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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON D.C. 20460

OFFICE OF THE ADMINISTRATOR  
SCIENCE ADVISORY BOARD

Date to be inserted

Honorable Stephen L. Johnson  
Administrator  
U.S. Environmental Protection Agency  
1200 Pennsylvania Avenue, N.W.  
Washington, DC 20460

Subject: Review of EPA's, "Toxicological Review of Acrylamide".

Dear Administrator Johnson:

In response to a request from EPA's Office of Research and Development (ORD), the Science Advisory Board (SAB) convened an expert panel to conduct a peer review of EPA's draft Integrated Risk Information System (IRIS) assessment entitled, "*Toxicologic Review of Acrylamide*". This draft document updates EPA's current evaluation of the potential health effects of acrylamide.

The SAB was asked to comment on the hazard characterization and dose-response assessment of acrylamide, including the Agency's selection of the most sensitive non-cancer health endpoint, the use of a pharmacologically-based toxicokinetic (PBTk) model, the derivation of a proposed oral reference dose (RfD), an inhalation reference concentration (RfC) for non-cancer endpoints, as well as the cancer descriptor, oral slope factor, and inhalation unit risk for acrylamide. The SAB Panel's report contains a number of recommendations that are aimed at making the assessment more transparent and improving the scientific bases for the conclusions presented. The Panel's key points and recommendations are highlighted below:

- The Panel agreed with the EPA's conclusion that based on the existing toxicity data base for acrylamide, neurotoxicity does appear to be the most sensitive non-cancer endpoint, and therefore, the most appropriate for developing the RfD and RfC for non-cancer health effects.
- The Panel believed that the use of the benchmark dose methodology in this assessment was deemed scientifically supportable, given the nature and robustness of the data sets available on the endpoint of concern.
- The Panel supported the Agency's conclusions that exposure to acrylamide in animals leads to heritable gene mutations and that these results indicate that it may also pose a hazard to humans. In addition, the Panel supported the Agency's conclusions that the available data on heritable gene mutations are not adequate to conduct a robust assessment of this endpoint at this time. The

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Panel urges further research on acrylamide-induced heritable germ cell mutations, given the serious nature of such effects.

- The Panel concluded that the rationale and justification for acrylamide being a “*likely human carcinogen*” via a mutagenic mechanism was well described and the conclusion was scientifically supportable, although it should be further elaborated.
- The Panel encouraged the Agency to use the two main chronic bioassays in rats for deriving the oral cancer slope factor and include an in depth discussion of the strengths and limitations of both studies.
- The Panel commends EPA for using the PBTK model for developing the RfD, RfC and cancer slope factor for acrylamide. The Panel did however provide some recommendations to the Agency for improving the model as they revise their draft document. The Panel notes that the use of internal dose metrics combined with a fairly robust understanding of the mechanism of action may replace the use of the default interspecies factor for toxicokinetic differences. Internal dose may be derived using the PBTK model or through application of other pharmacokinetic approaches indicated in the Panel report.
- The Panel agreed with the use of PBTK modeling to conduct dose-route extrapolation and commended the EPA for using the PBTK model to fill the gap resulting from the absence of robust animal toxicology studies investigating neurotoxicity via the inhalation route that would support the development of an RfC. In estimating the cancer slope factor and unit risk, human-rodent differences in pharmacokinetics were taken into account with the PBTK model, whereas pharmacodynamic differences were not, but should be, through the application of a standard factor.
- Finally, the Panel agreed that the use of the age-dependent adjustment factors (ADAF) to adjust the unit risk for early life exposure is well justified and transparently and objectively described.

The Panel appreciates the opportunity to provide EPA with advice on this important subject. A more detailed description of the technical recommendations is contained in the body of the report. We look forward to receiving the Agency’s response.

Sincerely,

Dr. Deborah Cory-Slechta, Chair  
SAB Acrylamide Review Panel

Dr. Deborah Swackhammer, Chair  
EPA Science Advisory Board

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1 **U.S. Environmental Protection Agency**  
2 **Science Advisory Board**  
3 **Acrylamide Review Panel**  
4  
5  
6

7 **CHAIR**

8 **Dr. Deborah Cory-Slechta**, Professor, Department of Environmental Medicine, School of  
9 Medicine and Dentistry, University of Rochester, Rochester, NY  
10  
11

12 **SAB MEMBERS**

13 **Dr. Lauren Zeise**, Chief, Reproductive and Cancer Hazard Assessment Branch, Office of  
14 Environmental Health Hazard Assessment, California Environmental Protection Agency,  
15 Oakland, CA  
16  
17

18 **OTHER SAB MEMBERS**

19 **Dr. Penelope Fenner-Crisp**, Independent Consultant, North Garden, VA  
20

21 **Dr. Jeffrey Fisher**, Professor, Department Environmental Health Science, University of  
22 Georgia, Athens, GA  
23

24 **Dr. Gina Solomon**, Senior Scientist, Health and Environment Program, Natural Resources  
25 Defense Council, San Francisco, CA  
26

27 **Dr. Anne Sweeney**, Professor of Epidemiology, Commonwealth Medical Education, The  
28 Commonwealth Medical College, Scranton, PA  
29  
30

31 **CONSULTANTS**

32 **Dr. Alfred Branen**, Associate Vice President, University of Idaho, Coeur d'Alene, ID  
33

34 **Dr. James S. Felton**, Senior Biomedical Scientist, University of California, Lawrence  
35 Livermore National Laboratory, Livermore, CA  
36

37 **Dr Timothy Fennell**, Senior Research Chemist, Drug Metabolism and Pharmacokinetics, RTI  
38 International, Research Triangle Park, NC  
39

40 **Mr. Sean Hays**, President, Summit Toxicology, Allenspark, CO  
41

42 **Dr. Steven Heeringa**, Director, Division of Surveys and Technologies, Institute for Social  
43 Research, University of Michigan, Ann Arbor, MI

**Dr. Richard M. LoPachin**, Professor of Anesthesiology, Department of Anesthesiology,  
Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY

**Dr. Lorelei Mucci**, Assistant Professor, Harvard Medical School, Channing Laboratory, Boston,  
MA

**Dr. Jerry M. Rice**, Distinguished Professor, Department of Oncology, Lombardi Cancer Center,  
Box 571465, Georgetown University Medical Center, Washington, DC

**Dr. Dale Sickles**, Professor and Vice-Chair, Department of Cellular Biology and Anatomy,  
Medical College of Georgia, Augusta, GA

#### **FEDERAL EXPERTS**

**Dr. Daniel R. Doerge**, Research Chemist, National Center for Toxicological Research, Food and  
Drug Administration, Jefferson, AR

#### **SCIENCE ADVISORY BOARD STAFF**

**Dr Suhair Shallal**, Designated Federal Officer, EPA Science Advisory Board, Science Advisory  
Board Staff Office, Washington, DC

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## EXECUTIVE SUMMARY

This report was prepared by the Science Advisory Board (SAB) Acrylamide Review Panel (the “Panel”) in response to a request by EPA’s Office of Research and Development (ORD) to review the Draft IRIS Toxicological Review of Acrylamide (hereafter referred to as the draft document). The Panel deliberated on the charge questions (see Appendix A) during a March 10-11, 2008 face-to-face meeting and discussed its draft report in a subsequent conference call on July 16, 2008. There were 26 charge questions that focused on the selection of the most sensitive non-cancer health endpoint, the use of a PBTK model, the derivation of a proposed oral reference dose (RfD), an inhalation reference concentration (RfC) for non-cancer endpoints, as well as the cancer descriptor, oral slope factor, and inhalation unit risk for acrylamide. The Panel encourages the Agency to review relevant data which has been published since their draft assessment was completed as they revise and finalize the IRIS document.

This Executive Summary highlights the Panel’s major findings and recommendations as a result of their deliberations. The responses that follow represent the views of the Panel.

### *Selection of Endpoint*

In the draft document, EPA identified neurotoxicity as the most sensitive non-cancer effect from exposure to acrylamide. This endpoint was based on an extensive database of animal and human studies. Other endpoints were also considered, such as reproductive toxicity and heritable germ cell effects. The Panel agreed that based on the existing toxicity data base for acrylamide, neurotoxicity does appear to be the most sensitive non-cancer endpoint, and therefore, the most appropriate for developing the RfD and RfC for non-cancer effects from exposure to acrylamide.

### *Mechanism of Action*

The Panel discussed two hypotheses regarding the mechanism of acrylamide neurotoxicity. The Panel did not attempt to resolve the debate over a definitive or single MOA for neurotoxicity; however, there was agreement that the discussion of MOA is important for inclusion in the draft document. The Panel found the separation of the discussion of MOA(s) for

1 neurotoxicity in two different sections of the document confusing and recommended their  
2 incorporation into a single section. A more complete presentation by the Panel of these MOAs  
3 has been appended (see Appendix B) to this report for EPA's consideration as they revise their  
4 draft document.

#### 5 6 *Derivation of RfD*

7 EPA's proposed RfD (0.003 mg/kg-day) for acrylamide is based on a benchmark dose  
8 analysis of the dose-response relationship for neurotoxicity in two chronic drinking water  
9 exposure bioassays using Fischer 344 rats. Uncertainty factors and a PBPK model were used to  
10 extrapolate the animal dose-response to a human equivalent dose-response in the derivation of  
11 the RfD. The Panel afforded considerable discussion to the question of whether the Friedman et  
12 al. (1995) and Johnson et al. (1986) studies were the best choices for derivation of the  
13 quantitative RfD (and RfC). The main concerns with these studies are that they were primarily  
14 designed as cancer bioassays and therefore did not include the most sensitive measures of  
15 neurotoxicity. Nevertheless, the Panel agreed that the selected studies did have some important  
16 strengths, including reasonable statistical power due to the relatively large number of animals,  
17 chronic dosing, and the fact that the NOAELs for the endpoint in the two studies were similar,  
18 implying some precision in the effect estimate measured. Several Panel members noted that the  
19 lack of sensitive functional/behavioral assessments is a significant data gap that should be  
20 considered in the context of setting a database uncertainty factor. Use of the benchmark dose  
21 methodology in this assessment was deemed scientifically supported, given the nature and  
22 robustness of the data sets available on the endpoint of interest. The calculations and choices  
23 made were described clearly and at an appropriate level of detail.

#### 24 25 *Heritable Germ Mutations*

26 EPA's draft document concluded that data also exist that reveal acrylamide's capacity to  
27 induce heritable germ cell effects at doses somewhat above those at which neurotoxicity has  
28 been observed, but that there are as yet no studies providing an in-depth examination of dose-  
29 response or identification of credible no-effect levels. The Panel supports the Agency's  
30 conclusions that exposure to acrylamide in animals leads to heritable gene mutations and that



1 these results indicate that it may also pose a hazard to humans. In addition, the Panel supports  
2 the Agency's conclusions that the available data are not yet adequate to conduct a robust  
3 assessment of this endpoint at this time. There is still uncertainty about the mode of action of  
4 acrylamide and its metabolite, glycidamide, in the induction of heritable genetic effects. The  
5 potential for DNA adducts of glycidamide to play a role is an attractive hypothesis for the mode  
6 of action. The Panel found the discussion in the document on heritable germ cell effects useful  
7 and presented in a clear, transparent manner reflective of the current science. However, the Panel  
8 suggested that, given the serious consequences of heritable germ cell effects, the considerable  
9 deficiencies of the database should be identified and the significance of this endpoint  
10 emphasized.

