats. Dr. Gargas summarized the WOE for acrylonitrile carcinogenicity, noting that there is a robust human database that found no causal association for any cancer type, and a robust data set in animals that shows clear association for multiple tumor sites. Acrylonitrile is a weak genotoxicant *in vitro* and largely negative in animal tests. The sponsors requested help from the panel on the WOE statement for the assessment.

At the request of the AN Group, Dr. James E. Klaunig, Professor and Director of Toxicology at the Indiana University School of Medicine, then presented information on mechanistic studies on acrylonitrile-induced rat brain neoplasia. He recommended that the panel move beyond thinking about genotoxic versus non-genotoxic mechanisms, and noted the wide range of potential mechanisms of action. He also noted that the presence of an adduct does not necessarily mean a mutational event has occurred: a mutation is a heritable event observed after cell division. For acrylonitrile, Dr. Klaunig stated that the MOAs may include oxidative stress, oxidative DNA damage, decreased GJIC, altered gene expression, and decreased apoptosis. He noted that several of these alterations may be secondary to oxidative stress. Dr. Klaunig described the *in vitro* and *in vivo* data showing that acrylonitrile causes oxidative stress, increases oxidative DNA damage, and decreases GJIC in glial cells but not hepatocytes. He indicated that acrylonitrile behaves like other promoters in the SHE assay (1- versus 7-day results). Dr. Klaunig noted that the data do not support a conclusion that there is no evidence of direct genotoxicity, but he believes that the evidence is strong for an indirect MOA. Noting that questions remain about the genotoxicity of the acrylonitrile metabolite 2-CEO, he expressed a willingness to conduct additional experiments evaluating the genotoxicity of CEO, noting that such studies, if positive, could give additional weight to a MOA involving direct DNA damage. Overall, Dr. Klaunig expressed the belief that CEO itself or one of its metabolites plays a key role in acrylonitrile tumorigenesis. Key hazard characterization issues identified by Dr. Klaunig include: (1) identification of the MOA(s); (2) consideration of the implications if there is more than one MOA; (3) determination of whether low-dose extrapolation should be linear or nonlinear; and (4) consideration of confidence in the MOA. He stated that he did not see a need for separate route-specific hazard characterization statements.

Clarifying questions from the panel

Peer reviewers asked a number of clarifying questions regarding these initial presentations on the available data and the hazard assessment. In response to questions regarding how smoking is accounted for in the studies, Dr. Collins stated that there is potential for confounding from smoking because people in the high exposure groups tended to smoke more, perhaps because these higher exposures occurred at earlier times when smoking rates were higher. Thus, smoking may explain some of the lung cancer cases, particularly in the high exposure categories. Some studies did try to

statistically separate the effects of smoking from the acrylonitrile exposure, but they could not evaluate the potential for interactions or a multiplicative effect. The attempts to control for smoking were rather crude, since most of the data had to be collected from relatives.

In response to a question from the panel, Dr Collins confirmed that all of the high-exposure population in the DuPont cohort,[8] was included in the evaluation, and that in the past in acrylic fiber plants like those in the Dupont study, there were episodes of very high exposures that produced intolerable acute symptoms. He also noted that exposure diminished with time, but there are still episodes of occasional high exposures. A panel member noted that it is important for the assessment document to more fully describe the exposure history and it should note that some of the workers were exposed to very high concentrations, including episodic exposures to the highest acutely tolerable levels.

Several peer reviewers asked Dr. Collins for clarification on the graph comparing RR based on the animal and human data (slides 9 and 10 of his presentation). Dr. Collins explained that a number of assumptions had to be made for this comparison. Both animals and humans were assumed to be exposed to 2 ppm in air for an occupational lifetime; this exposure is lower than the exposures in the animal bioassays. The human data were adjusted for partial lifetime exposure to be comparable to the animal studies. It was noted that the graphs represent *P*-value plots that represent various CLs around those estimates, and the estimates assumed that bias and confounding would not be operating. Dr. Collins noted that if bias and confounding were included, the human CL would be broader, but he did not think that it would expand to overlap the animal curve. Another panel member asked how the animal curve in slide 9 would change if one were to include all tumors attributed to acrylonitrile exposure, rather than only brain tumors. Dr. Collins said the curve would shift to the right, indicating a higher RR and greater difference between rats and humans.

A peer reviewer noted that when smokers quit smoking their lung cancer risk is lowered, and wondered whether there are data on workers who were exposed briefly to acrylonitrile, but who continued to smoke. Dr. Collins responded that the data have not been analysed in that way, and that the degree of confounding from smoking is not known.

Another peer reviewer asked whether one can identify separate groups (e.g., acrylonitrile users or producers) with higher or purer exposures. Dr Collins responded that fiber operation involved higher exposures and fewer exposures to other chemicals than the monomer operations, but that workers move around and some may have moved from monomer to cyanide to acrylamide, etc. In addition, the workers may have worked at other companies and exposed to other chemicals, but information on other exposures was not available.

A peer reviewer clarified a point made in slide 13, stating that IARC concluded that there are *inadequate* data in humans to support a causal relationship. This is different from 'no consistent findings of causal association'. The IARC panel had considered a conclusion that there is evidence of lack of carcinogenicity, but could not agree to it.

Several questions were asked about the MOA and supporting mechanistic studies. A peer reviewer asked whether the authors evaluated the MOA using the Hill criteria only for brain tumors or for all tumor types. Mr. Kirman confirmed that they only conducted the evaluation for brain tumors. Dr. Klaunig confirmed that the gap junction studies used acrylonitrile (not CEO), and that it appeared to be metabolically activated; there was no effect when cytochrome P450 was blocked. In response to a question about the effect of blocking apoptosis, Dr. Klaunig noted that clonal expansion can result from blocking cell death, if growth continues at the same rate.

A peer reviewer noted that studies of tumor promotion have focused on epithelial cells (e.g., cells with gap junctions that are involved in GJIC). Tumor promotion via this mechanism is not clearly established for other cell types. The reviewer noted that data are lacking on whether these phenomena are relevant for brain tumors of any type. To date, all attempts to demonstrate promotion in the brain have failed. Dr. Klaunig agreed, but noted that there is a tendency to associate increased cell proliferation with promotion, and this is not correct. Tumor promotion requires selective clonal expansion of initiated cells, and populations of initiated preneoplastic cells have not been identified in the brain.

Public comments

There were no public comments.

Panel discussion

The charge questions are listed at the beginning of the discussion for each major issue. Please note that the discussion ranged broadly over all of the charge

questions and associated issues related to each major topic, rather than following the charge questions one by one.

Adequacy of literature search

Was the literature search approach appropriate and adequate? Are there additional published studies or published data that you think should be considered for this risk assessment of acrylonitrile?

In general, the panel thought that the literature search was appropriate and adequate. Several panel members submitted premeeting comments noting additional relevant studies (see studies listed in Appendix B). These included studies on acrylonitrile toxicity, and studies on acrylonitrile and on related compounds that provided additional information relevant to evaluating the MOA, particularly the identification of DNA adducts. Individual studies noted by panel members that provided key supplementary information are noted in the context of the relevant discussion below.

Cancer hazard characterization

Have the appropriate mode(s) of action been identified? Have the appropriate data been adequately considered in the discussion of the mode(s) of action? Is the proposed MOA defensible?

Specifically, can oxidative stress be accepted as the MOA for brain cancer in rats? If so, using EPA's current (1999 and 2003) guidelines for carcinogen risk assessment, does the assessment make an adequate case that this mechanism is not relevant in humans?

More generally, are the tumors observed in animals biologically significant and relevant to human health? Points relevant to this determination include whether or not the choice follows from the dose–response assessment, and if the effect (including tumors observed in the cancer assessment) and the species in which it is observed is a valid model for humans.

Can acrylonitrile be considered not to demonstrate mutagenic or other activity consistent with linearity at low doses, so that a nonlinear extrapolation should be conducted under the EPA cancer guidelines? Does the evidence for a nonlinear MOA meet the standard for nonlinearity set in, for example, the IRIS assessment for chloroform?

How do you interpret the overall occupational database? Is the proposed WOE that epidemiological studies 'do not support identifying acrylonitrile as a human carcinogen' supportable under the EPA cancer guidelines? Is the estimated carcinogenic potency from the results of the epidemiology studies, even though they failed to reach significance, consistent with the available animal data?

Is the WOE for cancer from both oral and inhalation exposure assigned at the appropriate level and does it follow the EPA guidelines? Does the WOE statement present a clear rationale and accurately reflect the utility of the principal studies, the relevancy of the critical effects to humans, and the comprehensiveness of the database?

Hazard characterization – epidemiology evidence

The panel discussed several key issues related to the epidemiology data, including the strengths and weaknesses of the overall database, how these strengths and weaknesses should relate to the overall interpretation of the data, and initial thoughts on the weight of the epidemiology evidence and possible reasons for apparent differences from the animal data. Several panel members noted that it is hard to reach absolute conclusions, even with epidemiology data as strong as that for acrylonitrile. This is reflected in the sometimes differing interpretations and conclusions reflected in the following discussion.

In reviewing the epidemiology data, panel members noted that the acrylonitrile database contains unusually extensive data. There are several large, well-conducted epidemiology studies, several including good exposure data and a long and complete follow-up of the cohort. In particular, the NCI cohort,[9,12] contained detailed exposure information using several different exposure metrics, with smoking data on a subset of the cohort, and some analyses that adjusted for smoking. The DuPont cohort studied by Wood *et al*.,[8] also included some workers who were exposed to very high acrylonitrile levels (>100 ppm), although the sponsor noted that these high exposures were for very short durations. The cohort also included workers who were exposed to acrylonitrile for >20 years.

Panel members also noted several limitations to the data that are common in epidemiology studies. Although there were some smoking data, such data were available for only one cohort. The risk may have been over-estimated if the workers smoked more than the general population. Another potential confounder is asbestos exposure. Mesotheliomas were observed in the DuPont cohort, and asbestos may have also caused some of the lung cancers. Most of the epidemiology analyses were based on cancer mortality, with few analyses based on incidence. This is unlikely to be an issue for lung and

brain cancer, but could be an issue for prostate cancer, which has a high survival rate Higher diagnosis rates with better medical screening can also be an issue for prostate cancer, but Wood *et al*.,[8] used an internal unexposed worker control group, which would control for screening bias.

Several panel members noted the narrow confidence interval in the larger studies (increasing the confidence in the results), with the RRs near unity The decreasing magnitude of the RRs reported by more recent studies was also noted. These panel members stated that there was considerable consistency across studies, with the lung cancer data consistent with no effect or a modest increase They stated that the overall data do not support a dose–response relation between acrylonitrile exposure and cancer The meta-analyses,[13] were useful in pulling together the data across the studies, and show no evidence of risk for lung cancer (meta RR for the workers in the highest exposure category from seven studies where exposure estimates existed 1.2, 95% confidence interval 1.0–1.5).[10] One reviewer considered the epidemiology data to be negative, and considered the overall data as showing a lack of evidence of acrylonitrile carcinogenicity in humans, but noted power limitations in all but the largest study. Based on the large sample sizes and the narrow confidence intervals, this panel member stated that one can conclude statistically that the tumors observed in animals are not found at a similar level in humans, although MOA needs to be considered.

Panel members also emphasized the importance of looking at the pattern of risk. The epidemiology database for acrylonitrile includes a number of different cohorts from several different countries, and several of the studies included analyses using several different measures of exposures Generally, consistent results were obtained across these various studies. It was also noted that, because the epidemiology database includes a large number of different studies and endpoints (i.e., approximately five response measures in each of 32 studies), some statistical elevations (as well as decreases) in various cancer incidences at the 95% CL would be expected based on chance alone

One panel member noted that the Blair study,[9] found a two-fold increase in lung cancer risk in the highest exposure quintile. This increase was not observed when the data were analysed by decile, but cutting the data so finely results in a loss of power Thus, this reviewer stated that one cannot completely rule out an effect in the high exposure group. It was noted that the apparent effect could be due to confounding by smoking, but there are no data to support this one way or the other. Other panelists agreed that there is some indication of an increased risk of lung cancer, based on this positive response in the high exposure group of the Blair study,[9] but the evidence from the epidemiology studies is not persuasive for an increased risk of brain cancer.

One panel member pointed out that animal studies involve controlled exposure, while humans are exposed to a variety of chemicals, including both promoters and anti-promoters For example, the incidence of lung cancer in Japanese men is lower than that in American men, even though the Japanese have a higher incidence of smoking, perhaps because green tea consumed by the Japanese is an anti-carcinogen There are also metabolic differences in different ethnic groups. Another panel member noted that for acrylonitrile, the finding of a similar absence of response across populations in different cohorts in different countries indicates that the interplay with diet and other factors is not a significant concern, and strengthens the overall conclusion

One panel member noted that standard animal bioassays test primarily for a chemical's potential to act as a complete carcinogen, and do not evaluate separately whether a chemical is an initiator or a promoter. Promoters can also produce cancer in the absence of an applied initiator by acting on cells that have already been initiated Another panelist suggested that epidemiology studies act best at detecting complete carcinogens, since promotion may be missed if the population of interest did not have a high degree of initiation. The panelist suggested that the animal and human data are consistent with the hypothesis that acrylonitrile is a good promoter, but not a good initiator The evidence supporting acrylonitrile as a promoter is discussed in greater detail later in this section.

The panel then addressed weaknesses in the human data, and aspects of the data that would weaken a conclusion that acrylonitrile is not carcinogenic in humans. One reviewer cited the IARC standard for evidence of lack of carcinogenicity in humans:

> "There are several adequate studies covering the full range of levels of exposure that human beings are known to encounter, which are mutually consistent in not showing a positive association between exposure to the agent, mixture or exposure circumstance and any studied cancer at any observed level of exposure A conclusion of 'evidence suggesting lack of carcinogenicity' is inevitably limited to the cancer sites, conditions and levels of exposure and length of observation covered by the available

> studies. In addition, the possibility of a very small risk at the levels of exposure studied can never be excluded."[11]

This panelist noted that IARC, in its 1999 re-evaluation of acrylonitrile,[11] did not believe that acrylonitrile meets these criteria.

The panel discussed the possibility of a healthy worker effect. It was noted that the healthy worker effect is usually not a significant problem for cancer (unlike for such endpoints as cardiovascular risk), because factors that make one susceptible to cancer are not easy to exclude at the time of hire. Another panelist countered that people who are obese or who have depressed immune function or other factors that could make them more susceptible to cancer might be less likely to be hired or employed for long periods in these types of jobs, and so might not be included in the occupational cohorts. In particular, this panelist noted that in the Wood study,[8] the standardized mortality ratios (SMRs) for many of the cancer types were higher for the comparison with unexposed workers than when the general population was the referent (i.e., unexposed workers had a lower cancer risk that the general population). Panel experts in the area did not have an explanation for this phenomenon, but they noted that the epidemiology community does not consider the healthy worker effect to be an important confounder for cancer studies. In addition, although most of the studies used the general population as the reference population, the Blair study analysed risk by comparing exposed to unexposed workers,[9] which avoids concerns about the healthy worker effect.

One reviewer noted the lack of concordance between target organs even between the rat and mouse studies with acrylonitrile, with the data in mice weaker than that in rats, and cautioned against focussing only on one target organ. A panel member noted that the studies with acrylonitrile did evaluate the effect on total cancers, and did not see an effect. It was noted that if the epidemiology results are negative, it can be useful to consider what risks may not have been detected.

One panelist asked what would be required for the epidemiological data to be considered negative. Another panel member stated that the overall pattern of risk for brain cancer provides little evidence to support a relation between acrylonitrile and brain cancer (e.g., the magnitude of RRs in individual studies tend to be near 1.0 and do not increase with increasing exposure and latency), but suggested that a definitive answer would include an evaluation of the meta RR for the highest exposure groups for all tumor types; and a lack of an elevated risk in that group would be persuasive. While such an analysis was conducted for lung cancer in the meta-analysis,[10] that study did not include a meta-analysis for brain cancer in the highest exposure group. It is possible that a brain cancer meta-analysis for the highest exposure group was not carried out due to small numbers.

The panel discussed how to integrate the human data with the animal data, and how to explain the apparent differences between the animal and human results. Panel members noted that a key question is whether the MOA data are informative regarding why animals are different from humans; this issue is addressed in detail in the next section.

A reviewer noted that some of the differences in conclusions reached by different scientists evaluating the acrylonitrile database relates to the dose metric used for exposure. The standard dose metric for EPA cancer assessments is the lifetime average daily dose (LADD). Use of the LADD results is very different in comparisons between animal and human exposures than if one compares cumulative exposure in ppm per years. For example, the low concentration in the Quast *et al*.,[2] inhalation study was 20 ppm. Exposure for 2 years (considered a 'lifetime' for a rodent study) would result in cumulative exposure of 40 ppm/years.[b] In contrast, occupational exposure to 10 ppm for 10 years would result in a higher cumulative exposure (100 ppm/years), but a much lower LADD, after adjusting for the time actually exposed (8/24 hours/day, 240/365 days/year) and years exposed over the entire lifetime. This reviewer stated that once the data are normalized to LADD, the animal exposures were at least an order of magnitude higher than the occupational exposures. This issue is addressed in further detail in 'Choice of POD and uncertainty factors for inhalation cancer assessment, using nonlinear extrapolation'. The sponsor noted that an attempt was made to adjust for this sort of difference, but acknowledged that it is necessary to include some assumptions in making the adjustments. A panelist noted that this concern is partially addressed by the use of different exposure metrics.