#### 11 12 *Physiologically-Based Toxicokinetic (PBTk) modeling*

13 A physiologically-based toxicokinetic (PBTk) model originally developed by Kirman et  
14 al. (2003), and recalibrated by EPA with more recent kinetic and hemoglobin binding data in  
15 rats, mice, and humans, was used in the derivation of the RfD to extrapolate from the animal  
16 dose-response relationship to derive a human equivalent concentration. The Panel commends  
17 EPA for their efforts to adapt the PBTk model of Kirman et al. (2003) for acrylamide and  
18 glycidamide, recognizing that this was a complex and challenging task. The Panel believes,  
19 though, that the documentation is not adequate to determine whether the recalibrated Kirman  
20 model is appropriate for its intended use. While the Panel considered that the model structure  
21 was reasonable, the parameter estimates require greater justification. The Panel was concerned  
22 about the ability of the model to adequately simulate the kinetics of acrylamide and glycidamide.  
23 Several alternatives to the PBTk model have been proposed for making the estimates of internal  
24 dose in rats needed for both the non-cancer and cancer assessments and for calculating the  
25 Human Equivalent Dose (HED).

#### 26 27 *Uncertainty Factors*

28 EPA has proposed to use the default 10X uncertainty factors (UF) to account for  
29 intraspecies (i.e., human) differences. The Panel concurred with this choice, noting that there  
30 were insufficient data on inter-individual differences, based upon lifestage, gender or genetic

characteristics, to support departing from the default. Consensus was not achieved on the issue of the inclusion of an UF to account for deficiencies in the existing database.

EPA has suggested that the acrylamide IRIS document include a Table that lists points of departure for various endpoints to facilitate a Margin of Exposure (MOE) evaluation by EPA's Regional or Program offices, or by other end users of the assessment. The Panel recommends the inclusion of such a table, to the extent possible, in all IRIS documents which provides information that may be used to conduct a variety of MOE analyses for specific endpoints of interest and/or for other than lifetime durations of exposure and for windows of increased susceptibility early in the life cycle, in addition to the traditional lifetime focus. Agency risk assessments would benefit from the inclusion of transparently-developed, peer-reviewed consensus hazard values.

### *Carcinogenicity*

The Panel believes that the rationale and justification for acrylamide being a "*likely human carcinogen*" has been well described and the conclusion is scientifically supportable based on the fact that it produces tumors in rodents in both sexes, that there are multiple tumor sites, and tumors are induced via multiple routes of exposure. Acrylamide is also clearly and reproducibly carcinogenic in both rats and mice. Nonetheless, the draft document can be improved by expanding the discussion of biological plausibility and coherence beyond DNA adducts. The weight of evidence supports a mutagenic mode of action for carcinogenesis, and overall the rationale has been clearly and objectively presented. Significant biological support and data on any putative alternate MOAs are not sufficient for either explaining cancer findings or quantifying dose response relationships. More than one MOA may operate for a given carcinogenic chemical, and the likelihood that more than a single MOA is operative increases as levels of exposure increase.

EPA used two chronic drinking water exposure bioassays in Fischer 344 rats (Friedman et al., 1995; Johnson et al., 1986) to derive the oral cancer slope factor, and to identify the tumors of interest for the MOA discussion. The Panel agrees that the two chronic bioassays in F344 rats are the main studies to consider in dose response analysis, but the rationale for using only the Friedman et al. study for derivation of the oral cancer slope factor should be improved with the

1 strengths and limitations of both studies discussed in greater depth The use of the Weibull-in-  
2 time multistage-in-dose analysis is a reasonable and scientifically justifiable way to take into  
3 account the early mortality in the high dose group in the male study. The decision not to employ  
4 this analysis, in the case of the female because mortality across treatment and control groups did  
5 not differ and the overall survival appears to be fairly good, is also reasonable.

6 The draft document used area under the curve (AUC) in the blood for the putative  
7 genotoxic metabolite, glycidamide, as the dose metric for the PBTK model analysis to derive the  
8 human equivalent concentration. The Panel agreed that the AUC for glycidamide is the best  
9 choice for estimating the human equivalent concentration to derive the oral slope factor. One  
10 consideration in using this as the dose metric, however, comes from some of the human studies  
11 in which variability is not accounted for adequately. Consideration of additional human data can  
12 provide an improved basis for adjustments for cross-species differences in pharmacokinetics, as  
13 well as human variability in glycidamide formation from acrylamide.

#### 14 15 *Derivation of the RfC*

16 As with the RfC, EPA concluded that there were insufficient inhalation data to derive an  
17 inhalation unit risk (IUR). The PBTK model was used in a route-to-route extrapolation of the  
18 dose-response relationship from the oral data, and to estimate the human equivalent  
19 concentration for inhalation exposure to acrylamide. The Panel agreed with the use of PBTK  
20 modeling to conduct dose-route extrapolation and commended the EPA for using the PBTK  
21 model to fill the gap resulting from the absence of robust animal toxicology studies investigating  
22 neurotoxicity via the inhalation route that would support the development of an RfC. The Panel  
23 agreed that the absence of evidence for route of entry specific effects would allow route-to-route  
24 extrapolation for deriving an RfC based on using the PBTK model to calculate the human  
25 equivalent concentration (HEC).

26 The Panel agreed that the recommendation to use the age-dependent adjustment factors is  
27 well justified and transparently and objectively described. Additionally the Panel believed that  
28 the discussion of uncertainties is adequate, but that human variability could be more completely  
29 addressed. There is no characterization of sensitive populations, and this should be explored and  
30 discussed to a much greater extent.

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1  
2       The Panel commends EPA for using the PBTK model for developing the RfD, RfC and  
3 Cancer Slope Factors for acrylamide. The Panel notes that the use of internal dose metrics  
4 combined with a fairly robust understanding of the mechanism of action may replace the use of  
5 the default interspecies factor for toxicokinetic differences (i.e.,  $10^{1/2}$ ), but not the default  
6 interspecies factor for pharmacodynamics. This factor is still needed in deriving the RfC and  
7 RfD. Further the Panel strongly encourages the Agency to move forward with revising and  
8 finalizing their assessment.

## INTRODUCTION

### *Background*

This report was prepared by the Science Advisory Board (SAB) Acrylamide Review Panel (the “Panel”) in response to a request by EPA’s Office of Research and Development (ORD) to review the Draft Toxicological Review of Acrylamide (hereafter referred to as the “draft document”). The IRIS Toxicological Review(s) is a compilation and summary of the available information on the potential for cancer and non-cancer hazardous effects in humans from exposure to acrylamide.

The SAB was asked to comment on (1) whether the document is logical, clear and concise, (2) if the discussion is objectively and transparently represented, and (3) if it presents an accurate synthesis of the scientific evidence for non-cancer and cancer hazard. The SAB was also asked to identify any additional relevant studies that should be included in the evaluation of the non-cancer or cancer health effects of acrylamide, or in the derivation of toxicity values. In addition, the SAB was asked to provide advice on 26 specific charge questions related to the derivation of a proposed oral reference dose (RfD) and an inhalation reference concentration (RfC) for non-cancer endpoints, as well as the cancer descriptor, oral slope factor, and inhalation unit risk for acrylamide.

The Panel deliberated on the charge questions during a March 10-11, 2008, face-to-face meeting and discussed their draft report in a subsequent conference call on July 16, 2008. The responses that follow represent the views of the Panel. The specific charge questions to the Panel are available in Appendix A.

## RESPONSES TO THE CHARGE QUESTIONS

**Charge Question 1. *Please comment on the selection of neurotoxicity as the most appropriate choice for the most sensitive endpoint (in contrast to reproductive toxicity, heritable germ cell effects, or other endpoint) based upon the available animal and human data.***

Based on the existing toxicity data base for acrylamide, neurotoxicity does appear to be the most sensitive endpoint, and therefore, the most appropriate for developing the (non-cancer) RfD and RfC. Animal studies report microscopically-detected degeneration in peripheral nerve cells at doses of 1-2 mg/kg day, as compared to levels of 3-13 mg/kg day to detect impaired male reproductive performance. Animal studies provide a clear mechanistic understanding whereby low-dose, subchronic exposure leads to toxicity with concomitant nerve damage. Acrylamide has a direct or indirect effect on the motor protein kinesin or nerve terminals, producing damage in the peripheral and central nervous systems, which leads to sensory and motor disease. Correspondingly, reports of central-peripheral neuropathy, ataxia and muscle weakness in exposed human cohorts have been documented since the early 1950's. Acute occupational exposure to acrylamide can lead to an immediate neurologic response, e.g., sweating, nausea, myalgia, numbness, paresthesia, and weakened legs and hands. Following termination of short term exposure, these acute effects disappear.

There were issues of concern that should be noted:

- 1) As detailed in the response to Question 4, the determination of accurate benchmark doses (e.g., LOAELs, NOAELs, RfDs) from the Friedman et al. (1995) and Johnson et al. (1986) studies may be compromised by their lack of functional testing of neurotoxicity and the use of a relatively insensitive measure, peripheral axonopathy, as the primary index neurotoxicity.
- 2) There was concern that axonal degeneration observed under light microscopy was the endpoint chosen from the Friedman et al. (1995) and Johnson et al. (1986) studies for derivation of the RfD and RfC. Animal studies indicate that nerve terminal degeneration can occur prior to axonal degeneration at some doses. This would suggest that all of the cited

1 studies, including the subchronic Burek study and the 2 year bioassay studies of sciatic nerve  
2 (Friedman et al, 1995) and tibial nerve (Johnson et al, 1986) axons, in looking at axonal  
3 degeneration, may have missed a preceding terminal degeneration at a lower dose,  
4 particularly as no specific mention of terminal degeneration is provided and  
5 functional/behavioral measures of neurotoxicity were not included.

- 6 3) It should be noted that future studies may demonstrate effects of acrylamide exposure on  
7 male reproductive function, as currently evidenced in animal studies by increased pre- and  
8 post-implantation losses and decreased litter sizes, at even lower doses than those currently  
9 associated with neurotoxicity after acrylamide dosing in animal studies. The draft document  
10 states that “associations between human exposure to acrylamide and reproductive effects  
11 have not been reported” (p. 187 and p. 224); rather, these associations *have not been studied*.  
12 The lack of human data is a major limitation in this regard. As noted in the draft document,  
13 data also exist that reveal acrylamide’s capacity to induce heritable germ cell effects at doses  
14 somewhat above those at which neurotoxicity has been observed, but there are as yet no  
15 studies providing an in-depth examination of dose response or identification of credible no-  
16 effect levels. The heritable germ cell effects are very worrisome and deserve even more  
17 consideration, including perhaps the use of this endpoint to generate an independent RfD.
- 18 4) Although still controversial and recognizing that cigarette smoke is a complex mixture made  
19 up of hundreds of compounds, there is growing evidence that supports an association  
20 between cigarette smoking, a known source of acrylamide exposure, and altered semen  
21 parameters, including concentration, morphology, motility, and DNA fragmentation  
22 (Richthoff et al., 2008; Sepaniak et al., 2006; Marinelli et al., 2004). The lack of data  
23 regarding potential interactions between acrylamide and other exposures, including cigarette  
24 smoke, alcohol use, and cosmetics (another source of acrylamide exposure) has been cited as  
25 a major limitation in studies of human acrylamide exposure and adverse health effects (Rice  
26 2005; draft document p.194; p. 224). The investigation of altered semen parameters among  
27 occupationally exposed males, controlling for smoking and alcohol consumption, should be a  
28 high priority.

29  
30 New References

1 Richthoff J, Elzanaty S, Rylander L, Hagmar L, Giwercman A. Association between  
2 tobacco exposure and reproductive parameters in adolescent males. Int J Androl 2008; 31:31-9.  
3 Sepaniak S, Forges T, Foliguet B, Bene MC, Monnier-Barbarino P. The influence of  
4 cigarette smoking on human sperm quality and DNA fragmentation. Toxicol 2006; 223:54-60.  
5 Marinelli D, Gaspari L, Pedotti P, Taioli E. Mini-review of studies on the effect of  
6 smoking and drinking habits on semen parameters. Toxicol 2004; 207:185-92.