A reviewer observed that a large fraction of the brain tumors in the animal studies was microscopic. These tumors did not cause the death of the experimental animals, and were only detected as part of the complete necropsy. This means that the animal data are not directly comparable to the human data, since the endpoints for the epidemiology studies were mortality and tumor incidence, not microscopic tumors observed on autopsy.

[b]For comparison with the LADD from the occupational studies, this cumulative exposure would need to be adjusted by exposure for 5/7 days/week, 6/24 hours/day.

One reviewer noted that no chemical has been associated with brain tumors in people; the only identified causative agent is radiation used for medical treatments. Another panel member also noted that brain tumors are very rare in people, and occur primarily in children and very old people. The panelist stated that he personally does not believe acrylonitrile causes brain tumors in people.

The panel also considered what the epidemiology data say about tumors in organs other than the lung and brain. Prostate tumors were investigated in four cohorts, of which two (the DuPont cohort and the Marsh study,[14] of an individual cohort) showed a statistically significant increase. For the Dupont cohort, which experienced the highest ppm exposures, several early studies reported a significant increase in prostate tumors, but this increase was not observed in later studies. A panel member asked whether this could be due to the increase in background with age, and whether age-adjustment would correct for the increasing background. Others noted that mortality from prostate cancer is low. Another responded that one needs to be careful because the prostate data are for mortality. Although some of the panel members considered evaluating the prostate cancer data as part of a dose–response assessment, others did not think that a quantitative assessment should be conducted on an overall database that does not support an association between acrylonitrile exposure and cancer in humans.

Another reviewer noted that epidemiology data do not include people exposed for a full lifetime, and information is lacking regarding any increased risk following early life exposure. To address this issue, the panel considered the results of the three-generation rat study, which included an evaluation of cancer.[15] In this study, rats were exposed to 0, 100 or 500 ppm acrylonitrile in drinking water. Because the F0, F1 and F2 generations were each exposed for 1 year, and tissues were evaluated histopathologically, this study provides some information on age-related differences in susceptibility to acrylonitrile-induced carcinogenesis. There was no clear effect; tumor incidence was increased in the F1 generation but not in the F2 generation. One panelist stated that observation of an age-related increase would be meaningful, but the absence of such an increase is not, because exposures were only for 1 year. Another noted that the sample size was relatively small, only 20 per group.

It was also noted that, although most of the epidemiology data are for males, the Blair study included approximately 5000 females,[9] and the authors did look for an increased risk of breast cancer. Based on the Blair study,[9] panel members did not consider lack of data on women in other studies to be a significant data gap.

Overall, in this initial discussion, the sense of the panel was that, within the limitations of the epidemiology data, the hazard characterization data do not support an association between acrylonitrile and increased cancer risk in humans. However, it was noted that an association could not be ruled out completely. As discussed below, this initial conclusion was further refined in the development of the cancer WOE statement.

Hazard characterization – animal evidence The panel noted that acrylonitrile is clearly carcinogenic in rats and mice, based on the finding of increased tumors at multiple tissue sites in multiple oral (rats and mice) and inhalation (rats) studies. Therefore, discussion of the animal data focussed on considerations of MOA and how to integrate the apparently disparate animal and human data.

Hazard characterization– MOA The charge contained a number of specific questions related to evaluating MOA in the context of the hazard characterization. Instead of addressing the questions one by one, the panel considered the data and issues related to MOA in a comprehensive discussion. The panel agreed that consideration of the MOA is important for the assessment of acrylonitrile carcinogenicity.

The discussion on MOA began with several panelists providing background on cancer biology. One panel member noted that the observation of cancer in the experimental animals means that there was an irreversible genetic change in a single cell, which was promoted by mitogenesis or the absence of apoptosis. Further genetic changes are needed for a tumor to become invasive. This panelist stated that a chemical can cause tumors by acting as a mutagen, a cytotoxin (causing necrosis or apoptosis and reparative cell division), or via an epigenetic mechanism. For mutagenesis to be evident, the chemical needs to damage DNA, and then the damage is 'fixed' into the DNA, so that it is transmitted to the next generation of cells. This panel member noted that initiators are considered not to have thresholds, but stated a personal belief that initiators also have theoretical thresholds, due to the existence of DNA protective mechanisms, recognizing that not all DNA lesions lead to mutations, and not all mutations lead to cancer. The panel member continued that fixation of the mutation in the DNA requires cell division (mitogenesis). However, there is also a small, but finite, chance of error in the act of

replication itself. This means that a chemical could act as a promoter by increasing the replication and/or blocking of apoptosis of a spontaneously-initiated cell. This occurs by mitogenesis or by blockage of apoptosis. By definition, promoters cause earlier and higher frequency of tumors that would occur naturally (at a lower incidence). However, it is difficult to classify a chemical as a pure promoter. The classical promoters, such as phenobarbital and polychlorinated biphenyls (PCBs), do cause some increase in tumors even when administered alone, presumably because they act on spontaneously initiated cells. A limitation of *in vitro* assays is that they evaluate a phenotypic marker, but one cannot distinguish whether the chemical selected a pre-existing mutation or directly induced a mutation. Promotion requires regular, sustained exposure, and promoters have thresholds. There are many different mechanisms of promotion (e.g., cytotoxicity resulting in compensatory hyperplasia), and these mechanisms can be specific to a species, sex, organ, and/or cell type. However, a mitogen is not necessarily a promoter or indirect genotoxin. For example, growth factors such as epidermal growth factor (EGF) cause proliferation, but would not be considered promoters. The panelist suggested that the panel consider mechanism of action in terms of the following four possibilities: (1) the chemical does nothing to the cell; (2) the chemical mutates the cell; (3) the chemical kills the cell by necrosis or apoptosis, followed by reparative cell division; or (4) the chemical acts epigenetically.

This panelist continued by stating that the data regarding the genotoxicity of acrylonitrile are not convincing. He stated that the positive results seen with acrylonitrile were obtained with systems that can lead to false positives. For example, positive results in mammalian *in vitro* assays evaluating gene mutations at the thymidine kinase (TK) and hypoxanthine guanine phosphoribosyl transferase (HGPRT) loci (e.g., the mouse lymphoma and CHO assays, respectively) can reflect gene mutations and chromosome alterations, but they can also reflect changes that turn off a gene at the transcription level; none of the *in vitro* studies with acrylonitrile verified that the observed changes were due to mutations. Another panelist pointed out that Recio *et al*.,[16] conducted a molecular analysis of *hgprt* mutations induced by CEO in human lymphoblastoid cells. The first panelist also noted that any observed DNA adducts could result from damage to mitochondrial DNA that is not reflected in nuclear DNA damage. The data from Dr. Klaunig's laboratory indicate that acrylonitrile blocks GJIC, and is associated with oxidative stress. Most, if not all, tumor promoters block GJIC. Based on this analysis, the reviewer stated a personal belief that acrylonitrile acts by an epigenetic promoting mechanism, and that oxidative stress plays a role.

Another panel member noted that there is a natural background of tumors in experimental animals that increases with age, although some tumors are common and others are rare. Naturally-occurring tumors cannot be distinguished morphologically or molecularly from chemically-induced ones. One cannot state that an increase in a specific tumor type is *de facto* promotion. In addition, increased tumors in rats and mice frequently do not predict the tumor site in humans, even for known carcinogens. For example, beta-naphthylamine is a human bladder carcinogen, but causes only liver tumors in mice. The general practice in risk assessment is that, as a health-protective approach, tumors in animals are considered an indication of human carcinogenic potential, unless the MOA is shown not to be relevant to humans. In general, agents that cause certain types of tumors at multiple sites most commonly have a 'DNA reactive' MOA. However, specific site concordance among species often does not occur. Many sites in animals where tumors occur do not have an anatomic equivalent in humans. For example, the Zymbal glands, mouse forestomach, and Harderian gland are very responsive to DNA-reactive carcinogens, but have no equivalent human sites. These animal tissues also have a low frequency of naturally-occurring tumors. Even for genotoxic carcinogens, site concordance is rare, although there are some striking examples, such as vinyl chloride and angiosarcomas. Despite this concordance, vinyl chloride also causes tumors in experimental animals at sites that are not increased in humans. The reviewer noted that there are some chemicals with a database resembling that for acrylonitrile, causing tumors in animals, but with an extensive human database finding no increase in tumors in humans. However, these chemicals differ from acrylonitrile in important ways. For all of these chemicals, single specific organs are affected in experimental animals, and mechanistic studies have shown that they act via non-DNA reactive mechanisms that are unlikely to operate in humans. For example, melamine causes urinary bladder tumors secondary to calculi production, and d-limonene causes kidney cancer in male rats secondary to alpha$_2$-microglobulin nephrotoxicity. This reviewer was unable to think of any multi-organ agents for which adequate human data exist, but which are positive in animals and negative in humans.

Several panelists observed that, for acrylonitrile, there is clear evidence of carcinogenicity at several organ sites in the rodent studies. One panel member stated that all of the observed tumor sites are associated with susceptibility to DNA-reactive carcinogens. Overall, the spectrum of tumors for acrylonitrile in animals, including the finding of tumors in Zymbal gland,[2,17,18] follows the pattern of a genotoxic DNA-reactive carcinogen. Therefore, one of these reviewers concluded that acrylonitrile acts through a DNA-reactive mechanism(s). Another suggested that the report should draw on the WOE for structurally related compounds. Those data support the conclusion that there may be both non-genotoxic and genotoxic components to acrylonitrile tumorigenicity. A third did not consider the *in vivo* data to support a hypothesis of a genotoxic mechanism, and stated that the evidence is stronger for indirect DNA reactivity. This reviewer thought that the hypothesis of indirect genotoxicity can be directly supported by the data, although the assessment document emphasizes this MOA too strongly. He suggested that other hypotheses cannot be ruled out, and there is compelling evidence that MOA(s) besides indirect DNA reactivity may be important, but these other arguments are not convincing until direct data are available.

Several panelists thought that the literature regarding DNA adducts and a possible genotoxic MOA for acrylonitrile was not given enough consideration in the assessment document. One of these panelists agreed that the *in vitro* evidence for acrylonitrile genotoxicity is stronger than is stated in the assessment document in some cases, but acknowledged that extrapolating doses from *in vitro* to *in vivo* is difficult. In considering the DNA adducts, panelists noted that CEO could be expected to form DNA adducts by direct reaction with DNA. The early studies with acrylonitrile looked for the adducts analogous to vinyl chloride, such as 7-(2′-oxoethyl)guanine (7-OEG). This adduct was found in the liver but not the brain of rats exposed to acrylonitrile or radiolabeled CEO;[19] the absence of this adduct in the target tissue suggests that it is not responsible for the observed tumorigenicity. However, adducts formed in DNA have not been sufficiently investigated for a number of reasons. Use of radiolabeled acrylonitrile for the evaluation of DNA adduct formation *in vivo* has been limited by the tendency of ^{14}C ACN to polymerize, even at a relatively low specific activity. The identity of the adducts detected from *in vitro* reaction of CEO with DNA appears to depend on the reaction conditions used, and the means of identification of the adducts, with differences observed between two different laboratories in the nature of the adducts formed. Cyanohydroxyethyl adducts could be observed *in vitro* with high performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR) analysis,[19,20] whereas hydroxycarboxyethyl and oxoethyl adducts were observed with mass spectroscopic (MS) analysis. Solomon *et al*.,[21] found DNA adducts after reaction of CEO with deoxyadenosine, deoxycytosine, deoxyguanosine, or deoxythymidine. Several of these adducts were also observed after reaction of CEO with calf thymus DNA. Yates *et al*.,[19,20] found that reaction of radiolabeled CEO with deoxythymidine or calf thymus DNA resulted in the formation of 3-(2-cyano-2-hydroxyethyl)deoxythymidine, as well as an adduct formed by reaction with the phosphate group of the nucleotide. Analysis of these adducts *in vivo* has not been reported.

The panelists stated that adducts formed by the reaction of CEO with adenine or guanine in DNA have not been sufficiently investigated. Adducts are formed with these bases by ethylene oxide, acrylamide, and 1,3-butadiene, all of which are epoxides or metabolized to epoxides, and all of which form brain tumors in rats, like acrylonitrile. Another panelist countered that there is no animal or human evidence that 1,3-butadiene causes brain tumors. The relatively new analytical approach possible for detection and quantitation of low levels of DNA adducts with liquid chromatography-mass spectroscopy (LC-MS) has not been applied to the investigation of CEO. This approach could help define the role or lack of role of DNA adducts from CEO in acrylonitrile-induced tumorigenicity.

It was noted that the product of reaction with the phosphate backbone can lead to non-enzymatic DNA strand breaks; Yates *et al*.,[20] reported strand breaks following incubation of plasmid DNA with CEO. CEO has also produced point mutations and DNA strand breaks *in vitro*. A key study that was not included in the assessment was that of Recio *et al*.,[16] who conducted a molecular analysis of CEO-induced HGPRT mutations in TK6 mouse lymphoma cells. A panelist also suggested that the unpublished adduct studies by Walker *et al*. be removed because of the preliminary nature of the data, and because the data need to be presented in context.

One panel member expressed a belief that, when all of the unpublished adduct data are considered, the data on concordance for genotoxicity of acrylonitrile is as strong as it is for ethylene oxide and vinyl chloride. Acrylonitrile produces a circulating metabolite that is stable enough to enter the brain. For vinyl chloride, the metabolite is too reactive, and so only liver tumors are found, not brain tumors. This panelist pointed out that adducts can

lead to oxidative damage, while another panelist noted that oxidative stress can lead to adducts being 'fixed' in the DNA so that they are passed on to daughter cells. Even if oxidative damage contributes to the formation of acrylonitrile-induced brain tumors, the first panelist suggested that markers for genotoxicity, such as appropriate DNA adducts, would need to be evaluated in order to determine the relative contribution of different MOAs. For example, the MOA for 1,3-butadiene was evaluated using *in vivo* analyses of DNA and hemoglobin adducts formed from the parent and direct exposure to metabolites. The second panel member cautioned against putting too much weight on structure activity relationships.

Several panelists concluded that it is clear that acrylonitrile and CEO form adducts *in vitro*, and the issue of adduct formation needs to be resolved in order to rule out genotoxicity as a MOA. They noted that absence of evidence (of adducts) cannot be considered evidence of the absence of DNA adducts, and it is necessary to show that DNA adducts are not formed in order to rule out genotoxicity. However, some panelists noted that adducts do not necessarily result in mutations or tumors (e.g., because they are readily repaired). One of these panelists noted that ethylene oxide causes the formation of many different types of DNA adducts, but there is not good site concordance between adducts and tumors. The reason for this lack of concordance is a significant uncertainty. Similarly, it is unclear if the adducts and DNA damage identified to date with acrylonitrile are related to the observed rodent tumors. Overall, several reviewers noted that many chemicals that are weakly genotoxic have multiple MOAs, and concluded by stating that the current data are insufficient to conclusively identify any one MOA for acrylonitrile.

Dr. Gargas stated that, in contrast to ethylene oxide and butadiene, for which there are positive epidemiology data, the epidemiology data for acrylonitrile are negative. A panel member noted that IARC considers that there is 'limited evidence of carcinogenicity',[11] for ethylene oxide in humans based on marginal increase in lymphoid tumors in workers.

A panel member remarked that the hazard characterization should provide guidance for the dose–response assessment. Identification of the MOA leads to a determination of how low-dose extrapolation should be done, and identification of the toxic moiety. This panelist did not believe there was any solid reason to dismiss the animal data. Possible reasons for the apparent differences between the human and animal data include differences in the life stage of the first human exposures, differences in the magnitude and/or duration of exposure, possible metabolic differences between animals and humans, differences in the oxidative status, and/or the microscopic nature of the brain tumors in animals (and associated lower sensitivity of the brain tumor evaluation in humans). The difference between animals and humans could be qualitative or quantitative, but the available toxicokinetic data cannot explain the interspecies differences. Based on the available genotoxicity data, results of the Yates studies,[19,20] and in line with comments from Dr. Cohen (see Appendix B), this panelist stated that the data are insufficient to show that the tumors can be attributed to indirect genotoxicity from oxidative stress. Even if indirect genotoxicity were the MOA, the panelist would favor using a non-threshold (linear) approach for low-dose extrapolation.

At the request of the Chair, several reviewers with particular expertise in MOA met over lunch for further discussion of MOA. For the purposes of their discussion, this subgroup defined genotoxicity as causing initiation, and oxidative stress as being a promotional process. They concluded that: (1) the assessment needs to include a statement that the possible MOAs are not mutually exclusive; multiple MOAs are possible (and even likely). (2) In several places, the assessment interpreted lack of evidence as negative evidence; instead, the authors should say that evidence does not exist. (3) Some papers support DNA reactivity and need to be included in the assessment document. (4) The group did not reach a consensus on a MOA, but it was not clear whether the data support a consensus on a single MOA. It was noted that epigenetic MOAs, such as hypermethylation of regulatory genes, have not been fully addressed for acrylonitrile.

The issue of MOA for sites other than the brain was then discussed by the full panel. It was noted that multiple MOAs may apply, but that the assessment document and available supporting data addressed MOA only for the brain. Written comments from Dr. Cohen also noted the lack of information about MOA in tissues other than the brain (see Appendix B). One panelist disagreed, stating that the WOE is overwhelming that acrylonitrile is a promoter. While this panelist could not prove a MOA, and expressed a willingness to live with other conclusions, a personal belief was expressed that the data show that acrylonitrile does not act via a genotoxic MOA.