7  
8 **Charge Question 2. *Please comment on the discussion of mode of action for acrylamide-***  
9 ***induced neurotoxicity.***

10  
11 The Panel found the separation of the discussion of MOA(s) for neurotoxicity in two  
12 different sections of the document (Section 4.6.1, pages 123-124; and Section 4.7.3, pages 134-  
13 136) confusing and recommends their incorporation into a single section.

14 Acrylamide is a member of the type-2 alkene chemical class, which includes acrolein,  
15 methylvinyl ketone and methyl acrylate. A weight of evidence evaluation of the current body of  
16 data now suggests that the type-2 alkenes produce toxicity via a common molecular mechanism;  
17 i.e., formation of adducts with essential sulfhydryl thiolate groups on proteins that play  
18 regulatory roles in cellular processes (LoPachin et al., 2007a,b, 2008a; reviewed in LoPachin and  
19 Barber, 2006b; LoPachin et al., 2008b).

20 Currently, there are two hypotheses regarding the mechanism of acrylamide  
21 neurotoxicity: 1) Acrylamide/glycidamide inhibits fast axonal transport by forming adducts with  
22 kinesin, the transport motor (reviewed in Sickles et al., 2002). 2) Acrylamide disrupts nerve  
23 nitric oxide (NO) signaling at the nerve terminal (reviewed in LoPachin et al., 2006a). The Panel  
24 did not attempt to resolve the debate over the MOA of neurotoxicity. It is also possible that both  
25 MOAs may be pertinent, and studies directly comparing the time course of the two proposed  
26 MOAs in a single model have not been carried out. However, the Panel agreed that the further  
27 delineation of MOAs will improve acrylamide risk assessment. Both of the proposed MOAs  
28 suggest that visible axonal degeneration on light microscopy is not likely to be the low-dose  
29 effect in the causal pathway. Regardless, it should also be evident that substantial, detailed



1 molecular information is available regarding mechanisms of acrylamide neurotoxicity and that  
2 these data should be included.

3  
4 Thus, the following deficiencies in the draft document were identified by the Panel:

- 5 1) As drafted, the document's coverage of research findings is incomplete and does not  
6 adequately reflect the current molecular understanding of the mechanisms of acrylamide  
7 neurotoxicity. Moreover, information in the document regarding the hypothesized MOAs is  
8 not presented in a sufficiently transparent manner consistent with the Agency's guidance on  
9 identification of the key events leading to the effect of concern, i.e., use of the modified  
10 Bradford Hill criteria with respect to dose-response concordance, temporal relationship(s),  
11 strength, consistency, specificity of association and biological plausibility and coherence, as  
12 is done for carcinogenicity.
- 13 2) There was insufficient discussion of acrylamide adduct chemistry and corresponding  
14 neuronal targets pertinent to understanding the MOAs.
- 15 3) There was lack of a discussion of residual questions surrounding the respective roles of the  
16 parent toxicant, acrylamide, and its epoxide metabolite, glycidamide, in the production of  
17 neurotoxicity.

18  
19 The Panel recommends that the Agency expand its discussion of the two MOAs. Panel  
20 members provided more specific text that describes the two proposed MOAs, and the Panel  
21 offers this text to EPA for consideration in revising the acrylamide assessment. The text is given  
22 in Appendix B of this report.

23  
24 **Charge Question 3. *Please comment on the qualitative discussion of acrylamide's heritable***  
25 ***germ cell effects and whether the discussion is clear, transparently and objectively described,***  
26 ***and reflective of the current science.***

27  
28 Discussion in the document of heritable germ cell effects, consisting of 5 heritable  
29 translocation studies, the 2 specific locus studies, 2 studies on acrylamide transformation to  
30 glycidamide and the importance of this metabolism to toxicity, is relevant and useful, and is

presented in a clear, transparent manner reflective of the current science. However, the discussion is a linear description of germ cell toxicity with little synthesis, analysis and scrutiny. While some SAB members considered the presentation objective, some expressed concerns over the lack of inclusion of all potential MOAs. Given the serious consequences of heritable germ cell effects, the considerable deficiencies of the database should be identified and the significance of this endpoint emphasized.

The entire section is prefaced and summarized with the perspective that DNA adduct formation and mutagenicity is the only operative mechanism for heritable germ cell effects of acrylamide. While adducts can certainly lead to the observations, there are alternative mechanisms for discussion. Clastogenic mechanisms, as well as, mitotic spindle defects are viable candidates for dominant lethal effects. There is a wealth of acrylamide studies reporting these alternative mechanisms that should be included in this discussion as well. They were briefly outlined in the carcinogenicity section, but should also be identified here. In regards to spindle defects, the effects of acrylamide on kinesin motors involved in cell division should be added to the document (Sickles et al., 2007).

Adequate response data are lacking in the existing heritable germ cell studies such that the shape of the dose response relationship cannot be ascertained. However, in Tyl et al (2000) dose responses are identified - a NOAEL of 2 mg/kg/d and a LOAEL of 5 mg/kg/d for a 13 week exposure. All of the dominant lethal studies were conducted at a dose of 50 mg/kg or higher and most with multiple exposures. The specific locus studies were conducted at 50 mg/kg/d for 5 days (Russel et al., 1991) or with a single 100-125 mg/kg exposure (Ehling and Neuhauser-Klaus, 1992). The discrepancy between the negative results of Russel et al. (1991) and the positive results of Ehling and Neuhauser-Klaus (1992) may be dose-related or due to other factors. The fact that heritable translocations appeared at high frequency at the lowest doses tested implies that even lower doses may produce such effects.

However, in the absence of these data, the uncertainty should be identified. As a consequence of these limitations in the database, there is some uncertainty related to the RfD. The Panel unanimously agreed that this is an extremely serious data gap that should be a top priority for further study. Additional studies to address the aforementioned database deficiencies in mechanisms and dose-responses would be desirable.

1           The document requires correction in that the NTP/CERHR report was published in  
2 February 2005, not 2004. Also, there appears to be a discrepancy in the text (Pg 117 indicates the  
3 historical controls were 6%, yet on pg 116 in the discussion of the Adler et al. (1994) study, the  
4 historical controls are listed as 5/9890 which is 0.05%).

5  
6   **Charge Question 4. *Please comment on whether the selection of the Friedman et al, 1995***  
7 ***and Johnson et al, 1986 studies as co-principal studies has been scientifically justified.***  
8 ***Although EPA considers Friedman et al and Johnson et al to be co-principal studies, the final***  
9 ***quantitative RfD value is derived only from the Johnson study. Please comment on this aspect***  
10 ***of the EPA's approach. Please comment on whether this choice is transparently and***  
11 ***objectively described in the document. Please identify and provide the rationale for any other***  
12 ***studies that should be selected as the principal studies.***

13  
14           The Panel afforded considerable discussion to the question of whether the Friedman et al  
15 (1995) and Johnson et al (1986) studies were the best choices for derivation of the quantitative  
16 RfD (and RfC). The main concerns with these studies included the fact that they were primarily  
17 designed as cancer bioassays rather than for evaluation of neurotoxicity. Specifically, the Panel  
18 contended that the endpoint of axonal degeneration visible under light microscopy is an  
19 insensitive measure of neurotoxicity. Alterations visible under electron microscopy or  
20 functional/behavioral alterations would have provided more sensitive endpoints.

21           Nevertheless, the Panel agreed that the selected studies did have some important  
22 strengths, including reasonable statistical power due to the relatively large number of animals,  
23 chronic dosing, and the fact that the NOAELs for the endpoint in the two studies were similar,  
24 implying some precision in the effect estimate measured. The Panel also noted that there are no  
25 studies yet available which include the sensitive functional/behavioral assessments that would be  
26 most desirable. Several Panel members noted that this issue is a significant data gap that should  
27 be considered in the context of setting a database uncertainty factor.

28           With respect to the Burek et al. (1980) study, the Panel notes that while the endpoint in  
29 this study (axolemmal invaginations under electron microscopy) is a highly sensitive one for use  
30 in risk assessment, the study was subchronic. One Panel member proposed that EPA consider

1 generating an RfD based on the data in Burek et al. (1980), but not use a subchronic-to-chronic  
2 uncertainty factor given the existence of the two chronic studies, to compare the resulting RfD to  
3 that based on the less sensitive endpoint of axonal degeneration. Such a comparison might begin  
4 to quantify the degree of potential under-estimate of risk due to the less satisfactory choice of  
5 endpoint in the Johnson and Friedman studies.

6       There was a brief discussion of the report of foot splay at 0.5 mg/kg in F<sub>0</sub> males in the  
7 Tyl et al. (2000a) two-generation reproductive toxicity/dominant lethal mutation study. The use  
8 of this gross functional endpoint could also serve as a point of departure, although it was  
9 considered questionable because: it was only observed in the F<sub>0</sub> generation, was found in control  
10 animals to some degree (raising questions about the methodology used in the lab), and did not  
11 follow a clear dose-response relationship. Overall, the Panel decided that the Tyl study was not a  
12 good choice for derivation of the RfD.

13       The Panel also considered the option of deriving an RfD based on human data. Both the  
14 Calleman et al. (1994) and the Hagmar et al. (2001) studies contain sufficient data to allow the  
15 Agency to calculate an RfC or potentially an RfD. In this regard, the Panel made the following  
16 observations: (1) in general, it is preferable to use human data when available; (2) the Calleman  
17 study included a measure of internal dose (adduct levels) and a fairly sensitive measure of effect,  
18 thereby making it appealing for risk assessment; (3) PBTK modeling could allow dose  
19 extrapolation based on adduct levels, such that an ingested or inhaled dose could be estimated for  
20 purposes of setting either an RfC or an RfD from the data.

21       However, the Panel also cautioned that there are a number of drawbacks to using the  
22 human studies, including the following: (1) the sample sizes are small; (2) the samples mostly  
23 include young adult males; (3) the healthy worker effect would tend to bias these studies  
24 (especially the Calleman study) toward the null, since workers with significant neurological  
25 symptoms would leave the workplace, thus selecting for individuals with lower genetic  
26 susceptibilities; (4) the workers in each study were exposed to other confounding neurotoxicants  
27 (acrylonitrile and *N*-methylolacrylamide (NMA)), but this would tend to generate a more  
28 conservative risk estimate because these other exposures would tend to result in an over-estimate  
29 of the effect; and (5) the exposure duration was relatively short and variable (1 month to 11.5  
30 years in the Calleman study with an average of 3 years, and 55 days in the Hagmar study). In the

end, the Panel suggested that EPA undergo the exercise of generating an RfD from the Calleman study for purposes of comparison with the RfD derived based on the animal data. The Panel stopped short of recommending that the human RfD be used in place of the one in the draft document, but instead saw this as a type of sensitivity analysis, to help determine whether the RfD based on the Johnson study appears to be adequately health-protective despite the insensitive endpoint used in that study.

**Charge Question 5. *Please comment on the benchmark dose methods and the choice of response level used in the derivation of the RfD, and whether this approach is accurately and clearly presented. Do these choices represent the most scientifically justifiable approach for modeling the slope of the dose-response for neurotoxicity? Are there other response levels or methodologies that EPA should consider? Please provide a rationale for alternative approaches that should be considered or preferred to the approach presented in the document.***

Use of the benchmark dose methodology has become the preferred approach and an acknowledged improvement over the historically traditional NOAEL  $\div$  UF procedure for the derivation of RfDs. Its application in this instance is scientifically supported, given the nature and robustness of the data sets available for the endpoint of interest. The calculations and choices made were described clearly at an appropriate level of detail.