To help push the panel members to reach more definitive positions, the Chair suggested that one way to interpret this discussion was that data are

available that support oxidative stress, but DNA reactivity is a hypothesis and not supported by direct data. A panelist responded that the data are insufficient to rule out DNA reactivity. Another panelist stated that the data implicating oxidative stress and altered GJIC are convincing, but insufficient data are available on other potential MOAs. One reviewer expressed the issue as how the panel interprets the *degree* of genotoxicity, in the absence of sufficient data to adequately address the issue. Another noted that the MOA determines how the animal data are used to estimate the cancer potency; under EPA's guidelines, if the MOA is not known, linear low-dose extrapolation is used. Overall, in this initial discussion on the MOA, the panel agreed that the genotoxic potential of acrylonitrile is an open question. The consideration of MOA was expanded in the later discussion, as the panel considered the overall WOE, including how to integrate the human and animal data.

Hazard characterization – MOA and integration of animal and human data In comparing the animal and human data, one panel member noted the study of Schulz *et al*.,[22] who calculated LADD values for the key worker cohorts and compared these values with the animal studies. According to Schulz,[22] the mean LADD for the worker cohorts ranged from approximately 0.01 ppm (for the NCI cohort) to approximately 0.1 ppm (for the DuPont cohort). In contrast, the LADD values for the inhalation study of Quast *et al*.,[2] were 3.57 and 14.37 ppm, for the 20 and 80 ppm groups, respectively. This reviewer noted that Quast reported some increase in tumors at 20 ppm,[2] but most of the increase was in the 80 ppm group. Based on this analysis, Schulz found that extrapolation using the multistage model from the inhalation bioassay resulted in a risk estimate that was not inconsistent with the epidemiology data.[22] In contrast, the risk estimate derived from the Quast *et al*.,[2] drinking water study was inconsistent with the epidemiology data. Using the same dose metric and adjustment for less-than-lifetime exposure, as used by Ward and Starr,[23] Schulz was also able to replicate the results of Ward and Starr.[22,23]

As Dr. Starr was in the audience, the Chair asked him to respond. Dr. Starr observed that there was a range of exposures in the different cohorts. The median exposure in the DuPont cohort was approximately 10 ppm for about 8 years. In the NCI cohort, the median exposure was about 0.6 ppm for approximately 10 years. The panel considered the implications of time-to-tumor data on these less-than-lifetime exposures. Panel members noted that brain tumors were observed in all three of the bio/dynamics assays,[24–26] at the interim sacrifice. One reviewer noted that the early onset of tumors supports a genotoxic MOA, but also increases the confidence in the negative epidemiology data. However, another reminded the panel that most of the brain tumors in the animal studies were microscopic, and a longer exposure period would be needed to affect mortality. Early mortality in the Quast inhalation study was high,[2] due to early sacrifice of rats with large benign mammary tumors; these tumors were observed earlier and at a higher incidence in the exposed rats. One reviewer observed that brain tumors induced by ethylene oxide have a late onset, typically occurring at 18–24 months. Another reviewer said that calculating risk based on tumor incidence treats tumors equivalently regardless of whether the tumor killed the animal at 14 months, or whether the tumor was discovered histopathologically, even though these situations have very different implications for human health. This reviewer did not have a solution in mind, but suggested that the authors acknowledge the difference.

The panel began trying to integrate the human and animal data into a summary WOE statement. One reviewer stated that, in order to fully characterize the cancer hazard, one needs to either reconcile the animal and human studies or state that such reconciliation is not possible. Another suggested stating that 'under conditions of human exposure, acrylonitrile has not been shown to increase cancer risk', noting that additional text could address the issue of exposure of children. A panel member suggested text along the lines of Health Canada's assessment, which stated that 'while there has been consistent evidence of a lack of association between exposure to acrylonitrile and cancer of a particular site in recent, well-conducted epidemiological studies, the power of the investigations is insufficient to rule out increases in particularly rare tumors, such as those of the brain'.[27] Others thought that the Health Canada assessment,[27] is more cautious than it needs to be, and stated that the larger studies could rule out an effect with reasonable certainty. These panel members expressed comfort that the epidemiology studies could have detected rare cancers, and that a doubling of response could be ruled out for several different types of cancers. The importance of looking at the overall pattern of data (namely the magnitude of RRs, which is not impacted by the study's statistical power), taking into account latency and exposure duration, was emphasized. They noted that a strength of the overall epidemiology database for acrylonitrile is

the consistency of response using several different exposure metrics. Another panel member noted that Health Canada capped the upper limit of risk of brain cancer at a 50% increase.[27] Another noted that, by definition, a 95% upper CL of 1.5 means that one is only 95% certain that the increase in cancer risk is <50%; one cannot rule out a RR of 1.1. Several panel members supported the phrasing that 'under the conditions of occupational exposure, no increased cancer risk has been demonstrated', rather than concluding that acrylonitrile was not carcinogenic in workers

For the MOA text, the panelist suggested noting that multiple MOAs may be involved, and these may include oxidative stress, genotoxicity, and interference with GJIC, but that lack of knowledge regarding the MOA at various tumor sites precludes definitive statements Another panelist suggested that the data in hand are strongest in support of oxidative stress

The Chair provided an initial WOE statement as a starting point for the panel to work from in forming their recommendation for the WOE statement. Panel members discussed the specific points and language to include in the statement. With regard to the MOA characterization, a panel member suggested that the WOE statement capture that (1) it is likely that multiple mechanisms are operative, with different levels of influence at different doses; and (2) CEO is a genotoxic metabolite of acrylonitrile. There was general agreement with these suggestions. Another panelist emphasized that the WOE should state that all of the potential MOAs are relevant to humans.

The panel discussed how best to characterize the epidemiology data in the WOE statement. With regard to this data, one panelist stated that the negative epidemiology data could be explained by the idea that acrylonitrile is a promoter. Because promoters require continuous exposure to cause tumors, the cessation of exposure at retirement stops the promotion process and tumors are not observed. Another panelist countered that it is inaccurate to state that the epidemiology data are negative Of 12 cohorts with data on lung cancer, this panelist considered three to be positive for lung cancer. The positive studies were the Blair study,[9] the Werner and Carter study in the UK,[28] which found statistical significance among the young workers, and Thiess *et al*.[29] This incidence of positives should not be considered a statistical fluke. On the other hand, this reviewer later observed that the Dupont cohort,[8] reported no increase in lung tumors, even though exposures were higher than in the Blair study.[9] This reviewer also noted that meta-analysis can aid in the evaluation of the data if there are several weak studies showing the same trend. However, meta-analysis can also eliminate a positive response if the same response is not seen in all studies. To this reviewer, the data indicate that high exposure groups or younger people may be at increased risk of lung cancer from exposure to acrylonitrile. It was again noted by another panelist that with approximately five response measures in each of 32 studies, some statistical elevations (as well as decreases) in various cancer incidences at the 95% CL would be expected based on chance alone. Several panel members agreed that the WOE statement should not say 'negative epidemiology data', but should use wording such as the epidemiology data are 'less clear' or 'equivocal' or 'generally do not support carcinogenicity' or 'provide little support for carcinogenicity'. The potential for confounding of the epidemiology data was also noted One noted that the meta-analysis did evaluate the high-exposure groups separately, and the meta RR was 1.1 However, he noted that smoking increases the risk of lung cancer, with the increased risk being as large as 10- to 20-fold in heavy smokers, and this increase has only been partially adjusted for As such, the true risk of lung cancer associated with acrylonitrile exposure may be overestimated due to the lack of complete adjustment for smoking.

In discussing the text of the concluding statements for the WOE narrative, one panelist suggested that the conditions under which acrylonitrile is carcinogenic should be addressed. This panelist stated that the evidence suggests that acrylonitrile is carcinogenic at very high levels, but is not likely to be carcinogenic at levels to which the general public is exposed, suggesting that the human data should be used to bound the characterization This panelist also suggested that acrylonitrile has a low level of genotoxicity, but genotoxicity may not be the driver, analogously to formaldehyde. Other panelists stated that cancer hazard can be differentiated based on exposure level only if 'high' versus 'low' is defined in terms of the MOA, and the data are insufficient to identify the exposure conditions or to identify the precursor event(s) ('key event' in EPA parlance) that is necessary for carcinogenesis. One of these panel members noted that the apparent differences between humans and rodents could also be due to differences in biology, and cannot be attributed only to exposure

Several panel members noted that the hazard characterization statement sets the stage for the quantitation approach One reminded the panel that all promoters have thresholds, and expressed a belief that acrylonitrile acts as a promoter,

but others considered the data insufficient to state that a specific mechanism of carcinogenicity has been identified. Another reviewer agreed that promotion is possible, particularly from the parent, but that direct DNA reactivity should also be considered.

Several panel members noted the interplay between the hazard characterization in the WOE statement, and the implications for the quantitative portion of the assessment. One suggested stating that the animal data show that acrylonitrile is a multi-site carcinogen, but the epidemiology data do not support a carcinogenic effect at ambient levels. Another noted that this sort of characterization would rule out linear extrapolation to low doses, and asked whether the panel would agree with doing so. This panelist noted that there are problems with linear extrapolation all the way to 1E-6. A panel member observed that acrylonitrile is unusual in having epidemiological data that can quantitatively influence the risk assessment.

A panel member suggested that the WOE statement conclude with a statement along the lines of 'based on this information, the WOE suggests that acrylonitrile is likely to be carcinogenic at high doses typical of animal studies, but may not be carcinogenic at exposures typical of occupational exposure'. Another panelist questioned where the threshold is for the tumor data. Several panelists noted that a threshold cannot be demonstrated in animal studies, with only 50 animals per group, and stated that the threshold is suggested by the epidemiology data. One panelist liked the suggested wording, while another questioned the statement that acrylonitrile is 'likely to be carcinogenic' at high doses. Another noted that most of the occupational studies did not include lifetime average exposures that were as high as those experienced by the animals. In response to a panelist question, Mr. Dale Strother of BP Chemicals, Inc. noted that the high end of past human exposures approaches or exceeds those shown to be carcinogenic in rats. In fact, the occupational exposure limits in the US were set at a level until 1978 that is now known to be carcinogenic to rats.

The panel agreed that the Chair should capture this discussion in a revised WOE statement that would be presented to the panel for post-meeting approval. After additional post-meeting exchanges, consensus was reached on the following WOE statement. However, some individual panel members disagreed with some individual statements, as noted after the consensus statement.

Weight of evidence statement

Epidemiology data do not support an increased cancer risk from acrylonitrile exposure in exposed workers. In contrast, the experimental animal data clearly support the conclusion that acrylonitrile is carcinogenic in rodents. The proposed cancer MOAs in rodents involve general processes (e.g., oxidative stress, GJIC, DNA damage) that are known to occur in humans, and so the data are presumed to support the use of the rodent data in establishing a quantitative cancer risk value. Although the data are insufficient to rule out any contribution due to direct DNA reactivity, an overall WOE evaluation does not support this as a predominant contributor to rodent carcinogenesis. Furthermore, linear extrapolation from the animal data is not supported by the available epidemiology data. Based on this information, the overall weight of the evidence suggests that acrylonitrile may be carcinogenic to humans at high doses based on extrapolation from rat studies, but the cancer risk associated with the low levels to which humans have been exposed in occupational settings is negligible.

This conclusion is based on the following key data. No increased cancer risk has been consistently observed in several different large, well-conducted epidemiology studies using several different occupational cohorts in several different countries. These epidemiology studies evaluated tumors of the lung, brain, prostate, and a variety of other organs. The epidemiology data are strong with narrow confidence intervals for most tumor types, and include large numbers of individuals ($n > 50\,000$ across all cohorts, with one cohort including $>15\,000$ men and >5000 women). Portions of these cohorts included exposures on the order of those associated with increased tumor incidence in rat studies, using some measures of exposure. In contrast, acrylonitrile is clearly carcinogenic in rats and mice, with a carcinogenic response in multiple tissues of both sexes exposed via the oral (rats and mice) and inhalation (rats) routes. The reason for the apparent difference between humans and rodents is not known.

Several MOAs for the observed animal carcinogenicity have been proposed, including acting as a carcinogenic 'initiator' or a 'promoter' of carcinogenesis. However, no animal experiments have been performed to test acrylonitrile in an initiation/promotion model. Conceivably, acrylonitrile and/or CEO could contribute to multiple mechanisms of the multi-stage model of carcinogenesis, and different mechanisms could predominate at different doses.

Varying degrees of data support the different proposed mechanisms. For brain tumors, the evidence is most compelling for oxidative stress, either through interactions of reactive oxygen species with DNA or through epigenetic effects. These epigenetic effects include changes in signal transduction and inhibition of GJIC, endpoints that might also result from effects besides oxidative stress. The evidence is less compelling for direct DNA damage caused by the acrylonitrile metabolite CEO, or even by acrylonitrile itself, although there are gaps in the available data on the DNA reactivity of acrylonitrile and its metabolites *in vivo*. The data at present do not allow unequivocal determination of acrylonitrile's MOAs as an animal carcinogen. The data are insufficient to rule out a direct DNA-reactive MOA for brain tumors, or to definitively identify a specific key event or MOA for brain tumors. Furthermore, there is little information on the MOA for the tumors produced in other tissues in the rat and mouse studies. All of the MOAs proposed for the observed animal carcinogenicity involve general processes known to occur in humans.

Additional panel thoughts regarding this consensus statement were:

- One panel member thought that oxidative stress is probably closely related to the epigenetic mechanism by inducing signal transduction and inhibition of GJIC, rather than damaging nuclear DNA to cause mutations or genotoxicity. This panel member expressed the opinion that oxidative damage to DNA probably occurs in mitochondria but not nuclear DNA, and so should not be linked to indirect DNA damage.
- Two panel members suggested that the WOE statement include a statement that linear extrapolation from the animal data is also not supported by the MOA data, based on the data for GJIC and oxidative stress, and the lack of evidence for acrylonitrile mutagenicity *in vivo*.
- Two panel members thought that the WOE is not sufficient to support low-dose nonlinearity, because it has not been shown that all MOAs are nonlinear, and because the data are insufficient to rule out a direct DNA-reactive MOA. One of these panel members also noted that the human lung cancer mortality data are compatible with a linear model.
- One panel member questioned the statement that acrylonitrile may be carcinogenic to humans at 'high doses', since some of the epidemiology data come from fairly high doses.

Cancer dose–response and PBPK model

Was the PBPK model appropriately developed and validated? What are the limitations to the model, if any, and are there any relevant conditions for which it should not be applied?

Was the correct dose metric chosen?

What are the implications of using a model validated in rats but not humans?

Sponsor presentations

Dr. Thomas B. Starr of TBS Associates made a presentation (on behalf of the AN Group) on quantification of cancer risk using the human data (see presentation slides in Appendix D). Dr. Starr noted that US EPA's 1983 assessment,[38] reviewed the available data and determined that only the O'Berg data on DuPont textile fiber plant workers was adequate for quantification.[5] This study reported five lung cancer deaths among workers exposed to at least moderate levels (~15 ppm). The average cumulative exposure was 135 ppm/years. For workers with a cumulative exposure of 50 ppm/years, a more realistic high-end exposure that might still occur today, the lifetime risk using EPA's potency factor (central estimated) is 28 per 1000. The Starr *et al.*,[30] assessment analysed the Blair *et al.*,[9] study data (from the NCI/NIOSH cohort) on >25 000 workers in eight plants, with 163 lung cancer deaths. The assessment used a semi-parametric Cox proportional hazard model versus age-specific cumulative exposure. Individual exposure histories were reconstructed from job/process description and monitoring data. Three plausible exposure scenarios leading to 50 ppm per working years total exposure were considered, and added risks (maximum likelihood estimates) were projected for the three scenarios. Using this approach, Starr *et al.*,[30] calculated 70-year lifetime risk estimates that are 18- to 41-fold lower than the value of 28 per 1000 calculated from the 1983 US EPA potency,[38] which was a central tendency estimate. Starr also stated that even the upper 95% confidence bound on the estimated risk is 2- to 4-fold lower than EPA's 1983,[38] central tendency estimate. Therefore, using a conservative, low-dose linear dose–response model, Dr. Starr concluded that the newer, more complete, and more extensive epidemiology data are statistically inconsistent with the 1983 potency factor.[38] He outlined the advantages to the approach taken in the updated assessment and noted that a similar analysis of the Wood *et al.*,[8] study of the DuPont cohort resulted in findings virtually identical to those for the NCI cohort, even though the

DuPont cohort had much higher exposures The analysis of the Wood study,[8] found a slightly negative central estimate of potency despite markedly higher cumulative exposures, but the upper bound potency estimates were virtually identical to those calculated from the Blair study.[9]

Dr. Michael L. Gargas and Mr. Christopher R. Kirman of the Sapphire Group presented information on the PBPK modeling and the cancer dose–response assessment for acrylonitrile (see presentation slides in Appendix D) Dr. Gargas noted that in calculating the inhalation unit risk based on human data, they used the Blair *et al.*,[9] study as analysed by Starr *et al.*[30] The dose measure was cumulative external exposure (ppm/years) while the response measure was RR and the Cox proportional hazard model was used. The POD selected was approximately the ED001. They used linear low-dose extrapolation for the calculation based on human data, because there was insufficient information to do differently. The resulting unit risk was 2.9×10^{-6} $(ug/m^3)^{-1}$ with a range of 1.3 to 2.9×10^{-6}.