EPA's Benchmark Dose guidance provides default criteria to be used for selecting the benchmark response (BMR). For quantal data, an excess risk of 10% is the default BMR, since the 10% response is at or near the limit of sensitivity in most studies. In this case, even though the BMR at 10% extra risk also was within the range of observation, the BMR<sub>5</sub> was selected for the point of departure. The choice of a BMR<sub>5</sub> makes sense and is well-justified: (1) the 95% lower bound of the benchmark dose (BMD), BMDL<sub>5</sub>, remained near the range of observation; (2) the 5% extra risk level is supportable given the relatively large number of animals used in the critical studies; and (3) the use of BMDL<sub>5</sub> is consistent with the Agency's technical guidance for BMD analysis which allows flexibility in making such a choice. One of the strengths of the Johnson study is that it is sufficiently large (i.e., numbers of animals/group) to allow the lower

5% bound to be identified with sufficient stability that it is usable for risk assessment purposes. Therefore, it is reasonable to use that strength in the underlying data set and choose this number. Such a choice is appropriately conservative (i.e., public health protective).

While alternative approaches such as averaging the BMDLs from each of the four data sets (Friedman and Johnson, male and female) rather than using just the one for males in the Johnson study were discussed, the Panel concluded that the steps described by the Agency in the draft document represented the preferred approach.

**Charge Question 6. *Please comment on the selection of the uncertainty factors (other than the interspecies uncertainty factor) applied to the point of departure (POD) for the derivation of the RfD. For instance, are they scientifically justified and transparently and objectively described in the document? [Note: This question does not apply to the interspecies uncertainty factor which is addressed in the questions on the use of the PBTK model (see PBTK model questions below)]***

The Agency has proposed to use a composite uncertainty factor (UF) of 30: 10X to represent human variability ( $10_H$ ) and 3X to reflect the toxicodynamic component of the default interspecies uncertainty factor ( $10_A$ ). The other half of the 10x interspecies UF, i.e., the 3X that would otherwise account for interspecies differences in toxicokinetics, is subsumed in the PBTK modeling.

Two points were raised about the use of 3X as a default to account for interspecies toxicodynamic differences. First, it was noted that the rodents are less sensitive to the neurotoxic effects of acrylamide than humans. The Panel concluded that the application of a UF for interspecies toxicodynamics was directionally correct. Second, there is insufficient information available to define a chemical-specific factor and the default factor of 3X UF for interspecies in pharmacodynamics is therefore appropriate. It was noted that recent International Programme for Chemical Safety guidelines divide the default  $10_A$  into 2.5X for toxicodynamic differences and 4.0X for toxicokinetics differences, based primarily upon a review of the literature published in 1993 -(WHO IPCS 2005. *Guidance Document for the Use of Data in Development of Chemical-specific Adjustment Factors (CSAFs) for Interspecies Differences and Human*

*Variability in Dose/Concentration-Response Assessment*). The use of the factor of 3 (or  $\sqrt{10}$ ) is consistent with current EPA practice: according to the recent EPA (2004) Staff Paper “a default UF of 10 for interspecies variability that can now be reduced to 3 when animal data are dosimetrically adjusted to account for toxicokinetics.” The Staff paper cites the EPA (2002) RfD/RfC methodology document. That document divides UFs “into toxicokinetic and toxicodynamic components that have assigned default values of 3.16 ( $10^{1/2}$ ) each.”

EPA has proposed to use the default 10X UF to account for intraspecies (i.e., human) differences. The Panel concurred with this choice, noting that there were insufficient data on interindividual differences, based upon lifestage, gender or genetic characteristics, to support departing from the default.

Consensus was not achieved on the issue of the inclusion on an UF to account for deficiencies in the existing database that would confound the derivation of the most scientifically-defensible RfD. EPA concluded that an  $UF_D > 1$  was not necessary, arguing that the existing database is sufficiently robust, even though they acknowledge there are some unresolved issues that warrant further research: describing the MOA(s) for neurotoxicity, the potential for behavioral or functional adverse effects not detected in the assays to date, and the uncertainty that heritable germ cell effects may occur at lower than previously reported doses. Some Panel members agreed with EPA’s position. One Panel member noted that additional UFs were implicitly, if not explicitly, incorporated into the RfD derivation. Using the output of the log-logistic model applied to the data set for the male rats in the Johnson study resulted in the lowest set of BMDs/BMDLs. According to one Panel member, it was perhaps conferring an extra UF of ~2X. In addition, using the BMDL<sub>5</sub> as the POD, rather than the default BMDL<sub>10</sub>, also could be seen as conferring an extra UF of ~2X.

Other Panel members, however, disagreed with the Agency’s position regarding the database UF, arguing that the remaining uncertainties have major implications that could result in effects at significantly lower doses and thus a lower RfD. Database deficiencies include the following:

- 1) EPA had to rely on the observation of axonal degeneration visible by light microscopy, an endpoint which is not likely to be the most sensitive. EPA is using studies that were

not designed to evaluate neurotoxicity robustly, e.g., histopathology coupled with systematic evaluation of functional or behavioral parameters at multiple time points with robust numbers of animals/treatment and robust number of treatment groups; these studies should be done in adult animals and in a developmental neurotoxicity study in order to determine whether or not critical lifestage differences exist;

- 2) Both existing chronic studies were done in the rat, creating some remaining uncertainty about interspecies differences that is not addressed by the interspecies UF. Based upon the comparison of results from the Tyl et al (2000) 2-generation study in rats and the Chapin et al. (1995) 2-generation study in mice, the NOAEL for (adult) neurotoxicity is essentially the same (0.5 mg/kg/day in rats vs. 0.8 mg/kg/day in mice), but the difference could potentially be driven by dose spacing rather than a true difference in response. The outcomes of long-term exposure in mice hold the possibility of yielding lower NOAELs/LOAELs/BMDs than observed/calculated from the rat data. If this were to occur, the RfD/RfC would be lower.
- 3) The germ cell effects have not been fully explored and have major intergenerational implications if they do occur at dose levels lower than those for neurotoxicity. There is a lack of adequate data to define the dose response for heritable germ cell effects. While the existing data describe adverse effects at doses somewhat higher than those at which neurotoxicity was observed, BMD modeling of robust dose-response data may yield results competitive with/lower than the neurotoxicity BMDs/BMDLs.

**Charge Question 7. *Please provide any other comments on the derivation of the RfD and on the discussion of uncertainties in the RfD.***

### **Acrylamide and Cumulative Risk Assessment**

The Food Quality Protection Act (FQPA) of 1996 mandates EPA to consider the “cumulative effects” of pesticides and other substances that have a “common mechanism of toxicity” when setting, modifying or revoking tolerances for food use pesticides. Were acrylamide registered as a food use pesticide, its activity as a type-2 alkene would support a cumulative risk assessment of it and other chemicals in the class. From a scientific standpoint



1 and particularly from a public health perspective, they should be subjected to a cumulative risk  
2 assessment (e.g., see Wilkinson et al., 2000). Evaluating the cumulative effects of the type-2  
3 alkenes is particularly germane since human exposure is pervasive; i.e. chemicals in this class are  
4 used extensively in the agricultural, chemical and manufacturing industries. Furthermore, they  
5 are well-recognized environmental pollutants (e.g., acrolein, acrylonitrile), food contaminants  
6 (e.g., acrylamide, methyl acrylate) and endogenous mediators of cellular damage (e.g., acrolein,  
7 4-hydroxy-2-nonenal) (see LoPachin et al., 2008b). Thus, the application of standard approaches  
8 may result in RfDs and RfCs which could be associated with risks in the population. At a  
9 minimum, a caveat in this regard should be included in the acrylamide assessment document.

## 11 **Charge Question 8**

### 12 *Use of the PBTK Model*

13 *A physiologically-based toxicokinetic (PBTK) model originally developed by Kirman et*  
14 *al. (2003), and recalibrated by EPA with more recent kinetic and hemoglobin binding data in*  
15 *rats, mice, and humans (Boettcher et al., 2005; Doerge et al., 2005a,b; Fennell et al., 2005)*  
16 *was used in the derivation of the RfD to extrapolate from the animal dose-response*  
17 *relationship (observed in the co-principal oral exposure studies for neurotoxicity) to derive a*  
18 *human equivalent concentration (HEC). The HEC is the external acrylamide exposure level*  
19 *that would produce the same internal level of parent acrylamide (in this case the area under*  
20 *the curve [AUC] of acrylamide in the blood) that was estimated to occur in the rat following*  
21 *an external exposure to acrylamide at the level of the proposed point of departure, and related*  
22 *to a response level of 5% (i.e., the BMDL<sub>5</sub>). The model results were used in lieu of the default*  
23 *interspecies uncertainty factor for toxicokinetics differences of 10<sup>1/2</sup>, which left a factor of*  
24 *10<sup>1/2</sup> (which is rounded to 3) for interspecies differences in toxicodynamics.*

25 *With respect to the RfC, there are presently insufficient human or animal data to*  
26 *directly derive an RfC for acrylamide. The PBTK model was thus used to conduct a route-to-*  
27 *route extrapolation (oral-to-inhalation) to derive an RfC based on the dose-response*  
28 *relationship observed in the co-principal oral exposure studies for neurotoxicity. In this case,*  
29 *the HEC was based on a continuous inhalation exposure to acrylamide in the air that would*  
30 *yield the same AUC for the parent acrylamide in the blood as that estimated for the rat*

1 *following an external oral exposure to acrylamide at the level of the proposed point of*  
2 *departure (i.e., the BMDL<sub>5</sub>).*

3  
4 *Please comment on whether the documentation for the recalibrated Kirman et al. (2003)*

5 *PBTK model development, evaluation, and use in the assessment is sufficient to determine if*  
6 *the model was adequately developed and adequate for its intended use in the assessment.*

7 *Please comment on the use of the PBTK model in the assessment, e.g., are the model structure*  
8 *and parameter estimates scientifically supportable? Is the dose metric of area-under-the-*  
9 *curve (AUC) for acrylamide in the blood the best choice based upon what is known about the*  
10 *mode of action for neurotoxicity and the available kinetic data? Please provide a rationale for*  
11 *alternative approaches that should be considered or preferred to the approach presented in the*  
12 *document.*

13  
14 The Panel commends EPA for their efforts to adapt the PBTK model of Kirman et al.  
15 (2003) for acrylamide and glycidamide, recognizing that this was a complex and challenging  
16 task. The modified Kirman et al. model was produced by changing the model initially described  
17 for the rat, and adapting it to fit updated data published since the original publication in 2003,  
18 and to describe pharmacokinetics in humans. Three major modifications were described to the  
19 partition coefficients for glycidamide, the metabolic rate constants for oxidation and conjugation,  
20 and the partition coefficients for acrylamide. The simulations of the modified Kirman model  
21 were presented as tables containing comparisons of AUC data, and the extent of metabolism of  
22 acrylamide to glycidamide, and the extent of conjugation of each with glutathione.

23 However, the Panel had a number of concerns about the description of the model, and its  
24 parameterization. The Panel believed that the documentation is not adequate to determine  
25 whether the recalibrated Kirman model is appropriate for its intended use. Among the items that  
26 the Panel would like to see to justify the performance of the model are: the model code;  
27 graphical presentation of the data for time course simulations; and graphical presentation of dose  
28 response simulated by the model. Side by side comparisons of the model parameters for the rat  
29 and human could be accomplished by combining Tables E-4 and E-6.

The Panel noted that the model with some changes has been described in a manuscript published in 2007 by Walker et al. If life stage considerations are planned for subsequent work, PBTK modeling is the recommended tool for dosimetry estimates across life stages. The Panel would like to see the model used to simulate or show the degree of consistency with data published since 2005.

The Panel also noted that there have been additional studies of acrylamide, its metabolites and adducts, with varying data quality, and varying understanding of exposures. For example, exposures in smokers are likely a composite of exposure from diet (oral) and smoke (inhalation). There are possible ambiguities in assignment of acrylamide and glycidamide metabolites (the acrylamide mercapturic acid sulfoxide and the glycidamide mercapturic acids are isomeric, and need to be resolved chromatographically for appropriate quantitation). The Panel suggests that EPA review these reports for data quality and suitability, and if appropriate use them in evaluation/refinement of the model.

The Panel noted discrepancies between the PBTK predicted and measured critical dose metrics for the non-cancer (acrylamide AUC) or cancer (glycidamide AUC) PODs following drinking water exposures in rats (see table below).

			EPA PBTK Model Predictions	Tareke/Doerge Measured Data (2005, 2006)
EGV	BMDL (mg/kg/day)	Critical Dose Metric	Internal dose (uM-hr)	Internal dose (uM-hr)
RfD	0.27	AA_AUC	18.1	4.2
oral cancer	0.3	GA_AUC	15.1	4.7

The draft document notes that the data of Doerge et al. (2005 a,b) were available (page E-5), but it is not clear if the data were actually considered in updating the model.

1           While the Panel concluded that the model structure was reasonable, the parameter  
2 estimates require greater justification. The review notes (Page E-18 last paragraph) that: “In  
3 comparing different versions of the model, it was also noted that the model parameters were  
4 underdetermined, that is, there is just not enough basic pharmacokinetic data to derive a unique  
5 set of optimal parameter values, given the number of “adjustable” parameters in the current  
6 model.”

7           The Panel was concerned about the ability of the model to adequately simulate the  
8 kinetics of acrylamide and glycidamide. There is little justification presented for the adjustment  
9 of parameters from the original Kirman model. The method of optimization was not well  
10 described. The comparisons provided between observed data and model simulations are largely  
11 for AUC in tables. Thus it is difficult to determine how the model would perform under the kind  
12 of tests usually applied to a model, including the ability to fit kinetic data. Table E-4 indicates  
13 that while AUC for acrylamide and glycidamide can be simulated reasonably well with the  
14 revised rat model, and AM-GSH is reasonably close, the extent of metabolism to GA-GSH is  
15 overestimated by 3 fold by the model. Approximately 40% of the urinary metabolites were  
16 reported as GA-GSH (Fennell et al., 2005), but the model simulates that 70% would be derived  
17 from GA-GSH.

18           Table E-9 indicates that almost 50% of acrylamide is converted to glycidamide in  
19 humans. The data reported in Fennell et al. (2005) indicate approximately 13.5 % of the  
20 urinary metabolites were derived from glycidamide. Some recent studies indicate a higher degree  
21 of glycidamide formation from acrylamide, and substantial variation among individuals in this  
22 formation (Vesper et al. 2008; Hartmann et al. 2008). The model simulations are based on the  
23 assumption that all of the acrylamide not accounted for by excretion in urine by 24 hours is  
24 converted to glycidamide. As noted above, there are data not modeled that could greatly  
25 improve the model parameter estimates, using human urine kinetic data for acrylamide,  
26 glycidamide and urinary metabolites (e.g., Fennell et al. 2006; Hartmann et al. 2008; Vesper et al.  
27 2006, 2008). Table E-7 cites the Ratio of GA-GSH to AA-GSH metabolite excretion at low  
28 doses reported by Boettcher et al. (2005) as 0.206 as a data point used for calibration. Yet the  
29 model simulation reports a value of 0.733 (Table E-9). The half-life estimated for acrylamide in  
30 the model is approximately 5.8 hours and the half-life estimated for glycidamide is

1 approximately 6.1 hours. The half life calculated from urinary excretion rate for acrylamide in  
2 humans by Fennell et al. (2006), who studied small groups of healthy infertile adult men, was  
3 approximately half this, ranging from 3.13-3.49 hours. The issue of adjusting the parameters for  
4 partition coefficients and the rates of glutathione conjugation and oxidation is a serious one. It is  
5 possible to simulate the same AUC in blood with different model parameters, but with wildly  
6 different extents of metabolism and dose to the tissues for acrylamide or glycidamide, by  
7 adjusting partition coefficients, and metabolic rate constants. In other words, there may not be  
8 unique solutions unless the full body of reported data can be used in model verification. It is  
9 exceedingly important to carefully consider the extent of metabolism as a key piece of  
10 information in making parameter selections.

11 The description of the parameters and calibration for the human Kirman model are  
12 generally presented clearly on pages E-17 and E-18. A possible exception is the very general  
13 description of the “iterative process” that was used to evaluate physiologically feasible options to  
14 best fit the Fennell et al. (2005b) and Boettcher (2005) human data on adult adduct levels and  
15 urinary metabolites. A rough comparison of the final rat and human values suggests increased  
16 values for a number of tissue binding and metabolic parameters in the human model. Many of  
17 these parameters that changed from rat to human increased roughly by a factor of 2 with the  
18 exception of the Cytochrome P-450 oxidation rate that decreased by a factor of almost 2.1. It is  
19 not clear from the description of the iterative process used to calibrate these values whether the  
20 process was designed to force these parameters to move as groups or exactly what logic was  
21 employed to adjust these multiple parameters. The general logic behind the iterative testing of  
22 permutations of values could be clarified here without going into extreme detail.

23 An alternative approach that should be considered is a re-evaluation of the revised PBPK  
24 model of Kirman et al. (2003). Determining how well it simulates the more recent data and  
25 adjusting the metabolic parameters as necessary is one approach. The Panel had an extensive  
26 discussion as to whether the dose metric of area-under-the-curve (AUC) for acrylamide in the  
27 blood was the best choice based upon what is known about the mode of action for neurotoxicity  
28 and the available kinetic data. A variety of opinions were expressed, ranging from the assertion  
29 that AUC for acrylamide in blood was a suitable dose metric, to the fact that it may not be the best  
30 choice, but may be expedient. The best choice would be to have compartments for the tissues of

1 interest, and to model the amount of acrylamide and/or glycidamide reaching the tissues. The  
2 Kirman model and the modified Kirman model are both limited by the tissue descriptions: liver,  
3 lung, blood and a single compartment for remaining tissues.

4       There was extensive discussion among the Panel members about whether the  
5 neurotoxicity of acrylamide could clearly be attributed to acrylamide alone, to glycidamide, or to  
6 a mixed mode of action. This question was raised in the review document (Page 136, last full  
7 paragraph). Therefore the choice of acrylamide in blood as the dose metric may need to be  
8 revisited as this question is clarified.

9       Several alternatives to the PBTK model exist for making the estimates of internal dose in  
10 rats needed for both the non-cancer and cancer assessments and for calculating the Human  
11 Equivalent Dose (HED). The data available in Doerge et al. (2005) and Tareke et al. (2006)  
12 provide measured serum acrylamide and glycidamide AUCs in rats exposed at drinking water  
13 concentrations and resulting doses near the PODs. Simple linear extrapolation could be used to  
14 calculate the critical internal dose metrics. The hemoglobin adduct and other data available in  
15 several recent publications (Fennell et al. 2005; Vesper et al. 2006, 2008; Hartmann et al. 2008)  
16 together provide a robust means of estimating HEDs. The Panel also discussed the alternative  
17 approach of using pharmacokinetic principles to interpret measurements of hemoglobin adducts  
18 of acrylamide and glycidamide and thereby model glycidamide formation.

19       The Panel also raised concerns about the population variability in the metabolism and  
20 pharmacokinetics of acrylamide, and how that could be incorporated in the model. It was  
21 recognized that there are some high quality human data sets that could be used for PBPK model  
22 development (e.g. Fennell et al., 2005, 2006). However, there are limitations with the small  
23 number of selected subjects compared with the general population, in describing the population  
24 variation. The Panel has identified some studies that suggest variation in the extent of  
25 metabolism of acrylamide to glycidamide (Vesper et al. 2006, 2008; Hartmann et al 2008), and  
26 differences in extent of conversion of acrylamide to glycidamide in children (Heudorf et al.,  
27 2008). There is a need for a better understanding of exposure route differences, inter-individual  
28 variation and life stage differences in the metabolism of acrylamide to glycidamide, and their  
29 clearance. The Panel encourages an evaluation of the available literature, and if possible,  
30 simulation of human variability within the PBPK model.

**Charge Question 9. *Is the Young et al model adequately discussed relative to structure, parameter values and data sets used in the model?***

The Young et al. paper does not provide citations or values for many of its physiological model parameters. This is an unusual situation for a PBTK modeling paper. For chemical specific model parameter values, the authors fitted the chemical specific model parameter values for each administered dose, creating a model that is calibrated for each dose. This results in an unwieldy model for use in risk assessment. The preferred approach is to use all the administered dose groups and create a model with one set of chemical specific model parameters that describes all the pharmacokinetic data sets. The model was based on the use of linear terms to describe chemical specific reactions (e.g., binding, DNA adducts, and metabolism). This approach may not hold (and non-linear terms will be needed) when developing one set of chemical specific model parameters to describe the kinetics over a range of doses.

***Do you agree with the conclusion that the recalibrated Kirman et al. 2003 model is the best for deriving toxicity values?***

In the opinion of the Panel, the recalibrated Kirman model was superior to the Young et al. PBTK model. However, the Panel noted that the recalibrated model requires updating to include new data sets in the rat and human. The concerns described in Charge Question 8 need to be addressed to use the recalibrated Kirman et al 2003 model. The Panel also noted that an approach to calculating internal doses at the non-cancer and cancer PODs is available that relies on measured data (and minimal linear extrapolation in a dose range that has been shown to be linear) instead of the PBTK model. This approach also affords the ability to calculate the HED corresponding with the critical internal dose metrics associated with the PODs (see response to question 8). If life stages are considered, the PBTK modeling or another pharmacokinetic approach is the preferred approach for determining a HED or HEC.

**Charge Question 10.** *According to US EPA's RfC Methodology (1994), the use of PBTK models is assumed to account for uncertainty associated with the toxicokinetic component of the interspecies uncertainty factor across routes of administration. Does the use of the PBTK model for acrylamide objectively predict internal dose differences between the F344 rat and humans, is the use of the model scientifically justified, and does the use of the PBTK reduce the overall uncertainty in this estimate compared to the use of the default factor? Are there sufficient scientific data and support for use of this PBTK model to estimate interspecies toxicokinetic differences and to replace the default interspecies factor for toxicokinetic differences (i.e., 10<sup>1/2</sup>)? Is the remaining uncertainty factor for toxicodynamic differences scientifically justified, appropriate and correctly used?*

The Panel commends EPA for using the PBTK model for developing the RfD, RfC and Cancer Slope Factors for acrylamide. The kinetics of acrylamide are well characterized and thus the use of internal dose metrics that are thought to represent the critical dose metrics for non-cancer (neurotoxicity) and cancer (various tumor types) is a preferred approach for extrapolating across species. The Panel agrees that the use of internal dose metrics (calculated using the PBTK model or other pharmacokinetic approaches alluded to above) combined with a fairly robust understanding of the mechanism of action and thus the critical dose metric replaces the use of the default interspecies factor for toxicokinetic differences (i.e., 10<sup>1/2</sup>).

The Panel agreed with the use of the remaining UFs representing interspecies differences in pharmacodynamics and intraspecies variability in both pharmacokinetics and pharmacodynamics.