In introducing the PBPK model, Dr. Gargas noted that there is a quantitative difference between rodents and humans with respect to acrylonitrile metabolism (both through the epoxide hydrolase (EH) and glutathione S-transferase (GST) pathways). Acrylonitrile is very reactive, making it difficult to evaluate its kinetics and metabolism. Acrylonitrile kinetics are nonlinear at high doses (due to saturation of metabolism and cofactor depletion). Slide 12 shows a diagram of the metabolic pathways for acrylonitrile. While flux through the pathways is known, the kinetics for cyanide are not known quantitatively. A PBPK model is available for both humans and rats, covering the majority of important compartments For the rats, parameters were estimated using oral and intravenous (i.v.) data and inhalation studies were used to test the ability of the model to predict blood, brain, and liver concentrations of acrylonitrile and CEO Because kinetic data are not available for humans *in vivo*, the human model,[4] was developed using the parallelogram approach, based on the ratio of *in vitro*:*in vivo* rates for rats, and comparison between *in vitro* data for rats and people. Although this reduces the confidence in the risk assessment, this approach has been used for other chemicals and is considered appropriate for validation. Dr. Gargas *et al.* performed a sensitivity analysis and found the mean and 95th percentile individuals varied by a factor of 1.8. This calculation is the basis for the authors' use of a factor of 1.8 for the uncertainty factor for intraspecies variability in kinetics, for both the nonlinear cancer assessment and in their proposed RfD and RfC.

Mr. Christopher Kirman presented the cancer dose–response assessment based upon the animal data. The assessment authors used the incidence of rat brain tumors from a number of studies. Calculations were conducted both by assessing the oral and inhalation data independently and by using pooled data (using the same dose metric for oral and inhalation). Both internal and external dose measures were used for the calculations. For the pooled data, the authors considered both the AUC and peak CEO concentration as possible dose metrics Since the dose metric is based on internal dose, Mr. Kirman suggested that the dose–response curves for oral and inhalation should be consistent when the correct dose metric has been identified For AUC, there was no concordance between the dose–response curves for inhalation and drinking water, but the inhalation and drinking water curves were generally very consistent when the dose metric was peak CEO (Slide 20). To calculate the cancer dose–response, the assessment authors used the default of extra risk and fit the gamma model to the pooled data; the multistage model was fit to the individual data sets. For the pooled data, the POD selected was the LED001 (lower bound on the dose causing a 1/1000 response). The gamma model fit to the pooled data for brain tumors in rats produced a good visual fit, but the fit was not statistically significant. A significant fit was obtained when spinal tumors from all studies in which data were available were removed. The pooled data allowed for a lower POD than any single data set, due to the greater sensitivity with the larger sample size In selecting a method for low dose extrapolation, the authors decided to present the results using both the linear and nonlinear approaches, due to uncertainties regarding the MOA.

Clarifying questions from the panel

A peer reviewer asked why 50 ppm/years was used when the exposure in the Blair study,[9] was 0.6 ppm. Dr. Starr noted 0.6 was the median, but that he chose to focus on the realistic high-end exposures (which would be expected to result in higher risk).

Another peer reviewer asked why the lack of DNA adducts in rat brains support a nonlinear, margin of exposure approach. Mr. Kirman explained that EPA's guidance is that if the MOA for acrylonitrile involves direct DNA reactivity, one should assume a linear dose–response; however, a genotoxic MOA could also produce nonlinearity The peer reviewer noted that ethylene oxide may provide an example of deviation from the assumption of linearity, in that

the adduct dose–response is not linear at low doses, but is linear at higher doses.

In response to panel questions, the presenters confirmed that the Kedderis model was used without modification, and that the lung was not included in the model because it was not significant in regard to overall metabolic capacity. The presenters also suggested that the poor fit of the combined dose–response curves using the AUC metric and good fit with peak CEO suggests that there may be a threshold in the dose–response.

There were no public comments.

Panel discussion

Benchmark dose modeling The panel discussed the poor fit obtained with EPA's benchmark dose software (BMDS),[31] using the pooled data. (The goodness-of-fit P value was 0.0007, while one usually wants $P > 0.1$.) One panel member suggested that the authors use the results of modeling the data individually, which resulted in decent model fits, and then average the BMDs. Dr Starr noted that a few outliers drive the overall goodness-of-fit for these data. Several panel members questioned the approach of removing the spinal tumors, since these tumors are of the same cell type as the brain tumors, but in a different part of the body. Panel members also recommended that the authors focus more on the visual fit, particularly in the low-dose region, since a limitation of the BMDS software is that as n increases, the chance of a low goodness-of-fit P value goes up dramatically.

PBPK model In assessing the PBPK model and its use in risk assessment, one of the panel members stated that the model structure, development, and validation were appropriate. In particular, the physiology and partition coefficients are appropriate. The panelist expressed some concerns about the choice of the metabolic constants for glutathione (GSH) conjugation, but did not see a need for further refinement of these parameters, because metabolism in humans is flow limited. The model was not validated in humans; the parallelogram approach was used. Limitations of the model were noted by panel members. Most of the studies used to build the model were based on acute scenarios, and any changes in metabolic rates under chronic exposure conditions were not included. However, since metabolism is flow limited, this would not have a significant impact. While several panelists would have preferred to analyse variability using a Monte Carlo analysis of the parameter distributions, a meaningful result can be obtained with the approach used by the authors (based on the coefficient of variation and assuming a normal distribution). The authors used the oral exposure rate constant in rats for humans. This is consistent with the current practice in the modeling field. Overall, one panelist concluded that it is appropriate to use a model that has been validated in rats and not humans, that the approach for rat to human extrapolation was logical, and that the calculations of blood concentrations appear to be correct. Another panelist commended the authors on the work that went into the modeling, and expressed confidence in the model, since one set of constants could be used to describe multiple data sets.

A panelist found it surprising that the variability was the same for the oral and inhalation routes of exposure. The authors responded that the uncertainty factors calculated for human kinetic variability were only slightly different for the oral and inhalation routes, and so these two values were averaged to obtain the kinetic variability uncertainty factor (UF) of 1.8. The panel asked that this point be clarified in the revised assessment document.

One panelist expressed concern about the absence of *in vivo* validation of the human model. Additional documentation of the calculations was also requested, particularly the calculation going from the animal dose to the dose metric and then human dose, and as well as the adjustment for lifetime exposure, and calculation of the BMD, unit risk, and slope factors. Clarity would be improved by presenting additional intermediate values.

There was substantial discussion about the appropriate choice of dose metric for carcinogenicity. The assessment authors chose peak levels of CEO in the brain. A panelist noted that other possible dose metrics might be related to measures of acrylonitrile, cyanide, oxidative stress, or adduct levels. The panelist agreed that CEO is the best measure, but questioned what measure of CEO is most appropriate. An alternative would be the rate of CEO production from acrylonitrile, but this may not be an appropriate measure, given the interspecies differences in hydrolysis to CEO. Another alternative would be metabolism of CEO to cyanide, but that would require showing that cyanide is implicated in tumorigenesis. Another panelist suggested that if cyanide is implicated, both the parent (acrylonitrile) and the metabolite (CEO) may be appropriate possible dose metrics. Overall, given these uncertainties, the CEO level reflects both the rate of production and metabolism of CEO, and so these panel members agreed that the CEO level is an appropriate dose metric. They questioned, however, why peak concentration was used instead of steady state or AUC,

suggesting that AUC or steady state concentration of CEO may be more appropriate. One reviewer noted that peak CEO would be appropriate if tumorigenesis follows a threshold mechanism. Use of the steady state concentration would remove the issue of AUC versus peak, and would also make the results more relevant to a chronic, lifetime exposure, although the authors would need to identify the time span that would need to be simulated. On the other hand, the importance of comparing the inhalation and oral dose–response in evaluating the dose metric was noted.

The assessment authors explained that they used daily peak CEO and daily CEO AUC for calculating the LADD. Doses were simulated based on exposures in the animal bioassay, and then the human 24-hour concentration that would result in the same internal dose was calculated. Insufficient data were available to model cyanide levels. Use of peak CEO as the dose metric normalized the dose–response curve, compared to use of external dose, so that the oral and inhalation dose–response curves were consistent, as would be expected for a systemic endpoint. In addition, the tendency of the model to overestimate peak CEO concentrations in the brain,[32] was one of the reasons that medium confidence was placed in the PBPK modeling.

The panel noted that a major portion of the rationale for choosing peak CEO over CEO AUC as the dose metric was based on figure 5 of Kirman *et al*.,[33] (page D-15 of Appendix D), which showed a consistent dose–response for oral drinking water studies and the inhalation studies together using peak CEO, but not CEO AUC. (The Maltoni *et al*.,[34] gavage study was not included in this analysis, due to the bolus nature of the dosing.) In light of the importance of this analysis, the panel considered in detail the data on which the figure was based. One panelist noted that if all of the studies on the figure were 2-year studies, the study duration would not be a factor. In that case, this panelist would accept peak CEO, based on the fit with this dose metric and lack of fit using AUC. Another panelist noted that one 1-year inhalation study was included,[34] and questioned whether it is appropriate to include this study in the comparison. It was also noted that this study exposed the animals for 4 hours/day, while exposure in the Quast *et al*.,[2] study was for 6 hours/day. In addition, female rats in one of the drinking water studies,[18,24] were terminated at 19 months due to poor survival. The study authors replied that the model simulated the number of hours/day of exposure. In addition, in preparing the Kirman *et al*. paper,[33] the authors did consider using an adjustment to account for the differences in the number of months of exposure, but the adjustment did not improve the model fit or consistency across routes. (This information was not included in the published paper.) The panel recommended that the authors clarify in the assessment document how differences in exposure duration were addressed in the calculation of AUC.

In analyzing whether AUC or peak CEO is a better dose metric, the panel considered the results of Kedderis *et al*.,[32] in some detail. One reviewer stated that the model fit was good for the blood concentration of acrylonitrile but not for CEO following i.v. or oral dosing (figures 2 and 3 in Kedderis *et al*.[32]). Another noted that the concentration of acrylonitrile was modeled well in the brain following oral dosing, but the CEO concentration in the brain was overestimated by approximately a factor of 10; both acrylonitrile and CEO concentrations in the liver were fit well by the model. A reviewer suggested that the reason for the apparent difference in the dose–response for the oral and inhalation routes may be related to how well the CEO levels were predicted for the oral route, and reflect a model deficiency, rather than the appropriate choice of dose metric. Dr. Gargas agreed that the model tended to over predict CEO levels following the oral route. The reviewer suggested that the authors adjust the model for the degree of over prediction and then consider whether the oral and inhalation dose–response curves would be consistent for AUC. It was also noted that the Kedderis study,[32] suggested that the CEO concentrations in the brain are probably falsely low, because the levels decreased between the sacrifice time and the time the brain was removed. Since the concentration in the brain parallels that in the blood, Mr. Kirman noted that he could redo the potency calculation based on the AUC in blood, although he would expect a similar number. Reviewers observed that if brain and blood levels are similar, and CEO was also overestimated for blood, this suggests that the discrepancy is due to issues with the model, rather than an analytical problem with the brain. In contrast with the oral data, the model fit the data well for the inhalation route, for concentrations in blood, brain, and liver (figures 5–7 of Kedderis *et al*.[32]). Based on these comparisons, a reviewer initially suggested that if the model estimated the CEO data more accurately, the oral AUC dose–response might be more consistent with the inhalation data, and the peak measurements would be less consistent between routes. As addressed below, the authors noted upon further consideration that more

accurate estimation of CEO would not help in making the oral and inhalation AUC curves more consistent.

In response to panel questions, the authors presented additional details about the PBPK model development and validation on the second day of the meeting. Flexible parameters were optimized in the model development, and the model was tested using data from gavage dosing.[32] The model results matched the acrylonitrile data well, but CEO concentrations were consistently over-predicted. The assessment authors attributed this poor match for CEO concentrations to the short time frame of the exposure and modeling. In contrast to the inhalation route, for which model validation was carried out for data obtained after 3 hours of exposure, all of the oral time points were obtained within 15 min of gavage dosing; use of gavage dosing also meant that saturation of metabolism was an issue at these early time points. In contrast, the PBPK model is being applied for chronic risk assessment, so the authors proposed that the poor match for the acute oral data is not a problem. The authors noted that the PBPK model predictions for the inhalation route matched the data very well for blood and brain levels of acrylonitrile and CEO, although there was some scatter in the data for the liver.

The authors also explained that the AUC for the oral route was calculated over a 24-hour period, using six drinking episodes. The model was verified and refined for the oral route using gavage dosing, with measurements taken over a very short time frame (30 min).[32] It is extremely rare to collect kinetic data from drinking water studies due to the inconsistent intake rate. The authors noted that study-specific body weight and intake data were used, or the actual dose based on measured intake. This approach accounted for changes in water consumption with dose.

In further discussions of the choice of dose metric, panel members expressed the preference for using AUC or the steady state concentration of CEO, rather than peak concentration. If the panel were to recommend the use of a different dose metric, this would require recalculation of the cancer risk values. The authors observed that peak and steady state levels may be proportional if metabolism is flow limited, although these different metrics would not be proportional at exposures resulting in GSH depletion.

In considering the implications of the Kedderis data,[32] a reviewer suggested that if the dose is overestimated, the potency would be underestimated. This was confirmed by the assessment authors. They stated that if the brain dose of CEO were overestimated (consistent with the concern regarding the Kedderis gavage data),[32] this would mean that the potency following drinking water exposure would have been underestimated, making the AUC curves for inhalation and drinking water more disparate, rather than more similar. Thus, technical difficulties in measuring CEO could not have explained the difference between the oral and inhalation routes when using AUC as the dose metric.

Based on this understanding regarding the implications of overestimating the oral tissue dose, the panel reconsidered (1) whether peak CEO is an appropriate dose metric; and (2) whether the data can be pooled, in light of the differences in study duration. One panelist agreed that the difference between the dose–response for inhalation and oral using AUC as the dose metric argues that either the *rate* of CEO getting to the site of action is important, or that CEO is not the right dose metric. Another panelist agreed that peak CEO is an appropriate dose metric for the inhalation route, but questioned its appropriateness for drinking water, since the size and frequency of spikes in tissue concentration are determined by the frequency of drinking. One panel member was bothered that a difference in the response curve was not seen between a 6-hour inhalation exposure and drinking water exposure over 24 hours, when using peak CEO as the dose metric, and suggested that these data argue against a genotoxic MOA. Another noted the uncertainties in kinetic modeling for drinking water ingestion, and suggested that these uncertainties and the effect of the number of drinking episodes (sips) could explain the difference between inhalation and oral for AUC. The authors replied that AUC is not sensitive to the number of sips, although peak levels are very sensitive to this parameter. One panelist suggested that a paper by Osterman-Golkar *et al*.,[35] could provide useful data. In this study, rats were exposed to acrylonitrile in drinking water for 7 days/week for 105 days, and levels of the cyanoethylvaline hemoglobin adduct were monitored. This study provides useful data on AUC of acrylonitrile following drinking water administration. One panel member requested that the authors describe in more detail the effect on the AUC of the pattern of drinking water consumption. Because of the competing metabolic pathways, it is difficult to predict the implications from a general understanding of acrylonitrile metabolism.

The panel also considered other factors that could affect tissue dose. One panelist suggested that the rate of absorption into the liver could affect the rate of formation of metabolites, but the authors

said the difference based on different assumptions was only of the order of 10–20%. In response to a panelist question about the metabolic capacity of the lung, the authors noted that individual cells have metabolic capacity, but the total contribution to metabolism is small compared to the liver. Local metabolite levels could, however, be affected by lung metabolism. A panel member countered that lung metabolism could affect endpoints observed in the lung, and could also affect delivery of CEO to the brain, because blood from the lung is distributed directly to the brain (and other organs), while blood from the stomach is delivered to the liver before traveling to the brain. This panel member expressed a personal interest in acrylonitrile metabolism via the inhalation route, and in evaluating how this metabolism differs from the oral route. In considering the overall discussion of the PBPK model, one panelist noted that metabolic clearance of acrylonitrile is not flow-limited in the rat. Taking into account the 60% extraction ratio, and the negligible pulmonary clearance, and the insensitivity of AUC to the frequency assumptions used in the PBPK model for drinking water events, this panel member expressed comfort with the use of peak CEO as the dosimeter.

After discussion of these issues, the panel concurred with the authors' use of the PBPK model and choice of peak CEO as a dosimeter. This concurrence also mollifies the concern expressed earlier over the difference in LADD between the experimental animal cancer bioassays and human epidemiology studies, because the LADD would be expected to be more associated with the AUC, whereas the direct comparisons of exposures would be more associated with peak levels. However, the panel recommended that the assessment document address the issue of AUC of CEO in greater detail, noting that it is insensitive to the frequency assumptions used in the PBPK model for drinking water events (i.e., unlike peak concentration, values for AUC are very similar when oral occurs as a single bolus dose or as multiple smaller doses).

Other issues related to the PBPK model One panelist noted that the effect of melatonin could complicate the interspecies extrapolation. Humans produce the antioxidant melatonin at night, unless they are working at night. The panelist questioned whether rats produce melatonin at night, since they are nocturnal animals, and suggested that if oxidative stress is the mechanism of tumorigenesis, a lack of melatonin in rats could explain the differences between the rat and human studies. The panelist suggested checking on whether any of the workers in the epidemiology cohorts were night workers (and whether they had increased risk). Another panelist noted that the PBPK model describes kinetics, not dynamics, and so cannot address this sort of question. The potential for GSH depletion to act as a surrogate for the loss of an antioxidant such as melatonin was also noted. The sponsor observed that acrylic fiber workers do work at night and during the day. It was observed that many shift workers do 2 weeks of night work, and then switch to 2 weeks of day work. Melatonin also alters endocrine cycles. In response to the observation that dietary antioxidants are believed to play a role in cancer, a panel member noted that different rat chows can have a dramatic effect on the spontaneous tumor rate. In light of the possible effect of melatonin, the panel suggested that the authors look more closely at shift workers.

The panel addressed other improvements and investigations with regard to the PBPK model. In response to a panelist question, the authors noted that GSH depletion and resynthesis were not incorporated into the model, although the authors assumed that GSH is not significantly decreased. A panelist observed that GSH depletion may explain differences between the rat (in which acrylonitrile causes brain tumors) and the mouse (in which acrylonitrile is associated with tumors of several organs, but not with brain tumors). GSH levels would also assist in evaluating oxidative stress in the brain. An author also noted that a mouse PBPK model for acrylonitrile does not exist. The author also questioned what degree of GSH depletion would be of concern, noting that a decrease to 25% of control levels is needed to cause an effect in the liver, because there is a large reserve of reduced GSH. It was suggested that 50% GSH depletion may cause an effect in non-hepatic tissues. No mass balance has been carried out for GSH following acrylonitrile dosing, but the assessment authors did express a belief that GSH has been depleted. A panelist asked whether scenarios have been modeled where GSH levels are rate-limiting. The authors agreed that they could look at what doses are needed before GSH depletion is significant in the liver and in other tissues. This was considered a general research need. Another panelist stated that a decrease in GSH in a few target cells or cell types may be important, but could be missed if analyses are on the whole-brain level. It was noted that Klaunig *et al*.,[36] evaluated several markers of oxidative stress in acrylonitrile-dosed rats, and observed a decrease of only about 15–20% in studies with dosing up to 200 ppm in drinking water for up to 90 days. Overall, it was

agreed that the potential for GSH depletion is meaningful and is a research need, in light of the link to oxidative stress, but that the data were not available to resolve the issue at the peer review meeting

Noncancer assessment – RfC

What do the data on acrylonitrile absorption, distribution, metabolism, elimination, and mode-of-action tell us about identifying the critical effects and dose–response assessments in humans and animals?

Are the choice of the critical effects for RfC and the rationale for those choices appropriate? (The critical effects are those adverse effects appearing first in a exposure–response continuum.)

Is the choice and rationale of the principal study for RfC appropriate? (The principal study should present the critical effects in the clearest exposure–response relationship.)

Are there other issues to consider in determining noncancer hazard?

What is the appropriate POD for an acrylonitrile RfC? (appropriate calculation of HEC?) If a BMC based on the animal data is used as the POD, was the benchmark dose modeling appropriately conducted in determining this POD?

Are the uncertainty factors applied to derive the RfC for acrylonitrile appropriate and the rationale for the selections adequate? Do they follow EPA practice? Is the RfC derived appropriately?

Due to time limitations, the sponsor presentation was eliminated and the charge questions for the noncancer assessments were not addressed point by point. Instead, the panel addressed key issues related to the development of the RfC.

One panel member questioned the need for separate cancer and chronic noncancer values, if the cancer risk assessment would be protective for noncancer effects. Another suggested that a noncancer value be developed in order to compare with the cancer value and ensure that the cancer value is health protective. Based on the latter rationale, the panel discussed the noncancer quantification in detail. The assessment authors proposed two different approaches for deriving the RfC. The first was based on irritation observed with a NOAEL of 10 ppm in a human occupational study,[1] as the upper end of the range that did not cause notable irritation. The second option was based on nasal lesions in a 2-year rat study.[2]

Focusing first on the human study, one panel member questioned whether the NOAEL in the Sakurai *et al.*,[1] study of workers exposed to acrylonitrile should be 10 ppm, as chosen by the assessment authors, or whether it should be 1 ppm, the lower end of the exposure range. The panelist stated that 10 ppm is the upper end of the exposure range, but the highest NOAEL may have been lower. This panelist suggested that it might be more appropriate to use either the geometric mean or the lower end of the range (1 ppm) as the basis for the RfC. Another panel member questioned whether enough subjects were used to identify the no-effect exposure range as 1–10 ppm. If enough people were exposed, this panelist agreed with 10 ppm as the NOAEL. Several panel members agreed. The authors replied that the EU assessment,[13] reported that irritation is seen in factories where acrylonitrile exposure is not well-controlled. One panel member suggested that the authors' document improve on how they identified a NOAEL of 10 ppm from the Sakurai paper.[1] Based on this discussion, the panel reached consensus that the NOAEL in the Sakurai study,[1] was 10 ppm, but recommended that the text should be modified to better characterize the NOAEL.[1]

One panelist suggested that, because skin, nasal, and eye irritation are local effects directly related to exposure concentration, no adjustment is needed for exposure duration or for the occupational breathing rate for these endpoints. Several panel members agreed with eliminating the adjustment for discontinuous exposure, as long as continuous exposure to the same concentration would not cause irritation. One member agreed in general, but suggested that a duration adjustment may be appropriate for acrylonitrile, since GSH depletion may be involved in the irritation, as is seen for effects of acrylates on the skin. This panel member expressed a preference for maintaining the adjustment for discontinuous exposure, but noted that the difference is only a factor of 2 (after taking into account the occupational breathing rate). It was also noted that duration does matter at extremely short or long exposure durations, but the question is whether it matters at 8 versus 24 hours/day. The study authors stated that the adjustment was maintained because of concern about the potential for a mechanism where irritation does have a duration component. Overall, the panel recommended that the authors reconsider the adjustment for discontinuous exposure, and that the decision be supported by mechanistic arguments.

The panel discussed the selection of uncertainty factors in derivation of the RfC. The uncertainty factor for human variability (UF_H) can be divided into two components – an adjustment factor for

human dynamic variability (AF_{HD}), and an adjustment factor for human kinetic variability (AF_{HK}) The authors used the default of 3 for the adjustment factor for AF_{HD} and a factor of 1.8 for AF_{HK}, based on the variability in systemic dose calculated using the PBPK model of Sweeney *et al* [4] Several panelists questioned the use of 1 8 for human kinetic variability, since this factor represents variability in systemic dose, not dose to the respiratory tissue. One panelist also suggested that the intraspecies (human variability) uncertainty factor be composed solely of the adjustment factor for AF_{HD} of 3, or that the kinetic component reflect variability in breathing rate and surface area, noting that these latter parameters form the basis for the regional gas deposition ratio (RGDR) used to extrapolate from rats to humans for the RfC developed from the animal data. Another panel member preferred the default value of 10 for UF_H, noting the report of Grunske,[37] of a child who died after exposure overnight to a concentration that caused only minimal eye irritation or no effect in adults sleeping in the same room (and presumably exposed to the same concentration and duration). The first panelist countered that this would not be a concern because irritation would have occurred long before these systemic effects. One panel member noted that computational fluid dynamic (CFD) models are available to assist in comparing rat and human sensitivity to nasal lesions, although such a model is not available for acrylonitrile Another stated that, based on analogy to effects on the skin, interindividual differences would be expected to be much larger than the differences between adults and children; therefore, a UF that adequately addresses the heterogeneity of the population would not need to be adjusted further to address children. The assessment authors stated that they do not know the mechanism of the nasal irritation; it could be tissue binding or GSH depletion. A panel member suggested that the uncertainty regarding the mechanism supports using the default of 3 for the kinetic portion of UF_H The authors stated that acrylonitrile is proportional to CEO at low concentrations, so variability in CEO is reflected in acrylonitrile, but concurred that the variability for nasal irritation is not necessarily related to systemic acrylonitrile. The authors suggested conducting a variability analysis for deposited acrylonitrile. Other panel members argued for using a factor of 1 for AF_{HK}, reasoning that the irritation is due solely to external concentration, and not internal dose It was noted that this is how the Sakurai paper,[1] described the observed irritation, and this approach is consistent with the approach used for acute exposure guidance levels (AEGLs).[1] Overall, a majority of the panel recommended that the total factor for human variability should be 3.2 (based on an AF_{HK} of 1 and an AF_{HD} of 3.2). The panel recommended that the rationale for this choice should be described better in the assessment document

The panel considered the uncertainty factor for extrapolation from a LOAEL to a NOAEL (UF_L). Again focusing on the RfC derived from the human data, the panel agreed that this factor does not apply because a NOAEL was used as the basis for the RfC

The panel addressed the appropriate value for the database uncertainty factor (UF_D). One panel member expressed concern about noncancer effects that could result from disruption of GJIC (a mechanism that is proposed to contribute to the tumors). For example, atherosclerosis due to irritation of the endothelial wall of arteries would be detected only if investigators analysed this endpoint. Others noted that cardiovascular effects were not seen histopathologically in animal studies, and that increased mortality from cardiovascular disease was not reported in the epidemiology studies. The first panel member also noted that blocking intercellular communication could cause developmental toxicity and affect brain function, analogous to the situation with phenobarbital. Evaluation of children born to exposed mothers was suggested. With regard to the standard set of five studies for a complete database (chronic studies in two species, developmental toxicity studies in two species, and a multigeneration reproduction study), the database for acrylonitrile is missing only a developmental toxicity study in a second species. A panel member noted that the three-generation study,[15] provides data regarding the susceptibility of children. In this study, each generation was exposed for approximately 1 year. The study included some neurohistopathology and cageside analyses, and so included some consideration of neurodevelopmental toxicity. Based on these considerations, the panel recommended that a value of 1 be used for UF_D, including the three-generation study as part of the rationale for this value.

The panel discussed the uncertainty factor for extrapolation from less-than-lifetime exposure, (UF_S). The assessment used the default value of 10, because exposure was described only as '5 years or more'. Because the critical effect was irritation, several panelists recommended that this uncertainty factor is not needed for the RfC based on the human study. One panelist noted that this uncertainty factor addresses the question of whether an effect would occur if exposure were to the same concentration for a longer duration Based on the previous discussion, the panelist agreed that the factor would

not be needed if irritation were the only effect of concern, but questioned whether exposure for longer durations would result in systemic effects. Another calculated the systemic daily dose resulting from exposure to 10 ppm, and compared it with the oral dose resulting in systemic toxicity (in animals). This calculation indicated that irritation of the upper respiratory tract would occur at exposures lower than those that would be expected to cause systemic toxicity, even at longer exposure durations. Based on these considerations, and the conclusion that the critical effect has been appropriately identified, as discussed in the context of UF_D, the panel recommended that a value of 1 should be used for UF_S.

In summary, the panel reached consensus on the following approach for deriving the RfC based on human data:

1) Derive the RfC based on the NOAEL of 10 ppm (22 mg/m^3) in the Sakurai study,[1] but characterize the NOAEL better.[1]
2) Consider eliminating the dosimetric adjustment (e.g., not adjusting for intermittent exposure and the occupational minute volume), but justify the decision toxicologically.
3) Use an intraspecies variability factor of 3.2 (instead of a factor of 10), based on a kinetic subfactor of 1 for irritant effects, and provide the rationale.
4) Use a factor of 1 for UF_S (instead of 10).
5) Use a factor of 1 for UF_D, but further justify this with the three-generation study and the minimal neurotoxicity assessment in that study, particularly in light of the neurotoxicity seen with acrylonitrile given orally, and with its metabolite cyanide.

The panel then considered the derivation of an RfC based on nasal lesions in the study of Quast *et al*.[2] The assessment authors calculated a LEC10 (also called BMCL10) of 0.38 mg/m^3 based on nasal lesions in rats. Panel members noted that the RfC currently on EPA's IRIS was also based on Quast, using a LOAEL (human equivalent concentration) [LOAEL (HEC), which adjusts for the RGDR] of 1.9 mg/m^3, rather than the BMCL10. A second difference between the RfC on IRIS and the one proposed here is that a database UF of 10 was used on IRIS, due to the lack of an inhalation bioassay in a second species and 'the lack of reproductive data by the inhalation route with the existence of an oral study showing reproductive effects'. The panel member stated that this database UF is no longer needed, in light of the availability of the three-generation study. The current RfC on IRIS is 2 ug/m^3, or about 1/6 the RfC derived in the following paragraph.

The assessment authors used an uncertainty factor of 3 for interspecies extrapolation using RfC dosimetry; the panel concurred with this choice. For the intraspecies uncertainty factor, one panelist suggested using the variability in the RGDR (based on variability in the ventilation rate and surface area) as the basis for human kinetic variability, as discussed in the context of the RfC based on the human data. Another panelist noted that the panel had agreed earlier to use a factor of 1 for human variability in kinetics for the RfC derived from human data. However, because the nasal lesions were overt histopathology lesions, and not just irritation, panel members agreed that a composite UF of 10 should be used for intraspecies variability for an RfC based on the animal data. The panel further agreed that a factor of 1 is appropriate for UF_L, with several panelists noting that the RfC based on the nasal lesions was derived from a BMCL10 for a minor effect, although one panelist considered the BMCL10 to be closer to a LOAEL. The assessment authors noted that EPA considered the nasal lesions mild in its 1983 assessment ('a minimally adverse LOAEL').[38] The panel also recommended a value of 1 for UF_S and UF_D, based on the same rationale as used for the RfC based on human data. Overall, the recommended composite UF was 30, based on 10 for UF_H and 3 for UF_A.

Inhalation cancer quantification

In light of the hazard characterization for cancer, should cancer risk be calculated? If so, what is the appropriate data set (i.e., experimental animal or human) to use as the basis of the cancer dose–response?

What is the appropriate POD?

If experimental animal data are used, is the use of the gamma model, which was determined to provide an acceptable visual, but not statistical, fit to the dose–response assessment, reasonable? Does the suggestion that the lack of a statistically acceptable fit is due to scatter in the data appear correct? If so, should this scatter in preclude the use of a pooled dose–response data in the assessment?

If experimental animal data are used, what is the proper choice of LED for brain tumors in rats? How model dependent is this estimate?

What is the appropriate low dose extrapolation procedure?

If nonlinear, what is the best approach for estimating cancer risk at low doses?

If the judgment is that a linear extrapolation is appropriate, is the choice of dose–response model and rationale reasonable?

If a cancer risk is calculated, are the calculated oral and inhalation unit risks appropriate?

Use of pooled versus individual data sets to determine the POD

The panel considered whether the pooled or individual data sets should be used to determine the POD for the cancer quantification. One panelist noted that EPA's guidance (based on work by Stitler *et al*., 1993) is that one should show statistical compatibility of data sets prior to pooling. It was suggested that if pooled data are used, to note that this is different from the standard EPA approach. It was also noted that goodness-of-fit *P* values can be used to compare BMD models only within the same family of models. One panel member suggested the use of the average or geometric mean from the best studies. Another of the peer reviewers suggested that the gamma model fitted to pooled data for brain tumors in rats might attain statistical significance if only the data from the F344 rats were modeled, as this strain is more sensitive than the Sprague–Dawley rats. The assessment authors conducted this modeling at the meeting. Focusing on the Sprague–Dawley rat data, since all but one of the studies were in this strain, the *P*-value for the gamma model, excluding the F344 data, increased to 0.09, indicating better fit. The authors also stated that uncertainty due to differences among studies increases the confidence bounds. A reviewer noted that differences among studies (e.g., diet, strain, histopathology methods) is reflected in the low *P* value for the pooled data.

A number of different alternatives for identifying the POD were discussed. Several panel members recommended that the authors calculate the individual BMD from each study, calculate the weighted average from those studies (another suggested a geometric mean), and then put a CL on the weighted average. They noted that the bottom line may not be very different from that obtained with the pooled data, but this approach would remove the complications associated with poor fit from using a large data set. Using a weighted average would result in the Quast study,[17] being given more weight; this study also resulted in the lowest ED10/LED10 values. One panel member suggested using the central tendency, rather than a lower bound, because multiple measures are available. Another panel member expressed a preference for being able to see the results of individual studies.

The sponsor pointed out that an advantage to pooling the data for the dose–response analysis is that it allows one to combine data across routes. It also allows one to ask questions of the data that cannot be addressed with the individual study analyses, such as whether AUC or peak is a better dose metric. An additional problem with conducting the oral and inhalation analyses separately, rather than pooling, is that separate analyses could result in inconsistent dose–response values, even though the same dose metric is used. The sponsor also noted that using the dose metric as the normalizing factor across routes makes good biological sense.

The assessment authors agreed that the pooled data and the individual studies agree in the LED001 range, but noted that there is a clear difference in the range of doses when the model is used to extrapolate to a 1E-6 risk. Several panel members noted that an argument for using the LED001 could be made based on overall sensitivity of the data to detect an effect (based on the total number of animals), but it is not correct to use the LED001 based on agreement at the lowest dose. The authors replied that they used the LED001 for the pooled data, because this response level is in the range of observations for the pooled data. In contrast, the LED001 was not calculated for the individual studies because that was outside the range of the data for any individual study, as is normal for cancer studies in animals. One panelist noted that in the 0–0.01 mg/L CEO dose range, the responses are all in the background range, so this panelist was more comfortable with a POD of LED05, so that the POD is different from the control. Alternatively, an author suggested that the POD could be chosen based on the dose where different models diverge. Overall, it was noted that the slope factors calculated using either the pooled data and LED001, or the geometric mean of the individual data sets (LED10) are within a factor of 2 if one uses a linear extrapolation, so the difference with this approach would be minimal. However, if one uses the margin of exposure (MOE) approach, the difference would be a lot larger. Either way, a consistent rationale for the choice of data set and POD needs to be presented. Some panel members suggested using standard statistics to determine what dose is statistically significant.