**Charge Question 11.** *Please comment on whether the PBTK model is adequate for use to conduct a route-to-route extrapolation for acrylamide to derive an RfC in the absence of adequate inhalation animal or human dose-response data to derive the RfC directly. Was the extrapolation correctly performed and sufficiently well documented?*

The Panel discussed the lack of inhalation toxicology and PK studies. One Panel member who has conducted inhalation PK exposure studies noted the difficulty with conducting



controlled rodent exposure studies and the difficulty in maintaining stable exposure concentrations because of the low volatility of acrylamide and its propensity to sublime. The Panel agreed with the use of PBTK modeling to conduct dose-route extrapolation. Additionally, the Panel commends the EPA for using the PBTK model to fill the gap resulting from the absence of robust animal toxicology studies investigating neurotoxicity via the inhalation route that would support the development of an RfC. The Panel agreed that the absence of evidence for route of entry specific effects would allow route-to-route extrapolation for deriving an RfC by using the PBTK model to calculate the human equivalent concentration (HEC). This would yield an equivalent internal dose (Acrylamide AUC) associated with those achieved at the POD from the oral sentinel (Johnson et al.) studies. The Panel noted that few inhalation PK studies exist to allow a robust parameterization of the inhalation component of the PBTK model for either rats or humans. Despite this, the Panel noted that acrylamide is very water soluble and non-volatile, and the compound has a relatively long half-life. Therefore, the absorption of acrylamide via inhalation should be nearly complete, and first pass effects are negligible, thereby making the pharmacokinetics of acrylamide via inhalation easy to extrapolate from the oral case, using simple principles of pharmacokinetics. The Panel agreed that the application of pharmacokinetic approaches (e.g., the use of the PBTK model) reduces uncertainty associated with animal to human extrapolation and thus warrants replacing the default UF associated with interspecies extrapolation for pharmacokinetic differences as was done for deriving the RfD.

The Panel noted that the air concentration one would derive using the default approach (multiply the HED by body weight [70 kg] and dividing by daily inhalation rate [20 m<sup>3</sup>/day] yielding 0.266 µg/m<sup>3</sup>) is very similar to the HEC derived using the PBTK model (0.25 µg/m<sup>3</sup>). Therefore, if the EPA also decides to provide an extrapolation based on measured data (as described in the response to charge question 8), the default approach of extrapolating from an absorbed oral dose to an equivalent intake from the inhalation route (multiplying by 70 kg and dividing by 20 m<sup>3</sup>/day) can be used with confidence to calculate the RfC.

**Charge Question 12. *Please provide any other comments on the derivation of the RfC and on the discussion of uncertainties in the RfC.***

1           The Panel has no further comments beyond those already discussed above.

2  
3   **Charge Question 13. *Would you suggest that EPA include a Table that lists points of***  
4 ***departure (e.g., NOAELs, BMDs, etc.) for various endpoints that could be used, in***  
5 ***conjunction with exposure assessments, to conduct a MOE analysis?***  
6

7           To the extent permitted by the available data, the Panel supports the concept of the  
8 inclusion of a table in the IRIS acrylamide document which provides information that could be  
9 used to conduct a variety of MOE analyses for specific endpoints of interest and/or for other than  
10 lifetime durations of exposure, in addition to the traditional lifetime focus. In doing so the  
11 magnitude of the MOE that represents a negligible risk should be reported for each point of  
12 departure tabulated.

13           Currently, for those environmental agents for which sufficient data exist, IRIS documents  
14 will present the derivation of a Reference Dose (RfD) and a Reference Concentration (RfC), as  
15 traditionally defined, to be used in the assessment of scenarios which assume that long-term or  
16 lifetime exposures are occurring to non-carcinogenic hazards. Additionally, in those cases where  
17 the agent of interest has been shown to have carcinogenic potential, an oral cancer slope factor  
18 (CSF) and/or an inhalation unit risk (IUR) may be derived, in order to estimate lifetime cancer  
19 risks. Whether or not this step is included is determined by a weight-of-evidence evaluation of  
20 the body of evidence supporting carcinogenic potential and an understanding, or lack thereof, of  
21 the mode(s) of action by which the carcinogenic responses are mediated. These four values (the  
22 RfD, RfC, CSF and IUR) are applicable in situations where the assessment is focused on the  
23 general population exposed over a lifetime, and may have more limited utility in the assessment  
24 of specific subpopulations and/or less-than-lifetime exposure durations.

25           EPA Program and Regional offices and other end-users of IRIS documents often must  
26 develop risk assessments for specific populations and/or less-than-lifetime exposure scenarios in  
27 order to carry out their respective legislative and regulatory mandates. These risk assessments  
28 would benefit from the inclusion of transparently-developed, peer-reviewed consensus hazard  
29 values.

A comprehensive table would, for example, include NOAELs, LOAELs, BMDs and BMDLs at the 1%, 5% and 10% risk levels (as the default) for those studies deemed the most appropriate for the assessment of specific endpoints and for acute, intermediate and long-term exposure scenarios, data permitting. It is recognized that it will typically not be possible to fill in every cell for every endpoint and all exposure durations of interest and that a different BMD<sub>R</sub>/BMDL<sub>R</sub> may better reflect the study's results. Some EPA program offices have extensive experience in the selection of study types and durations that best lend themselves to the assessment of specific endpoints, exposure durations and subpopulations.

For this draft acrylamide assessment, such a table would display the relevant outcomes of a review of the reliable and well-performed studies which evaluated the potential for neurotoxicity in the adult and developing organism, reproductive toxicity including heritable germ effects, developmental toxicity, and general systemic toxicity following acute, intermediate and long-term exposure, as appropriate.

**Charge Question 14. *Please comment on the discussion of methods to quantitate the dose-response for heritable germ cell effects as to whether it is appropriate, clear and objective, and reflective of the current science. Has the uncertainty in the quantitative characterization of the heritable germ cell effects been accurately and objectively described?***

*[It should be noted that the section under review is 5.5 rather than 5.4. In addition, page 215 which includes figures 5-2 and 5-2a, was inadvertently omitted in the draft EPA report and thus not available for review by the Panel. Correction of this error, however, is not expected to impact the recommendations of the Panel on this question as outlined below.]*

Although reservations were expressed about the lack of data to quantify dose-response, it was the consensus of the Panel that the discussion of the methods should be retained in the report. The report adequately characterizes the current science, reflects historical attempts to estimate these risks and notes that the quantitation methods are based only on the Dearfield et al. (1995) publication. Concerns about the validity of the data and methods are given throughout the section and it is appropriately noted on page 217, " these uncertainties in the assumptions and

1 data gaps warrant further research to improve the usefulness of the following quantitative  
2 estimates of risk of acrylamide-induced heritable effects.”

3 Some specific observations/recommendations/concerns are outlined below:

4 • The parallelogram models were clearly described and the rationale for the decision to use  
5 the modified direct and doubling dose approach appears appropriate.

6 • Clearly, there is considerable uncertainty regarding the validity of the underlying  
7 assumptions for these methods and these methods may underestimate risk since they do not take  
8 into account all elements that may contribute to the risk.

9 • The extrapolation of exposure is based on animal studies using high dosages (50 to 100  
10 mg/kg or even higher)

11 • The risk extrapolation factors (REFs; pg. 217) should be explained in more detail and  
12 information included on how each number is derived (range, etc).

13 • In agreement with the report, given the differences in glycidamide production in different  
14 species, an REF of 1 for the metabolic and dose rate variability is likely incorrect. There appear  
15 to be significant dose-rate and species-dependent variations in acrylamide metabolism to  
16 glycidamide (e.g., see Barber et al., 2001; Fennell and Friedman, 2005).

17 • An REF for uncertainty in the mode of action was recommended since the doubling dose  
18 is dramatically higher when generated using specific locus studies which are clearly point  
19 mutations (53.1 mg/kg using Ehling and Neuhauser-Klaus, 1992) versus using heritable  
20 translocation data that could be based on clastogenic mechanisms (1.8, 3.3, 0.39 mg/kg for  
21 Shelby et al., 1987, Adler et al., 1994 and Adler, 1990).

22 • The implementation of the modified direct approach was difficult to understand when, in  
23 the absence of the number of human loci capable of mutating to dominantly expressed disease  
24 alleles, it was assumed to be 1000. Clarification of how this number was derived would be  
25 helpful (i.e. how do we know the number of mutable genes?).

26 • In the doubling dose approach it was not clear how the four data sets, each of which used  
27 high acrylamide dosing rates without significant dose ranges, could accurately predict the  
28 number of new diseases in the offspring at low doses.

Lack of current research in this area is a major concern and little has been done to update the research and data collection based on the Dearfield et al. (1995) methods. The Panel is in agreement with the report that recommends further research and data to fill the critical data gaps and reduce uncertainties including gaps in interspecies extrapolation factors, the quantitative relationship between genetic alterations in germ cells and heritable disease, and the shape of the low-dose response relationship. Research might include multiple dose studies, including dose selection comparable to that employed in the repeated dose studies which identified neurotoxicity as a critical effect. It is also recommended that impacts on different cell types be determined and that biomonitoring data be utilized in any models developed.

**Charge Question 15. *Please comment on the scientific support for the hypothesis that heritable germ cell effects are likely to occur at doses lower than those for neurotoxicity? What on-going or future research might help resolve this issue?***

The Panel unanimously agreed that germ cell-induced effects should be taken very seriously, as their implications are highly significant from a public health perspective. There is an absence of data on these effects in lower dose ranges, making it very difficult to speculate about the relevance of this endpoint at or below the dose levels that cause neurotoxicity. Panelists did point out that heritable translocations appeared with very high frequency at the lowest doses tested (i.e., 5 x 40 mg/kg resulted in 24% translocation carriers, Shelby et al., 1987). The high frequency of germ cell effects at these doses implies that these studies were far from identifying a LOAEL or NOAEL, and that there would likely be germ cell effects at much lower doses. However, the combination of lack of testing at lower doses, and the narrow dose range in which testing has been done, makes it very difficult to extrapolate down to a low dose range. The Panel agreed that it is a high priority to extend the heritable translocation studies down into lower dose ranges, and that this information would be very useful for risk assessment once it is completed.

**Charge Question 16. *The risks of heritable germ cell effects (i.e., number of induced genetic diseases per million offspring) for some estimated exposure in workers and the population are***

1 *presented in Table 5-11, and are based on the quantitative methods and parameter estimates*  
2 *discussed in Section 5.4 of the Toxicological Review. Please comment on whether or not the*  
3 *quantitation of heritable germ effects should be conducted, the level of uncertainty in the*  
4 *results, if Table 5-11 is useful for risk assessment purposes, and if the RfD should be included*  
5 *in the Table as one of the exposure levels.*

6  
7 The Panel supports the Agency's conclusions that exposure to acrylamide in animals  
8 leads to heritable gene mutations and that these results indicate that it may also pose a hazard to  
9 humans. In addition, the Panel supports the Agency's conclusions that the available data are not  
10 adequate to conduct a robust assessment of this endpoint at this time.

11 The Panel's deliberations regarding quantifying heritable germ cell mutations centered on  
12 the importance of including data such as those presented in Table 5-14 (not Table 5-11, as noted  
13 in the final question), the potential significance of these endpoints to human risk assessment, and  
14 the paucity of new data developed since the Dearfield et al. (1995) review upon which this  
15 section relied heavily (including Table 5-14). A majority of Panel members were supportive of  
16 the inclusion of this table in the document and for including the RfD and RfC among the  
17 concentrations in the table as this would facilitate comparison with the neurological endpoints.  
18 Suggestions also included adding more information into the review regarding the role of CYP  
19 2E1 in the dominant lethal effects of acrylamide, which indicated a requirement for metabolism  
20 to glycidamide. While the caveats from the Dearfield et al. (1995) review were recapitulated in  
21 the document, the Panel discussed the need to further elaborate the limitations in the underlying  
22 data and to include reference to the new relevant studies that pertain to uncertainty and dose-  
23 response.