Several panelists noted the importance of the biological perspective in addressing whether to pool the data. One observed that strain differences could be important if one study is picked, because

F344 rats are more sensitive than Sprague–Dawley rats. Differences between males and females were also suggested, but another panelist stated that the points of departure based on individual data sets for brain tumors do not exhibit large differences between males and females in the same study. This reviewer suggested pooling all of the data for a best estimate. Other reviewers suggested the authors also clarify that some of the animal studies included more detailed histopathology, which could have led to finding more microscopic tumors. One reviewer noted that male and female tumor data are not usually pooled. This reviewer stated that while it is reassuring that the male and female data result in similar points of departure, one should make the decision on pooling based on biological, rather than statistical grounds, as well as considering methodological issues, such as the number of brain sections evaluated.

Peer review panel members made a number of specific suggestions on how the POD should be calculated and presented. A reviewer suggested that the authors could make a valid argument for pooling, based on similar dosimetry for the oral and inhalation routes, but noted that there is a minimal impact of pooling versus using the individual studies. Another panel member suggested that the authors provide the PODs for the individual studies, note the range and geometric mean, and note that removing the Fisher rat study results in a tighter fit (better goodness-of-fit P value) for the pooled data. The first panel member suggested that the authors provide a best choice and the scientific rationale for that choice, and put alternatives in an appendix, so that the reader is not distracted by too many numbers. A general difficulty with the draft assessment document was that the authors sometimes recommended approaches in which they had lower confidence than alternative approaches that were not recommended. One reviewer also suggested conducting the inhalation cancer risk calculation in terms of the uptake of acrylonitrile in mg/kg per day, to allow an alternative comparison with the oral route. The sponsor noted that this calculation was presented in the Kirman *et al* paper.[33] Overall, the panel agreed that the authors should use their best judgment regarding whether the POD should be based on the pooled data or individual studies.

Low-dose extrapolation approach – linear or nonlinear?

The panel then addressed whether a linear or nonlinear approach should be used to extrapolate to exposures below the experimental animal data. One panel member did not believe that the author's decision to use a 10% tumor response as a NOAEL equivalent for the nonlinear extrapolation could be justified. This panel member recommended that the slope factor calculated from the epidemiology data be used for the cancer quantification. Other panel members noted that a severity factor of 10 would be needed for extrapolating from tumor data and would compensate at least somewhat for the high response. One panel member expressed a preference for using a probabilistic approach, rather than uncertainty factors, for low-dose extrapolation. In the absence of a probabilistic approach, this panel member preferred using linear extrapolation, stating that even if there is a single, minor MOA involving direct DNA interaction, low dose linearity will result (at sufficiently low doses). Another questioned the appropriateness of using an MOE approach at all, because such an approach would assume that acrylonitrile acts exclusively as a promoter, and the mechanistic data are insufficient to show this if acrylonitrile acts via multiple mechanisms. This panelist stated that he does not like the linear approach, but would recommend it, because a non-genotoxic MOA has not been demonstrated, and other good alternatives do not exist. A different member noted that there is good evidence for a non-genotoxic MOA that is a likely driver for the dose–response, and weak evidence for a genotoxic MOA that may not be a driver. Based on the relative WOE and weight of contribution of these different MOAs, this panel member stated that a nonlinear extrapolation approach is justified.

Another panel member emphasized that the database for acrylonitrile is unique in that it includes robust epidemiological data, bioassay data from two rodent species by two routes of exposure, a PBPK model, and extensive mechanistic data, and stated that all of these sources of data should be integrated into the acrylonitrile risk assessment. This panel member agreed that the data cannot rule out a genotoxic component, but presented calculations on how to use the epidemiology data to provide perspective on the animal data. The panel member noted the calculations of Schulz *et al*,[22] who found that, after normalizing to the LADD, the human occupational exposures are fairly close to the rat exposures of Quast *et al*,[2] with the human exposures lower by only one or two orders of magnitude. Noting that the LADD is a conservative exposure metric, the panelist felt that comparison of the tumor data in the animal and human studies indicates that linear extrapolation would be overly conservative. The conclusion that the appropriate dose metric is peak CEO (rather than AUC), also suggests that LADD is overly conservative and that

short-term higher-level exposures contribute more to the cancer risk Finally, many analyses on the role of dose rate in cancer induction have shown that, on average, cumulative exposures in a shorter period of time are associated with a higher overall cancer risk than the same cumulative exposure spread out over a longer period of time. (Details of the normalized exposure calculations are addressed further in the cancer quantification section.)

Another panel member considered the animal and human data to be consistent, noting that linear extrapolation resulted in comparable estimates of risk from the animal and human data This panel member also stated that the magnitude of the expected increase in risk could have been missed even in the DuPont cohort (which had the highest exposures) The first panel member agreed that a very small increased risk in the exposed workers cannot be ruled out, but stated that the WOE is that the epidemiological data do not support a causal association between acrylonitrile exposure in workers and an increased cancer incidence. This panel member also stated that a conclusion that linear extrapolation is not appropriate is also supported by the WOE for the acrylonitrile MOA. These data suggest that, while direct DNA reactivity cannot be conclusively ruled out based on the existing data, it is likely not the primary determinant in the carcinogenic mechanism of action at low doses

In considering how to approach low-dose extrapolation, one panel member stated that the epidemiology data for acrylonitrile are the best one can have If these data cannot be used to modify how one extrapolates from animal data, then epidemiology data can never be useful unless they are positive. Other panel members agreed that the extrapolation approach needs to take the epidemiology data into account, but suggested that the POD should be identified from the animal data. They felt that the epidemiology data could be used to bound or put a floor on the quantification from the animal data. One panel member suggested that this could be done by using a linear model to extrapolate from the animal data, but using the central tendency rather than the lower bound on dose.

One panelist stated that there are times one can deviate from a linear model even for genotoxic carcinogens. For example, ethylene oxide shows a nonlinear dose–response for DNA adducts after one subtracts out background adducts. A similar situation could apply for acrylonitrile Other panelists offered that even genotoxicity can result in nonlinear tumor dose–response, due to the influence of DNA repair, and therefore these panelists considered the predominance of the data to support a nonlinear dose–response for acrylonitrile.

One panel member stated that the data are insufficient to show low-dose nonlinearity, because this would require that *all* MOAs must be nonlinear. Since the data are insufficient to rule out a direct DNA-reactive MOA, this panel member stated that the possibility exists for low dose linearity. This panel member stated that the issue is not whether low dose linearity exists; rather the issue is what data and procedures can be utilized to estimate the potential cancer risk at low doses

The panel was polled regarding whether both linear and nonlinear approaches should be presented in the assessment document The panel recommended by seven votes to six that both linear and nonlinear approaches be shown, with the linear described, perhaps, in an appendix. When asked whether they preferred a linear or nonlinear approach, 11 of the 13 panel members preferred a nonlinear approach The panel agreed that, regardless of whether the linear is presented in the main document or the appendix, the executive summary should present the authors' best judgement.

Different panel members favored different lines of reasoning for the nonlinear approach. Because time was too limited to reach agreement on a common rationale, and to ensure that all panel member opinions were captured in the meeting report, the Chair requested that panel members submit a post-meeting statement providing their individual rationales. Panelists provided the following reasons for supporting a nonlinear approach, with one panel member re-asserting that the data are insufficient to show low-dose nonlinearity.

Several panel members stated that, based on the WOE, linear extrapolation is inappropriately conservative. This is based on comparing the risk value that would be established from linear extrapolation with the extensive epidemiological data, including several large, well-conducted studies that generally did not show increased risk after exposure to relatively high levels of acrylonitrile. Using the tumor data in rats with a nonlinear approach and bounding the results using the human data was considered the only approach that reflected both the animal and human results.

Most panelists supporting this rationale also linked it to the mechanistic data with varying degrees of weight. Several noted that a genotoxic MOA could not be ruled out, that multiple MOAs are likely, and/or acrylonitrile has not been shown to act exclusively by a non-genotoxic MOA. One suggested that acrylonitrile tumorigenicity involves a significant contribution from non-genotoxic

mechanisms and/or indirect genotoxicity (esp oxidative stress), in addition to a potential minor contribution from direct genotoxicity, but a minor contribution from direct genotoxicity could not be ruled out. One cited evidence from other epoxide-generating compounds (e.g., ethylene oxide) that support a nonlinear dose–response (after background adducts have been accounted for). Another stated that the evidence for genotoxicity did not seem to be convincing. On the other hand, another panelist stated that use of the nonlinear model is not readily supported, given that acrylonitrile is not shown to act exclusively by non-genotoxic MOA, but the authors could use the overestimation of the risk using the linear model from the rodent cancer data (compared to using the human data from epi studies of sufficient quality and power) to indicate the inappropriateness of the linear model and justify the need to explore other approaches such as a nonlinear model.

Two panel members supported nonlinearity solely based on the mechanistic data. One stated that, although there are data showing acrylonitrile (and/or metabolites) binding DNA *in vitro*, these are at unrealistic concentrations, and there is no *in vivo* evidence of genotoxicity in any target organ, while there is very strong evidence for a reversible, threshold response *in vivo* in a target tissue (brain), suggesting a non-genotoxic MOA. The second stated that acrylonitrile was not shown to be a mutagen, but did have many characteristics of well-documented tumor promoters; particularly the inhibition of GJIC in a reversible fashion in the target cells.

Nonlinearities in the overall carcinogenic process were also cited by two panel members.

Choice of POD and uncertainty factors for inhalation cancer assessment, using nonlinear extrapolation

The panel considered the specifics of how to conduct low-dose extrapolation using a nonlinear approach, using an approach analogous to the derivation of an RfC to derive an RfC-equivalent value based on the cancer endpoint.

The draft assessment document provided several alternatives for the POD, but the authors' preference was 635 ug/m^3, based on the LED001 for the pooled data, with no additional factor to account for extrapolation from the tumor endpoint. The assessment proposed the following factors for nonlinear extrapolation from the tumor data: for interspecies extrapolation, a factor of 1 was used for kinetic differences (because a PBPK model was used to extrapolate based on tissue dose), and the default factor of 3 was used for dynamic differences. For human variability, a PBPK model was used to calculate a factor of 1.8 for human variability in tissue dose, based on the ratio for the 95th percentile to the mean;[4] the default factor of 3.2 was used for human dynamic variability. Thus, a total factor of 17 ($3 \times 1.8 \times 3.2$) was used in the draft assessment's margin of exposure (described here as nonlinear) analysis.

In considering the specifics of how to conduct the low-dose extrapolation, one panel member recommended that if the panel considers the data to support a nonlinear extrapolation, the human data should be used to estimate a BMD for cancer, based on an ED01 or LED01 from Starr's analysis or another analysis of the human data. Appropriate uncertainty factors would include UFs for effect level, for less than lifetime exposure, and for human variability. Another also suggested using the 95% upper CL on the Starr analysis of the human data to get the LED01. Dr. Starr noted that this could be calculated, although the Starr *et al*.,[30] analysis avoided the issue of non-exposure during childhood or after retirement, and so some extrapolation is needed to lifetime exposure. In contrast, another panelist recommended that the human data not be used for quantitative risk estimates. The panelist considered it to be scientifically inconsistent to state that the epidemiology data do not demonstrate an increase in cancer risk from acrylonitrile exposure, but then to use the data for quantitation. This panelist expressed concern that using the human data for quantification would imply that the epidemiology data show that acrylonitrile is a human carcinogen. The first panel member countered that one could not say that the human data do not support an increase in cancer risk, and instead one should state that the data *generally* do not support an increase. This panelist believed that one cannot be sure based on the epidemiology data.

The importance of having the quantification consistent with the hazard characterization statement was also noted. Several panelists suggested using the human data in some fashion to bound the animal data. Several panel members supported using the epidemiology data as a check on the animal data, and noted that they consider this different from using the human data as the basis for the quantification. Dr. Starr noted that his assessment did not ask the question what the highest potency was that human data could have missed. That potency estimate would have been much higher than the potency estimated from the data. Panelists noted this sort of question would need to be addressed in order to appropriately use the human risk data to quantitatively bound the animal data.

Dr. Gargas observed that linear extrapolation from the rat data and from the epidemiology data (when the latter data were converted from ppm per years) resulted in similar slope factors, although the slope for the human data was not statistically significant. He stated that the epidemiology data provide some indication of 'how bad things could be', but did not exhibit a trend, and the slope factor is determined primarily by one high-dose point. A panel member suggested that the human data be plotted on the graph presenting peak CEO versus tumor response. Dr Gargas replied noting that the dose metric would need to be calculated for the human data. A panelist suggested that the authors caveat any such calculation by noting that a positive response was not seen, even with the high-exposure cohort. This panelist also noted that precedents exist for using negative data to bound the potential risk, with appropriate caveats.

The panel addressed what level of response should be used for the POD for low-dose extrapolation in the cancer quantification. One panel member stated that EPA's 1999 draft cancer guidance is to use the LED10 but use of lower points of departure is allowed, when lower responses are in the range of the data. Another added that EPA's 2003 draft cancer guidance expresses a preference for the lowest POD adequately supported by the data. A reviewer stated that the choice of POD has a minimal impact (about two-fold) if a linear extrapolation is used, but this reviewer would not recommend linear extrapolation. The choice of POD becomes more important if a nonlinear approach is used. Several panel members noted that all of the points of departure under consideration are below saturating dose levels, removing saturation as a source of nonlinearity. The panel also noted the inter-relationship between the choice of POD for a nonlinear extrapolation and the choice of uncertainty factors.

Individual panelists suggested various options for combining different choices of the POD (e.g., LED001, LED01, LED05, LED10, corresponding to the lower bound on the dose corresponding to a 0.1, 1, 5 and 10% response, respectively), and uncertainty factors. Reasons provided for the lower PODs included that the LED001 was in the range of the pooled data, and that this value is more conservative (all other things being equal), reflecting the lack of precedent for the overall approach recommended by the panel. Other panel members supported an LED05, because this was in the range of the data for individual studies. Some of these panel members also expressed the opinion that a lower POD would require adjustment of the uncertainty factors, but since a method has not been developed for such an adjustment, the LED05 should be used. An LED01 was suggested as a compromise between these values of the POD. The panelist suggesting this compromise also expressed greater comfort with how the numbers compared with the epidemiology data when the LED01 was used as the starting point. The overall sense of the panel was that it is preferable to not use the default according to EPA's draft 1999 cancer guidelines (the LED10), because this is not a health protective approach, and because lower responses are in the range of the data. The panel noted that EPA recommends that a precursor effect be used for nonlinear extrapolation to obtain an RfC equivalent, but an appropriate precursor has not been identified for acrylonitrile.

Panel discussion regarding the choice and apportionment of UFs used in the nonlinear assessment initially focused on the factors for severity and human variability, but then was broadened to consider comparison with the human data. Some reviewers suggested that a *severity* factor of 10 be used regardless of the POD, but suggested that the factor for *human variability* should be reduced at lower values of the POD since, for non-stochastic processes (as implied by the use of a nonlinear extrapolation approach), moving to the left on the dose–response curve means that one is observing the response in the sensitive population for a noncancer effect. Others stated that adjusting only the human variability factor for different values of the POD does not adequately take variability into account. The panel agreed overall that, regardless of how the areas of uncertainty are apportioned, the total UF needs to take into account the response level at the POD, and that in the end, the calculated values must be compared with the epidemiology data.

Several panel members noted the importance of comparing the quantitation from the animal data with the epidemiology data. They suggested that the human data be used to 'influence' the dose–response determined from the animal data. Initially, one panel member suggested comparing with the floor from the epidemiology data, stating that the safe value should not be below the lowest exposures in the epidemiology data. However, as the discussion progressed, this 'influence' evolved to rough comparisons, in light of the uncertainties. One panelist suggested that the calculated risk value should be near, but, in fact, should be somewhat below the lowest average continuous lifetime exposures from the major epidemiology cohorts. Having the RfC-equivalent below these equivalent lifetime exposures is health-protective in light of the uncertainties in the epidemiology data (e.g., lack

of early-life exposure). This panel member suggested that the extrapolation approach recognize that there is little precedent for this sort of data set, and acrylonitrile is not clearly non-genotoxic. A comparison of the animal data and human data after normalization for duration of exposure by calculation of the LADD, as was carried out by Schulz *et al.*,[22] was recommended as an appropriate approach for comparing an RfC derived from the animal cancer data with the human exposures. Concern was also expressed about the need to avoid over-precision, given the uncertainties in the data set.

In light of these considerations, this panel member suggested that an uncertainty factor <200 would be problematic in extrapolating from a 10% tumor response. This panel member expressed concern that using an LED10 of 23 mg/m^3 and a factor of 170 would result in a risk value of approximately 60 ppb. As calculated by Schulz *et al.*,[22] this concentration is higher than the continuous lifetime exposure calculated for the NCI cohort (10 ppb) and the Dutch cohort (21 ppb). The panelist noted that even using the LED05 would result in a risk value above the lifetime exposures in these two cohorts. Another panel member noted that using the LED001 of 635 ug/m^3 divided by a composite UF of 17 would result in a risk value of 37 ug/m^3 (17 ppb). This lower value is in the range of the continuous lifetime exposure for the Dutch and NCI cohort, as calculated by Schulz.[22] Another panelist suggested that the decision be left to the sponsor, and the panel should just recommend that the authors look at the human data for bounding.

The panel considered whether risk to children was addressed adequately in the quantitation, or whether an additional uncertainty factor is needed to protect children. Panel members noted that the three-generation study,[15] compared the cancer incidence in rats exposed only as adults, and the risk in two generations exposed *in utero* through adulthood. There was an increase over adult-only exposure at the high dose in one generation but not in the other. Exposure was for the same duration in each generation, although the overall tumor rate was relatively low, because the animals were only exposed for 1 year. Recognizing the limitations in this study, the panel agreed that the available evidence did not suggest that children are more sensitive than adults. It was also noted that consideration of the overall database addresses the Food Quality Protection Act (FQPA) factor. Overall, the panel agreed that no additional factor is needed for protection of children.

The panel considered various ways of including the human data. Several panel members recommended that the human data be used in two ways, first in the overall WOE analysis, and then as a reality check after calculating the cancer risk, to make sure that the risk value derived from the animal data is credible. Another alternative approach considered was to use the Starr analysis,[30] to calculate an LED01 for humans and compare to the animal data, with caveats. Most panel members did not agree with this latter approach, considering it inconsistent with a statement that the human data are generally negative. Overall, most of the panel preferred to base the cancer quantitation on the animal data, using the human data only for a reality check and for perspective on the animal data.

The overall panel recommendation for the cancer quantification was not resolved until after the panel considered the cancer and noncancer data together, as described in the following section.

Choice of overall inhalation value

Having considered the inhalation risk values based on cancer and noncancer endpoints and data, the panel reviewed the values derived for overall consistency, and to consider whether only one inhalation value should be derived for both the noncancer and cancer endpoints. Derivation of only one value was an option due to the use of the nonlinear assessment for cancer.

A panel member summarized the inhalation values considered by the panel, as well as previous assessments:

As described above, the authors of the assessment calculated an RfC based on the animal data based on nasal lesions in the Quast *et al.*,[2] study, using a BMCL10 (or LEC10) of 0.38 mg/m^3, as shown in table 5-6 of the draft assessment. The panel concurred with the use of this LEC10, but recommended using a composite UF of 30, resulting in an RfC of 13 ug/m^3, or about 6 ppb.

For the nonlinear cancer estimate, based on modeling the individual animal studies and using internal dose, the POD for an LED10 is 23 200 ug/m^3. Using a composite UF of 17 (as suggested in the draft assessment), this would result in a risk value of 1400 ug/m^3, or about 636 ppb.

One reviewer expressed discomfort with a risk value of 636 ppb, considering it too high, based on comparison with the epidemiology data. Alternatively, using the same POD and a UF of 170 (including 10 for severity of effect), the risk value would be about 64 ppb, which is in the range of the epidemiology data after adjusting to continuous

lifetime exposure. The assessment authors did not present the LED01 based on the individual studies, but making the reasonable assumption that risk in this region is roughly linear, and using a UF of 170, the risk value would be 1/10 that calculated using the LED10, or about 6 ppb.

For comparison, based on EPA's current unit risk, the concentration corresponding to a one in a million risk (1E-6) is 0.007 ppb.

One panel member noted that, based on these calculations, the noncancer value of 6 ppb for the RfC would drive the setting of exposure limits.

The panel members were polled on whether the inhalation risk value should be based on cancer or noncancer, and what the POD should be. Twelve panel members preferred basing the value on cancer rather than the nasal lesions, while the thirteenth abstained. Ten members voted that a POD of 5% should be used as the basis of the assessment. Two panelists preferred a POD of 1% based on tumors, but one would then reduce an uncertainty factor. No members preferred a 10% POD as the basis of the assessment. Panel members preferring to use tumors based their preference on the greater importance of tumors over nasal lesions. Those who expressed a reason for preferring 5% as the POD noted that this number has a stronger basis than the 1% value, based on the response that could be detected in individual studies. Based on the results of the poll, a panel member noted that one would need to qualitatively argue that one should not evaluate the nasal lesions from a risk assessment perspective, because otherwise the nasal lesions would drive the assessment.

Based on these considerations, the consensus of the panel was that the authors derive an RfC-equivalent value for inhalation exposure based on the LED05 for cancer. The consensus was also that the composite uncertainty factor is 180, based on a default factor of 3.2 for animal to human toxicodynamics, a default of 3.2 for human toxicodynamic variability, and a factor of 1.8 for human variability in toxicokinetics, plus a factor of 10 for severity of response.

Panel members also suggested that, rather than showing several different approaches to the calculation, the authors present their best judgment of the approach, and the supporting rationale. Alternative approaches, including calculations based on external dose, should be moved to an appendix.

Oral cancer quantification

Due to time limitations, the panel did not address a separate oral cancer quantification. If the POD that the authors end up choosing is based on a pooled data set, the oral cancer quantification would be analogous to the inhalation assessment, and many, if not most, of the discussion points in the previous section would be applicable here.

Oral noncancer assessment

What do the data on acrylonitrile absorption, distribution, metabolism, elimination, and mode-of-action tell us about identifying the critical effects and dose–response assessments in humans and animals?

Are the choice of the critical effects for the RfD and the rationale for those choices appropriate? (The critical effects are those adverse effects appearing first in a dose–response continuum.)

Is the choice and rationale of the principal study for the RfD appropriate? (The principal study should present the critical effects in the clearest dose–response relationship.)

Has the benchmark dose modeling been used appropriately in the choice of the critical effects?

Are there other issues to consider in determining noncancer hazard?

What is the appropriate point-of-departure for an acrylonitrile RfD? If a BMD is reasonable, was the benchmark dose modeling appropriately conducted in determining this POD?

Are the uncertainty factors applied to derive the RfD for acrylonitrile appropriate and the rationale for the selections adequate? Do they follow EPA practice? Is the RfD derived appropriately?

Sponsor presentation and proposed RfD

The sponsor presentation was eliminated due to time limitations. Instead, the sponsor only noted a change since the preparation of the assessment document. The original assessment based the RfD on neurological measures, and presented data suggesting that both acrylonitrile and cyanide may be responsible for this endpoint. However, the authors noted that the principal study,[3] tested other organonitriles (which are also metabolized to cyanide) and did not see the same effects with these other related chemicals. Therefore, the assessment authors concluded that cyanide can be ruled out as a causative agent, and that the dose–response can focus on AUC for acrylonitrile. Taking this change into account, the proposed RfD was based on a human equivalent dose (HED) calculated using the PBPK model. The proposed principal study,[3] evaluated neurotoxicity in rats exposed to acrylonitrile or

other organonitriles for 12 weeks in drinking water. The proposed critical effect was a continuous neurotoxic endpoint, decreased amplitude for sensory nerve action potential (ASAP); an LED(HED) of 32 mg/kg per day was calculated, with the response level defined based on a one standard deviation change in the mean. A factor of 3 was used for interspecies extrapolation, to account for toxicodynamic differences with the use of the PBPK model. A factor of 5.8 was used for intraspecies variability, based on a dynamic factor of 3.2, and a kinetic factor of 1.8 based on the PBPK model, as described previously in the context of the cancer assessment. The assessment authors used a factor of 1 for UF_L for extrapolation from a BMD, and a factor of 10 for extrapolation from a subchronic study. As described in the context of the RfC, a factor of 1 was used for database uncertainties.

There were no public comments.

Panel discussion of RfD

Due to time limitations, only an abbreviated discussion of the RfD and its derivation was possible. The panel discussed and resolved the major decision points, but did not consider the assessment point by point. Furthermore, several panel members considered the oral RfD assessment unnecessary, since cancer would drive any risk management decisions.

One panel member agreed with the principal study,[3] and critical effect (neurotoxicity) identified by the assessment authors, and concurred that forestomach irritation and hyperplasia reported in several studies is not relevant. This panel member expressed a preference for the RfD based on a noncancer endpoint, and recommended if a cancer quantitation is carried out for the oral route, it be conducted using nonlinear extrapolation. Another commented that the critical effect has not been clearly characterized as adverse, and recommended that the authors explain the rationale for considering it adverse. The panelist also stated that neurotoxicity is usually due to the parent, not the metabolite, and so recommended that the parent chemical be used as the dose surrogate, in the absence of a clearly identified MOA. Others concurred. Several panel members requested that the authors better explain the calculations used in applying the PBPK model to derive the HED.

In discussing the HED derived from the PBPK dose metrics, panel members observed that the HED derived based on AUC CEO (as a surrogate for cyanide) is lower (more conservative) than the number that would be obtained by dividing the animal dose by 10 (the uncertainty factor for animal-to-human extrapolation). In addition, the PBPK model accounts only for kinetics, and the dynamic factor of 3 would still need to be applied. In contrast, the HED calculated based on AUC acrylonitrile is comparable to the animal dose. However, based on the absence of neurological effects in the Gagnaire study,[3] in animals dosed with other aliphatic nitriles that are metabolized to cyanide, the panel concurred with the recommendation to use AUC acrylonitrile as the dose surrogate. Thus, the panel concurred on deriving the RfD from the human equivalent dose calculated as a benchmark dose (BMD, or LED) of 32 mg/kg per day. The panel also agreed with a factor of 1.8 for human variability in kinetics, based on the ratio of the 95th percentile to the mean for acrylonitrile in blood,[4] a factor of 3.2 for human variability in dynamics, a factor of 3.2 for toxicodynamic differences between rats and humans, and a factor of 10 for subchronic to chronic extrapolation. As discussed above for the RfC, the panel concurred with a factor of 1 for the UF_D, but recommended that the authors support this factor better, using the three-generation study to show that young rats were tested adequately. Thus, the composite UF is 180 ($1.8 \times 3.2 \times 3.2 \times 10$).

With regard to UF_L, one panelist recommended that this UF is not needed because the endpoint is continuous and the default of a one standard deviation (SD) change is used to identify an abnormal response. Another countered that defining the benchmark response as a one SD change is equivalent to 10% of the animals being abnormal at the BMD. It was noted that EPA is currently discussing when and whether a factor analogous to UF_L should be used for RfDs and RfCs derived from BMDLs. One consideration is the severity of the endpoint, and a factor of 10 might be used for more severe endpoints. This issue has only been addressed relatively recently, and most RfDs on IRIS that are based on BMDs do not use this additional factor. One panelist suggested that the issue is not important, because cancer would drive the assessment, but another noted that there are times where people doing site risk assessments want a separate noncancer value, such as for mixture assessments.

Overall, the panel agreed with the RfD presented by the assessment authors based on AUC acrylonitrile as the dose surrogate, with the additional changes recommended above.

The resulting RfD would be 0.2 mg/kg per day (32 mg/kg per day ÷ 180). The panel also recommended that the authors note the issue regarding the factor for use of the BMDL, but also note that the overall assessment would be driven by cancer.

As a final resolution, the panel presumed that if the assessment is revised as recommended here, the assessment would be loaded onto ITER. The panel requested that they be given the opportunity to review the revised assessment document, including a summary table showing how the risk values were modified.

Final comments

In concluding statements, many panel members commended the authors on the thoroughness of the review, and in compiling an extensive database into a well-written, well-analysed, unified assessment document. Some felt that the assessment document could be improved by presenting the single, most scientifically supportable approach, rather than all possible options for a risk assessment. Strengths of the assessment document noted by panel members included that it harmonized the drinking water and inhalation routes, that it demonstrated the utility of PBPK modeling, and that it incorporated the epidemiology data. Several also commented that the meeting was very well conducted, and noted the value of bringing together panelists with opposing viewpoints. Many of the panel members also re-affirmed their support for the nonlinear extrapolation, and were pleased that the epidemiology data could be incorporated into the overall WOE conclusion. One panelist expressed concern about using the nonlinear approach in the absence of clear support from the MOA, and suggested that the revised document should clearly explain why a linear extrapolation was not used, in light of the data supporting a mixed MOA. In supporting the overall WOE text, one reviewer noted that the assessment incorporated three key pieces of evidence: the epidemiology data, tumor studies in animals, and *in vivo/in vitro* mechanistic studies. Other panel members noted the advantage of considering multiple approaches, but suggested that the authors place greater emphasis on the approach best supported by the science. One suggested that the authors consider the implications of acrylonitrile being (1) only an initiator; (2) only a promoter; or (3) both an initiator and a promoter, and consider how these different alternatives would explain the data. Another suggested that the text that appears to be dismissive of the animal data should be modified. Several panel members stated that the MOA is the major outstanding issue, and reminded the authors of the importance of considering multiple MOAs. Several also suggested further research that could be conducted to resolve the outstanding issues; these ideas were summarized in the section on panel member suggestions.

Panel consensus statements and recommendations

Consensus statements and recommendations

The panel reached the following overall consensus statements and recommendations:

1) The panel recommended that the appropriate WOE statement be as follows:

> Epidemiology data do not support an increased cancer risk from acrylonitrile exposure in exposed workers. In contrast, the experimental animal data clearly support the conclusion that acrylonitrile is carcinogenic in rodents. The proposed cancer MOAs in rodents involve general processes (e.g., oxidative stress, GJIC, DNA damage) that are known to occur in humans, and so the data are presumed to support the use of the rodent data in establishing a quantitative cancer risk value. Although the data are insufficient to rule out any contribution due to direct DNA reactivity, an overall WOE evaluation does not support this as a predominant contributor to rodent carcinogenesis. Furthermore, linear extrapolation from the animal data is not supported by the available epidemiology data. Based on this information, the overall weight of the evidence suggests that acrylonitrile may be carcinogenic to humans at high doses based on extrapolation from rat studies, but the cancer risk associated with the low levels to which humans have been exposed in occupational settings is negligible.
>
> This conclusion is based on the following key data. No increased cancer risk has been consistently observed in several different large, well-conducted epidemiology studies using several different occupational cohorts in several different countries. These epidemiology studies evaluated tumors of the lung, brain, prostate, and a variety of other organs. The epidemiology data are strong with narrow confidence intervals for most tumor types, and include large numbers of individuals ($n > 50\,000$ across all cohorts, with one cohort including $>15\,000$ men and >5000 women). Portions of these cohorts included exposures on the order of those associated with increased tumor incidence in rat studies, using some measures of exposure. In contrast, acrylonitrile is clearly carcinogenic in rats and mice, with a carcinogenic response in multiple tissues of both sexes exposed via the oral (rats and mice) and inhalation (rats) routes. The reason for the apparent difference between humans and rodents is not known.
>
> Several MOAs for the observed animal carcinogenicity have been proposed, including acting as

a carcinogenic 'initiator' or a 'promoter' of carcinogenesis. However, no animal experiments have been performed to test acrylonitrile in an initiation/promotion model. Conceivably, acrylonitrile and/or CEO could contribute to multiple mechanisms of the multi-stage model of carcinogenesis, and different mechanisms could predominate at different doses. Varying degrees of data support the different proposed mechanisms. For brain tumors, the evidence is most compelling for oxidative stress, either through interactions of reactive oxygen species with DNA or through epigenetic effects. These epigenetic effects include changes in signal transduction and inhibition of GJIC, endpoints that might also result from effects besides oxidative stress. The evidence is less compelling for direct DNA damage caused by the acrylonitrile metabolite CEO, or even by acrylonitrile itself, although there are gaps in the available data on the DNA reactivity of acrylonitrile and its metabolites *in vivo*. The data at present do not allow unequivocal determination of acrylonitrile's MOA(s) as an animal carcinogen. The data are insufficient to rule out a direct DNA-reactive MOA for brain tumors, or to definitively identify a specific key event or MOA for brain tumors. Furthermore, there is little information on the MOA for the tumors produced in other tissues in the rat and mouse studies. All of the MOAs proposed for the observed animal carcinogenicity involve general processes known to occur in humans.

2) For the quantitative cancer assessment, the panel consensus was that a nonlinear approach be used for low-dose extrapolation, although the panel recognized that the data are insufficient to rule out a role for genotoxicity. The panel concurred with the authors' use of the PBPK model and choice of peak CEO as a dosimeter. However, the panel recommended that the assessment document provide a better description of the inability of AUC of CEO to provide a consistent dose–response relationship following oral and inhalation exposures in the rat. The panel also requested that the authors clarify how differences in exposure duration were addressed in the calculation of AUC. The panel recommended by a simple majority that both linear and nonlinear approaches to low-dose extrapolation be shown, with the linear approach described, perhaps in an appendix. The majority recommendation to show both linear and nonlinear approaches is consistent with what the authors proposed in the draft assessment document.

For specific decision points in the quantitative cancer assessment, the consensus of the panel was:

- The authors should use their best judgment regarding whether the POD should be based on the pooled data or individual studies. This was based on the panel's recognition of the overall high quality of the authors' assessment document.
- A POD of 5% increased tumor incidence should be used as the basis of the assessment. Two members preferred a 1% POD as the basis of the assessment, but one of these panelists would then use a reduced uncertainty factor. No members preferred a 10% POD as the basis of the assessment.
- The authors should clarify that the uncertainty factor of 1.8 for human toxicokinetic variability was obtained by averaging the oral and inhalation values, which were very close.
- The composite uncertainty factor for the cancer assessment should be 180, based on a default uncertainty factor of 3.2 for differences between animal and human toxicodynamics, a default uncertainty factor of 3.2 for human toxicodynamic variability, a factor of 1.8 for human variability in toxicokinetics, and a factor of 10 for severity of response with the POD of 5%. An uncertainty factor for differences between animal and human toxicokinetics is not needed because a PBPK model is used.
- The rationale for the choice of POD and uncertainty factors in extrapolating from the animal data should use the epidemiology data to bound the quantitative cancer estimate, such that the resulting risk value (based on rat data) should not be inconsistent with the epidemiologic data.
- No additional factor is needed for protection of children.