24  
25 **Charge Question 17.** *Do you know of any additional data or analyses that would improve the*  
26 *quantitative characterization of the dose-response for acrylamide-induced heritable germ cell*  
27 *effects? Would these data also support the quantitative characterization of "total" male-*  
28 *mediated reproduction risks to offspring (i.e., lethality + heritable defect)? If data are not*  
29 *available, do you have any recommendations for specific needed studies?*

1 A concern raised by the Panel was that there is a lack of a suitable data set for dose  
2 response assessment for acrylamide-induced heritable germ cell effects. The majority of the  
3 studies reported have been conducted in mice, using relatively high doses.

4 Using wild type and Cyp 2E1 knockout mice, it has been demonstrated that oxidation of  
5 acrylamide to glycidamide is required for the dominant lethal effect (Ghanayem et al., 2005a)  
6 and for the induction of erythrocyte micronuclei and DNA strand breaks in lymphocytes, liver  
7 and lung using the Comet assay (Ghanayem et al., 2005b). The greater incidence of heritable  
8 translocation carriers in mice administered glycidamide (Generoso et al., 1996) compared with  
9 acrylamide (Adler et al., 1994) suggests that glycidamide plays a key role in the mode of action  
10 for heritable genetic effects.

11 The risk equivalent factors (REFs, page 217) need to be updated. There are profound  
12 differences between rats, mice and humans in the extent of metabolism of acrylamide to  
13 glycidamide, and the relative internal dose of acrylamide and glycidamide differs markedly  
14 between mice, rats and humans. The extension of the physiologically-based pharmacokinetic  
15 modeling approach to include the mouse should be a priority. The blood-testis barrier is thought  
16 to contribute to the reduction of internal dose in the testis compared with other tissues for  
17 ethylene oxide (Fennell et al., 2001). Testis should be included as a compartment in the model.  
18 Data permitting, including the testis as a compartment in the model could potentially improve the  
19 dose response characterization for this endpoint.

20 In reviewing data needs (page 220), it is noted that “The estimates do not take into  
21 account other potential genotoxic mechanisms such as effects in spermatogonia stem cells,  
22 effects in female germ cells, or induction of recessive mutations that would not appear in the first  
23 generation, but could lead to additional adverse effects in subsequent generations.” Studies to  
24 examine the dose response for heritable genetic effects, and the effect of long-term exposure to  
25 acrylamide are needed.

26 There is still uncertainty about the mode of action of acrylamide and glycidamide in the  
27 induction of heritable genetic effects. The potential for DNA adducts of glycidamide to play a  
28 role is an attractive hypothesis for the mode of action. With respect to the possible role for  
29 protamine modification in the generation of effects, there was extensive Panel discussion  
30 concerning the potential of glycidamide to form adducts with cysteine in proteins and peptides.

Adducts to protamine from acrylamide have been identified in late stage spermatids and suggested to mediate the dominant lethal effects (Sega et al., 1989). Whether glycidamide will form similar protamine adducts has not been determined. Kinesin motor proteins associated with cell division are an additional site of potential action leading to heritable germ defects (Sickles et al., 2007) that requires future consideration. Both AA and GA inhibit two kinesin motor associated with spindle formation and maintenance as well as separation of chromosomes. Loss of fidelity of chromosomal separation is related to aneuploidy, micronuclei formation and instability of the genome. The motor protein inhibitions occur at concentrations well below the occurrence of all heritable germ cell effects. Furthermore, glycidamide is more potent than acrylamide. Surveying populations occupationally exposed to acrylamide in manufacturing plants was suggested as an approach for evaluation in humans.

**Charge Question 18. *Have the rationale and justification for the cancer designation for acrylamide been clearly described? Is the conclusion that acrylamide is a likely human carcinogen scientifically supportable?***

Yes, the rationale and justification has been clearly described, although it should be further expanded (see below), and the conclusion is scientifically supportable. Acrylamide is clearly and reproducibly carcinogenic in both rats and mice. As outlined in the draft document, it produced tumors at multiple sites in the rat in multiple chronic studies, and was a skin tumor initiator in mice by multiple routes. To paraphrase the International Agency for Research on Cancer (IARC) Monographs Preamble, in the absence of tumor data in humans it is both reasonable and prudent to regard evidence of carcinogenicity in experimental animals as evidence for a probable cancer hazard to humans. This conclusion is consistent with both national and international guidelines for carcinogenic hazard identification. The U.S. National Toxicology Program (NTP) has long emphasized that chemicals that cause tumors at multiple sites or in more than a single species are reasonably anticipated to be human carcinogens. Both the NTP and IARC have placed acrylamide in cancer classifications similar to that of EPA's "likely human carcinogen" (This could be noted in the Toxicological Review).



1           When experimental exposure of rats or mice to known human carcinogens is via diet or  
2 drinking water, tumor sites observed in those species do not necessarily correspond to the same  
3 tumor sites in humans. Exposure to chemicals that cause tumors of the mammary gland or the  
4 liver in mice or rats, for example, does not necessarily correspond to increased cancer risk  
5 specifically for female breast or liver in humans. The essential point to be considered is that in  
6 any given case a tumor at these or any other site(s) results from an MOA known to operate in  
7 humans, such as somatic cell mutagenicity.

8           Primary CNS tumors as a group, which are discussed at considerable length in the draft  
9 document, should be restored to the list of experimental tumors produced by acrylamide and that  
10 are of interest for the MOA discussion. The Panel cautions that the viruses that can cause  
11 primary CNS tumors in hamsters and other non-human species are not relevant to this  
12 discussion.

13           It should be emphasized that the spectrum of tumors consistently seen in acrylamide-  
14 exposed rats is completely consistent with a DNA-reactive MOA, based on published data about  
15 other substances that induce or initiate the same kinds of neoplasms. The only agents known  
16 conclusively to induce tumors of the brain and peritesticular mesothelium in rats are all DNA-  
17 reactive, and in fact a single exposure to a direct-acting mutagenic carcinogen has been observed  
18 to suffice for tumor induction at either site. The concept that acrylamide acts by a mutagenic  
19 MOA is thus supported by the spectrum of acrylamide-associated tumors that occur in exposed  
20 rats and mice, as well as by the biotransformation pathway of acrylamide *in vivo*.

21           Tumor initiation – promotion data for mouse skin are perhaps not sufficiently emphasized  
22 in the draft document. First, only DNA-reactive chemicals or chemicals biotransformed to  
23 DNA-reactive metabolites are established tumor initiators. As acrylamide is an initiator, and by  
24 multiple routes of administration, it is a permissible inference that acrylamide is also acting by a  
25 DNA-reactive MOA in mouse skin, as do other initiators. It is most striking that, in mice,  
26 systemic exposure to acrylamide is more effective for skin tumor initiation than direct  
27 application to the skin. The order of efficiency, oral > ip > dermal application, for initiation of  
28 TPA-promotable squamous cell papillomas and carcinomas on mouse skin strongly supports the  
29 importance of systemic exposure and post-hepatic distribution of a reactive metabolite in the  
30 MOA for carcinogenicity at this site.

**Charge Question 19. *Do you agree that weight of the available evidence supports a mutagenic mode of carcinogenic action, primarily for the acrylamide epoxide metabolite, glycidamide (GA)? Has the rationale for this MOA been clearly and objectively presented, and is it reflective of the current science?***

A sound rationale and justification already supports the mutagenic MOA, and this evidence is further supported by additional new data as described below. The weight of evidence supports a mutagenic mode of action, and overall the rationale for this mode of action has been clearly and objectively presented. Some improvements to the presentation are as follows. The discussion of biological plausibility and coherence could be expanded beyond DNA adducts and the human relevance section could be somewhat more expansive without being repetitive. The argument on page 145 regarding the lack of relationship of cytogenetic damage to a mutagenic MOA should be carefully re-considered, as the literature is full of these correlations. Evidence for and against the arguments set out should be carefully evaluated, and much better referencing included. Reports from Bonassi and Hagmar are cited as supportive, yet contradictory findings from the same authors supporting an alternative argument could just as easily have been cited. The discussion includes strong generalizations that may not hold up to close scrutiny.

There has been one published study to date that has examined biomarkers of acrylamide exposure and human cancer risk. Olesen et al (2008) characterized hemoglobin adducts of acrylamide and glycidamide in a case-control study of post-menopausal breast cancer. The authors found no association between levels of glycidamide hemoglobin adducts and breast cancer risk. Moreover, they found no overall association between acrylamide adducts and risk. Upon adjustment for smoking status, however, they observed a 2.7-fold (1.1-6.6) increased risk restricted to ER+ breast cancer per 10-fold increase in acrylamide-hemoglobin level. With respect to this study design, the authors did not match or restrict the cases and controls on smoking status, which raises concern given the very strong link between smoking and acrylamide adducts. Interpretability of the Olesen study with respect to supporting the mode of carcinogenic action should be taken cautiously.

For very high levels of acrylamide exposure, the animal and other experimental data do support a mutagenic effect of acrylamide. It has been questioned whether such a mechanism might also apply to lower doses (and indeed, at the lowest doses to which humans are exposed), because of uncertainty about whether the compensatory mechanisms are in place to detoxify acrylamide. But data clearly indicate that glycidamide is formed. There are the consistent observations in humans of glycidamide-hemoglobin adducts (Bjellaas et al., 2007; Chevolleau et al., 2007; Vesper et al., 2006, 2007) or glycidamide urinary metabolites (Urban et al., 2006), including children (Heudorf et al. 2008), thus demonstrating the widespread internal exposure to the putative mutagenic metabolite of acrylamide at ongoing low levels of exposure in the general population.

The Panel did not consider the carcinogenicity to be hormonally-related. The existing short-term mouse studies in SENCAR, ICR (skin) and A/J (lung) show no such selectivity of carcinogenicity for hormonally regulated tissues. Also, the Panel discussed the fact acrylamide/glycidamide is not unique among DNA-reactive epoxides for carcinogenic action in thyroid, peritesticular mesothelium, and mammary tissue (e.g., glycidol, ethylene oxide). In addition, this argument does not consider the CNS tumors observed in both chronic acrylamide cancer bioassays, a site that was discussed by the Panel as representing strong evidence for a DNA-damaging mechanism (cf. Rice, 2005). Finally, a recent publication considered by the Panel of short-term exposures to high doses of acrylamide in male F344 rats found essentially no evidence for hormonal dysregulation in the hypothalamus-pituitary-thyroid axis based on measurements of gene expression, neurotransmitters, hormones, and histopathology (Bowyer et al., 2008). Some studies of chronic low dose exposure, such as the cohort study of acrylamide and ovarian/endometrial cancers (Hogervorst et al., 2007) and others (Khan et al., 1999) have shown positive associations with hormones. The Panel encourages the Agency to review all relevant new data that has been published since their completion of the current draft assessment as the revise and finalize this IRIS document

**Charge Question 20. *Are there other MOAs that should be considered? Is there significant biological support for alternative MOAs for tumor formation, or for alternative MOAs to be considered to occur in conjunction with a mutagenic MOA? Please specifically comment on***

1 ***the support for hormonal pathway disruption. Are data available on alternate MOAs sufficient***  
2 ***to quantitate a dose-response relationship?***

3  
4 No, there is not significant biological support for MOA alternatives to the mutagenic  
5 MOA, and data on any putative alternate MOAs are not sufficient to quantify dose response  
6 relationships. It must be emphasized that more than one MOA may operate for a given  
7 carcinogenic chemical, and the likelihood that more than a single MOA is operative increases as  
8 levels of exposure increase. Some well-documented non-DNA reactive MOAs appear to be  
9 high-dose phenomena. These are often important for understanding bioassay results in  
10 experimental animals, and sometimes for high-exposure situations in human experience, but they  
11 are usually less important because they represent negligible risks when cumulative human  
12 exposures to these and similarly acting compounds fall considerably below bioassay dosage  
13 levels. MOAs that can occur both in experimental rodents and in humans and that operate both  
14 at bioassay dosage levels in experimental animals and at lower levels as well, into the human  
15 exposure range, are most significant for humans. In general, for chemicals such as acrylamide  
16 where there is a compelling body of data to support a DNA-reactive MOA via biotransformation  
17 to glycidamide, the evidence for alternative or additional high-dose MOAs would have to be  
18 convincing to explore alternative approaches to dose response and risk assessment. One caveat  
19 that should be mentioned is that mutations induced by acrylamide are observed following high  
20 doses. There are similarly acting agents, such as methylmethanesulfonate (MMS) that create N7-  
21 Guanine, the same DNA adducts, as does glycidamide yet show a threshold for mutations. These  
22 data are consistent with robust repair mechanisms for the specific type of DNA adducts produced  
23 by glycidamide and MMS. However, it should also be noted that low dose exposures have not  
24 been tested in animal mutation studies and NOAELs have not yet been established. Therefore  
25 future research should include dose response analyses to stringently test the relationship between  
26 DNA adducts and mutations and gain a better understanding of the effects at lower doses. The  
27 Agency should mention the finding of inhibition of kinesin motor proteins as a newly-identified  
28 and potential site of action of AA or GA in the production of carcinogenicity (Sickles et al,  
29 2007).