3) The panel recommended that the overall quantitative assessment be based on the cancer endpoint, as the endpoint of concern, but also saw value in estimating RfCs and a RfD, based on noncancer effects, in order to ensure that the cancer value was protective of noncancer effects.

Thus, the panel reached consensus on the following approach for deriving an RfC for non-carcinogenic effects based on human data:

- Derive the RfC based on the NOAEL of 10 ppm (22 mg/m^3) in the Sakurai *et al.* study,[1] but characterize the NOAEL better.
- Consider eliminating the dosimetric adjustment (e.g., not adjusting for intermittent exposure and

the occupational minute volume), but justify the decision toxicologically, based on the fact that local effects such as nasal and eye irritation are determined more by concentration than by the product of concentration and time.
- Use an uncertainty factor of 3.2 for human variability (instead of a factor of 10), based on a toxicokinetic subfactor of 1 for irritant effects, and provide the rationale.
- Use an uncertainty factor of 1 for addressing extrapolation from subchronic to lifetime exposure (instead of 10).
- Use a database uncertainty factor of 1, but further justify this with the three-generation study. In light of the neurotoxicity seen with acrylonitrile given orally, and the neurotoxicity of the acrylonitrile metabolite cyanide, the panel also felt that it is important to note that the three-generation study included some consideration of neurodevelopmental toxicity.

For an RfC based on animal data, the panel reached consensus on using the lower bound on the concentration causing a 10% response (LEC10), or the lower bound on the benchmark concentration corresponding to a 10% response (BMCL10) of 0.38 mg/m^3 based on nasal lesions in rats in the Quast *et al* study.[2] The panel recommended an uncertainty factor of 1 for extrapolating from a LOAEL (based on the minimal severity of the lesion and use of the BMCL). A value of 1 was also recommended for the uncertainty factor for subchronic to chronic extrapolation, and for the database uncertainty factor, based on the same rationale as used for the RfC based on human data. Overall, the recommended composite uncertainty factor was 30, based on 10 for the uncertainty factor for human variability and 3 for the uncertainty factor for interspecies extrapolation, to account for toxicodynamic differences remaining after interspecies extrapolation using RfC dosimetry.

4) For the RfD, the panel reached consensus on the following points:

- As proposed by the authors, an RfD should be derived from the human equivalent dose calculated as a benchmark dose of 32 mg/kg per day, based on neurotoxicity in the Gagniere *et al* study.[3]
- The panel agreed with the use of an uncertainty factor of 1.8 for human variability in toxicokinetics, based on the ratio of the 95th percentile to the mean for acrylonitrile in blood;[4] a factor of 3.2 for human variability in toxicodynamics; a factor of 3.2 for toxicodynamic differences between rats and humans; and a factor of 10 for subchronic to chronic extrapolation.
- As for the RfC, the panel concurred with the use of a factor of 1 for the database uncertainty factor, but recommended that the authors support this factor better, using the three-generation study to show that young rats were tested adequately. Thus, the composite uncertainty factor is 180 ($1.8 \times 3.2 \times 3.2 \times 10$).
- The resulting RfD would be 0.2 mg/kg per day (32 mg/kg per day $\div$ 180).
- The authors should note in the text that the issue of whether to include a factor for use of the BMDL (which represents a defined *effect* level) is actively being discussed by EPA.

5) The panel noted that in several places, the assessment interpreted lack of evidence as negative evidence; instead, the panel recommended that the authors should say that evidence does not exist.

6) The panel requested the opportunity to review the revised assessment document, including a summary table showing how the risk values were modified.

Other suggestions from the panel and individual panel members

These suggestions reflect panel considerations that would enhance the quality of the overall assessment, but which do not necessarily need to be addressed in the revised assessment document. This section also includes specific suggestions made by individual panel members for improvements to the acrylonitrile assessment document. While the individual suggestions do not carry the same weight as suggestions by multiple panel members, they have been included in the report to provide additional guidance to the document authors as they consider revisions.

- In light of the possible effect of melatonin, one panel member suggested that the authors consider looking more closely at shift workers.
- One panel member suggested that the assessment document more fully describe the exposure histories for the epidemiology studies, and the document should note that some of the workers were exposed to very high concentrations, including episodic exposures to the highest acutely tolerable levels.
- One panel member suggested that the authors specifically note that increases in brain tumors have not been observed in humans.

- One panel member recommended that the RR for lung cancer quoted from the Collins/Acquavella meta-analysis,[10] should be for the seven studies where exposure estimates existed. These studies had a RR = 1.2 (95% CI = 1.0–1.5) for lung cancer.
- Two panel members suggested that the authors clarify that some of the animal studies included detailed histopathology, which could have led to finding microscopic tumors that would not be detected in the epidemiology studies.
- One panelist suggested that the report should draw on the WOE for structurally related compounds
- A panelist suggested that the unpublished adduct studies by Walker *et al.* be removed, due to the preliminary nature of the data, and because the data need to be presented in context.
- Several panelists suggested that the assessment document address the literature regarding DNA adducts and a possible genotoxic MOA more completely, including discussion of the work by Yates *et al.*,[19,20] regarding DNA adducts, and the molecular analysis of CEO-induced HGPRT mutations in mouse lymphoma cells by Recio *et al.*[16]
- Several panelists suggested that markers for genotoxicity, such as appropriate DNA adducts, would need to be evaluated in order to determine the relative contribution of different MOAs.
- One panel member suggested that the authors note that there are different public health implications for tumors that kill animals at early time points versus tumors that are discovered histopathologically at terminal sacrifice.
- One panelist suggested that the conditions under which acrylonitrile is carcinogenic should be addressed as part of the WOE consideration.
- One panel member suggested that the authors describe in more detail the effect of the pattern of drinking water consumption on the dose metrics.
- Several panelists suggested that moving alternative approaches and considerations (such as the linear alternative for low-dose cancer extrapolation) to an appendix would help make the assessment document more readable.
- Several panel members suggested that additional documentation of the calculations used in applying the PBPK model to derive the HED and HEC be included. In particular, additional documentation would be useful for the calculation from the animal dose to the dose metric and then human dose, and as well as the adjustment for lifetime exposure, and calculation of the BMD, unit risk, and slope factors. Clarity would be improved by presenting additional intermediate values.
- One panel member suggested conducting the inhalation cancer risk calculation in terms of uptake of acrylonitrile in mg/kg per day, to allow comparison with the oral route.
- A panel member suggested that the human data be plotted on the graph presenting peak CEO versus tumor response.
- A panelist suggested that the authors caveat the cancer risk calculations by noting that no increased cancer risk has been consistently observed in the epidemiology studies, even in the high-exposure cohort.
- One panelist suggested that the executive summary should footnote the origin of important calculations
- Several panel members suggested that, rather than showing several different approaches to the calculation, the authors present their best judgment of the approach, and the supporting rationale. Alternative approaches, including calculations based on external dose, should be moved to an appendix.
- One panelist suggested that the text that seems dismissive of the animal data should be modified.
- One panelist suggested that if the POD is from pooled data, the authors should note that this is different from the standard EPA approach.
- One panel member suggested that the authors further explain why a nonlinear approach to cancer quantification was used, in light of a mixed MOA.
- One panel member requested that the authors further explain the rationale for considering the critical effect for the RfD, amplitude for sensory nerve action potential (ASAP), to be adverse
- The panel noted that the potential for GSH depletion and the implication of such depletion is a research need.
- One panel member provided a number of suggestions for further research to clarify the acrylonitrile MOA:

  1) Studies are needed to characterize the adducts of CEO reaction with DNA (or immediate derivatives) *in vivo* in acrylonitrile-exposed rats and mice, and to determine

dose–response. This information could then be used to look for excretion of adducts in humans or identify analogous hemoglobin adducts.

2) Examine the oxidative stress endpoints in the systems used by Dr. Klaunig using ethylene oxide at concentrations equimolar to the acrylonitrile levels tested by Dr. Klaunig.
3) Need additional *in vivo* mutagenicity studies in the rat, including dose–response studies in acrylonitrile-exposed rats followed by dose–response studies in CEO-exposed rats at the same plasma levels as the selected acrylonitrile exposure levels. These data would provide information on the degree to which CEO is responsible for acrylonitrile-induced 'stochastic' effects.

   a) Need to develop a sensitive mutation assay in primary glial cells (measuring mutation frequency and mutation spectrum) for evaluating relative mutagenicity of epoxides including CEO.
   b) Need mutational spectra studies of mutants from rats exposed to acrylonitrile and CEO. These studies would be used to:

      i) Determine the frequency and type of DNA sequence changes versus epigenetic events.
      ii) Compare mutations from rats exposed to the parent compound versus epoxymetabolites.

4) Perform a cancer bioassay study with acrylonitrile in P450-2E1-null mice. This assay would allow one to determine whether CEO is major contributor to acrylonitrile carcinogenicity.

References

1 Sakurai H, Onodera M, Utsunomiya T, Minakuchi H, Iwai H, Mutsumura H. Health effects of acrylonitrile in acrylic fibre factories. *Br J Ind Med* 1978; **35**: 219–25.
2 Quast JF, Schuetz DJ, Balmer MF, Gushow TS, Park CN, McKenna MJ. *A two-year toxicity and oncogenicity study with acrylonitrile following inhalation exposure of rats*. Dow Chemical Co. Toxicology Research Laboratory, Midland Michigan for the Chemicals Manufacturing Association, Washington, DC (partially printed, complete report on disk), 1980.
3 Gagnaire F, Marignac B, Bonnet P. Relative neurotoxicological properties of five unsaturated aliphatic nitriles in rats. *J Appl Toxicol* 1998; **18**: 25–31.
4 Sweeney LM, Gargas ML, Strother DE, Kedderis GL. Physiologically based pharmacokinetic model parameter estimation and sensitivity and variability analyses for acrylonitrile disposition in humans. *Toxicol Sci* 2003; **71**: 27–40.
5 O'Berg MT. Epidemiologic study of workers exposed to acrylonitrile. *J Occup Med* 1980; **22**: 245–52.
6 Benn T, Osborne K. Mortality of United Kingdom acrylonitrile workers-an extended and undated study. *Scand J Work Environ Health* 1998; **24**: 17–24.
7 Swaen GMH, Bloemen LJN, Twisk J, Scheffers T, Slangen JM, Collins JJ *et al*. Mortality update of workers exposed to acrylonitrile in the Netherlands. *Scand J Work Environ Health* 1998; **24**: 10–16.
8 Wood SM, Buffler PA, Burau K, Krivanek N. Mortality and morbidity of workers exposed to acrylonitrile in fibre production. *Scand J Work Environ Health* 1998; **24**: 54–62.
9 Blair A, Stewart PA, Zaebst D, Pottern L, Zey J, Bloom T *et al*. Mortality of industrial workers exposed to acrylonitrile. *Scand J Work Environ Health* 1998; **24**: 25–41.
10 Collins JJ, Acquavella JF. Review and meta-analysis of studies of acrylonitrile workers. *Scand J Work Environ Health* 1998; **24**: 71–80.
11 International Agency for Research on Cancer (IARC). *Evaluation of carcinogenic risks to humans. Acrylonitrile. Monograph*, Volume 71, part I, pp 43–108 and preamble, 1999.
12 Marsh GM, Youk AO, Collins JJ. Reevaluation of lung cancer risk in the acrylonitrile Cohort study of the National Cancer Institute and the National Institute for Occupational Safety and Health. *Scand J Work Environ Health* 2001; **27**: 5–13.
13 European Union (EU). Risk assessment of acrylonitrile. CAS No. 107-13-1. EINECS No. 203-466-5. April, 2001. Draft.
14 Marsh GM. Mortality among workers from a plastics producing plant: a matched case-control study nested in a retrospective cohort study. *J Occup Med* 1983; **25**: 219–30.
15 Friedman MA, Beliles RP. Three-generation reproduction study of rats receiving acrylonitrile in drinking water. *Toxicol Letts* 2002; **132**: 249–61.
16 Recio L, Simpson D, Cocharane J, Liber H, Skopek TR. Mutational specificity of 2-cyanoethylene oxide in human lymphoblastoid cells. *Environ Mol Mutagen* 1989; **14**: 162.
17 Quast JF. Two-year toxicity and oncogenicity study with acrylonitrile incorporated in the drinking water of rats. *Toxicol Letts* 2002; **132**: 153–96.
18 Johannsen FR, Levinskas GJ. Comparative chronic toxicity and carcinogenicity of acrylonitrile by drinking water and oral intubation to Spartan(R) Sprague-Dawley rats. *Toxicol Letts* 2002; **132**: 197–219.
19 Yates JM, Summer SCJ, Turner MJ, Recio L, Fennell TR. Characterization of an adduct and its degradation product produces by the reaction of cyanoethylene

oxide with deoxythymidene and DNA *Carcinogenesis* 1993; **14**: 1363–69.
20 Yates JM, Fennell TR, Turner MJ, Recio L, Sumner SC. Characterization of phosphodiester adducts produced by the reaction of cyanoethylene oxide with nucleotides *Carcinogenesis* 1994; **15**: 277–83
21 Solomon JJ, Singh US, Segal A. *In vitro* reactions of 2-cyanoethylene oxide with calf thymus DNA. *Chem-Biol Interact* 1993; **88**: 115–35
22 Schulz MR, Herts-Picciotto I, Todd L, Ball L. Reconciling animal and human data in a cancer risk assessment of acrylonitrile. *Scand J Work Environ Health* 2001; **27**: 14–20.
23 Ward CE, Starr TB. Comparison of cancer risks projected from animal bioassays to epidemiologic studies of acrylonitrile workers *Regul Toxicol Pharmacol* 1993; **18**: 214–32.
24 Bio/dynamics. *A twenty-four month oral toxicity/ carcinogenicity study of acrylonitrile administered in the drinking water to Spartan rats* Bio/dynamics Inc., Division of Biology and Safety Evaluation, East Millstone, NJ, under project no. 77-1745 for Monsanto Company, St Louis, MO, 1980
25 Bio/dynamics *A twenty-four month oral toxicity/ carcinogenicity study of acrylonitrile administered in the drinking water to Fischer 344 rats*. Bio/dynamics Inc., Division of Biology and Safety Evaluation, East Millstone, NJ, under project no 77-1746 for Monsanto Company, St Louis, MO, 1980
26 Bio/dynamics *A twenty-four month oral toxicity/ carcinogenicity study of acrylonitrile administered by intubation to Spartan rats.* Bio/dynamics Inc., Division of Biology and Safety Evaluation, East Millstone, NJ, under project no 77-1745 for Monsanto Company, St Louis, MO, 1980.
27 Health Canada. Priority substances list assessment report. Acrylonitrile, 2000.
28 Werner JB, Carter JT. Mortality of United Kingdom acrylonitrile polymerisation workers. *Br J Ind Med* 1981; **38**: 247–53
29 Thiess AM, Frentzel-Beyme R, Link R, Wild H Mortalitatsstudie bei hemiefacharbeitern verschiedener produktionsbetriebe mit exposition auch gegenuber acrylonitril. *Zentralbl Arbeitsmed Arbeitsschutz Prophyl Ergon* 1980; **30**: 259–67.
30 Starr TB, Gause CK, Stone RA, Youk AO, Marsh GM, Collins JJ A risk assessment for occupational acrylonitrile exposure using epidemiology data *Risk Anal* 2003; **24**: 587–601.
31 United States Environmental Protection Agency (US EPA) Benchmark dose software (BMDS) version 1.3 2. 2003 Available at http://cfpub epa gov/ncea/cfm/ recordisplay cfm?deid = 20167
32 Kedderis GL, Teo SK, Batra R, Held SD, Gargas ML Refinement and verification of the physiologically based dosimetry description for acrylonitrile in rats *Toxicol Appl Pharmacol* 1996; **140**: 422–35
33 Kirman CR, Hays SM, Kedderis GL, Gargas ML, Strother DE. Improving cancer dose-response characterization by using physiologically based pharmacokinetic modeling: an analysis of pooled data for acrylonitrile-induced brain tumors to assess cancer potency in the rat. *Risk Anal* 2000; **20**: 135–51.
34 Maltoni C, Ciliberti A, Dimaio V Carcinogenicity bioassays on rats of acrylonitrile administered by inhalation and ingestion. *Med Lavoro* 1977; **68**: 401–11
35 Osterman-Golkar S, MacNeela JP, Turner MJ, Walker VE, Swenberg JA, Sumner SCJ *et al* Monitoring exposure to acrylonitrile using adducts to N-terminal valine in hemoglobin *Carcinogenesis* 1994; **15**: 2701–707.
36 Jiang J, Xu Y, Klaunig JE Induction of oxidative stress in rat brain by acrylonitrile (ACN) *Toxicol Sci* 1998; **46**: 333–41.
37 Grunske F. Ventox and ventox-poisoning. *Dtsch Med Wochenschr* 1949; **74**: 1081
38 United States Environmental Protection Agency (US EPA). Health assessment document for acrylonitrile. EPA-600/8-82-007F. October, 1983.

Appendix

The appendix is available at http://www.tera.org/peer/AN/ANWELCOME.htm