Occasionally high-dose or “unique rodent-specific” MOAs may be invoked or postulated to discredit bioassay results as irrelevant to humans, especially when such putative MOAs are observed uniquely in non-human species. Such a postulated MOA needs to be very precisely defined and its relevance thoroughly investigated and critically tested before the postulated MOA is accepted by the biomedical and risk assessment communities. Any MOA developed for a single substance is at best speculative until a general pattern can be rigorously demonstrated for a family of substances that operate via the same MOA. The hormonal disruption MOAs proposed for acrylamide as tissue-specific alternatives to a DNA-reactive MOA are highly speculative, are supported by at most limited evidence, and do not meet this standard as noted in response to charge question 19. The data are insufficient for characterizing dose-response relationships for any of these proposed alternatives.

**Charge Question 21. *Two chronic drinking water exposure bioassays in Fischer 344 rats (Friedman et al., 1995; Johnson et al., 1986) were used to derive the oral slope factor, and to identify the tumors of interest for the MOA discussion. Are the choices for the studies, tumors, and methods to quantify risk transparent, objective, and reflective of the current science? Do you have any suggestions that would improve the presentation or further reduce the uncertainty in the derived values?***

The two chronic studies bioassays in F344 rats are the main studies to consider in dose response analysis. Overall the document does a good job discussing these studies, but the rationale for using only the Friedman et al. study for derivation of the oral slope factor is problematic, and the strengths and limitations of both studies should be discussed in greater depth. The text describes the Friedman et al. study as “superior” and “larger and better designed” but the Panel does not agree that this is the case, and recommends that both studies should be subjected to modeling for the purposes of deriving oral slope factors. The two studies may have fairly similar oral slope factors. At a minimum, estimates for the second study should also be presented to clarify the impact of study selection in the uncertainty discussion.

The methods to quantify risk are transparently presented and reflective of current science, with the exception that a factor to scale for pharmacodynamic differences in potency between

humans and animals has not been applied. The development of unit risk based on HEC accounts for the pharmacokinetic but not pharmacodynamic differences, and in such situations EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (p. 3-7) indicates inclusion of a pharmacodynamic factor be considered. The potential human variability in cancer response attributable to human pharmacokinetic variability in handling acrylamide should be discussed qualitatively and analyzed quantitatively. Hemoglobin adduct data could provide the basis for such an analysis. The assumption underlying the modeling is that each and every individual of the same age exposed to the same external dose faces the same risk of cancer is inconsistent with these data.

With respect to study selection, one of the reasons for not using the Johnson study had to do with the rates of CNS tumors in this study, particularly in the controls. The Friedman et al. study was designed "to investigate whether glial tumors in the Johnson et al. study were significant." But, as Rice (2005) points out, the histopathological examination for glial tumors was incomplete. Only one-fifth of the 1.0 mg/kg-day dose females' spinal cords were subjected to histopathological examination, even though one-third of the glial tumors in the Johnson et al. study were seen in the spinal cord. The approach to the evaluation of CNS tumors in Friedman et al. was seen by the Panel as a significant study limitation.

Another improvement over the Johnson study noted in the document for the Friedman et al. study was different, presumably better dose spacing. The doses for males in the Friedman et al. and Johnson et al. studies were the same, except Johnson et al. had one additional lower dose group. The doses in Friedman for females were 1.0 and 3.0 mg/kg-day compared to 0.01, 0.1, 0.5 and 2.0 mg/kg-day for the Johnson study. The Friedman study did extend the high end of the dose response range for females and did offer a more complete dose response function for thyroid tumors, employed somewhat larger dose groups (100 per group and two control groups). But Johnson et al. did have 60 animals per dose group, did provide a complete histopathological evaluation, and had more dose groups than a standard bioassay.

Another limitation of the Friedman et al. study is that the degree of histopathological examination of oral tissue is unclear. The Friedman study does not tabulate findings for certain tumor sites seen in the Johnson study, so quantitative comparisons are not possible and the reader is not able to consider these sites or perform independent evaluations regarding the significance

1 of the findings. It appears EPA may have the data needed to do the analysis since it was able to  
2 do a time-dependent analysis for slope estimation using the Tegeris Lab report. EPA could then  
3 look at the data and analyze as appropriate the data for these sites.

4 A criticism about the possible impact of a sialodacryoadenitis virus on tumor findings  
5 had been raised and was another reason given for using the Friedman study. On the other hand,  
6 US FDA had raised some issues in auditing the Friedman et al. study regarding environmental  
7 controls at the lab facility and the possibility of some under-dosing of animals. Ultimately both  
8 studies have strengths and weaknesses and on balance neither seems clearly superior. Both are  
9 reasonably strong studies, and thus oral slope estimates should be presented for both studies.

10 Some comments regarding details on tumor data presentation and analysis in the EPA  
11 draft document follow:

12 Tests for dose-related trends should be conducted and presented for the all tabulated sites.  
13 By Fisher's exact test, the mammary tumors in the 0.5 mg/kg-d group in the Friedman et al.  
14 study are significant ( $p < 0.05$ ). The statistics used in the draft document that correct for  
15 intercurrent mortality should be re-checked. It appears this group has a treatment-related finding  
16 and this should be noted and the discussion that this group is devoid of treatment-related tumors  
17 (page 75) changed. The clitoral gland findings in the Johnson et al. study stand out because  
18 histology was done only on clitoral tissues observed with gross masses. This is worth an  
19 explanatory footnote. Also given the approach taken to collecting this tissue, the clitoral tumors  
20 in the 0.5 mg/kg dose group also appear worthy of note. All four masses analyzed indicated  
21 tumor compared to none in controls ( $p < 0.1$ ). In the Friedman et al. study, CNS tumors of glial  
22 origin should be combined for analysis as was done by WHO 2006. Considering the findings of  
23 glial tumors in females in the Johnson study, the dose related trend for both sexes in the  
24 Friedman study, although falling a hair short of statistical significance at the  $p \leq 0.05$  level,  
25 provide some evidence of a CNS glial cell effect in the Friedman study. This should be  
26 discussed. Also, the extent of examination of oral tissue in the Friedman study is unclear.  
27 Finally, the Friedman study employed two control groups for the male rats that do not differ  
28 from one another. For the statistical treatments, there is no apparent reason why these groups  
29 should not be combined. The Toxicological Review did this for the dose response analysis but  
30 may not have done the same for the pairwise comparisons.

1           The data choice for modeling to address the discrepancy between the Friedman et al. and  
2 the Tegeris laboratory reporting of thyroid tumors for the male noted in Appendix D of EPA's  
3 draft document was appropriate. A final minor point, in the discussion of the confidence in dose  
4 response analysis in chapter 6 (page 229), issues are raised that seem better placed in the  
5 discussion of the hazard characterization.

6  
7 **Charge Question 22. *The cancer slope factor (CSF) derivation includes an adjustment for***  
8 ***early mortality (i.e., time-to-tumor analysis). Is this adjustment scientifically supported in***  
9 ***estimating the risk from the 2-year bioassay data for increased incidence of tumors in the***  
10 ***rats?***

11  
12           The use of the Weibull-in-time multistage-in-dose analysis is a reasonable and  
13 scientifically justifiable way to take into account the early mortality in the high dose group in the  
14 male study. The decision not to employ this analysis in the case of the females is also reasonable  
15 since mortality across treatment and control groups did not differ and the overall survival appears  
16 to be fairly good.

17  
18 **Charge Question 23. *Please comment on whether AUC for glycidamide is the best choice of***  
19 ***the dose metric in estimating human equivalent concentration to derive the oral slope factor.***

20  
21           The Panel agreed that using the AUC for glycidamide is the best choice for estimating the  
22 human equivalent concentration to derive the oral slope factor. This decision was based on the  
23 strong evidence from experimental results that the AUC was linearly correlated with adduct  
24 levels in single/repeat dosing studies. There was agreement that glycidamide is the more  
25 mutagenic metabolite based on experimental studies. The Panel felt there was good  
26 documentation in the report regarding the correlation between levels of DNA adducts and extent  
27 of mutations *in vivo*. Moreover, the metabolic conversion of acrylamide to glycidamide supports  
28 the MOA.

29           One consideration in using this as the dose metric, however, comes from some of the  
30 human studies in which variability is not accounted for adequately, specifically, inter-individual



variation is not assessed and that the value used for cross-species comparisons is based on small numbers of healthy adult male humans. This is discussed at greater length in response to Question 8. Consideration of additional human data (e.g., Vesper et al., 2006) to evaluate the degree humans form glycidamide from acrylamide is clearly warranted. Such data may provide the basis for comparing human acrylamide and glycidamide AUCs, using methodology of Calleman, Bergmark and colleagues (Bergman et al., 1991). This in turn can provide an improved basis for adjustments for cross-species differences in pharmacokinetics, as well as human variability in glycidamide formation from acrylamide.

**Charge Question 24. *As with the RfC, there were insufficient cancer inhalation data to derive an inhalation unit risk (IUR). The PBTK model was used in a route-to-route extrapolation of the dose-response relationship from the oral data, and to estimate the human equivalent concentration for inhalation exposure to acrylamide. Please comment on whether this extrapolation to derive the inhalation unit risk was correctly performed and sufficiently well documented.***

The response to this question is nearly identical to the response to charge question #11. The Panel agreed with the use of PBTK modeling to conduct dose-route extrapolation and commended the EPA for using the PBTK model to fill the gap resulting from the absence of robust animal toxicology studies investigating neurotoxicity via the inhalation route that would support the development of an RfC. The Panel agreed that the absence of evidence for route of entry specific effects would allow route-to-route extrapolation for deriving an RfC based on using the PBTK model to calculate the human equivalent concentration (HEC). This would yield an equivalent internal dose (Glycidamide- AUC) associated with those achieved at the point of departure from the oral sentinel (Johnson et al.) studies. The Panel noted that few inhalation PK studies exist to allow a robust parameterization of the inhalation component of the PBTK model for either rats or humans. Despite this, the Panel noted that acrylamide is very water soluble and non-volatile, and the compound has a relatively long half life. Therefore, the absorption of acrylamide via inhalation should be nearly complete, and first pass effects are negligible, thereby making the pharmacokinetics of acrylamide via inhalation easy to extrapolate from simple

principles of pharmacokinetics. The Panel agreed that the application of pharmacokinetic approaches (e.g., the use of the PBTK model) reduces uncertainty associated with animal to human extrapolation and thus warrants replacing the default uncertainty factor associated with interspecies extrapolation for pharmacokinetic differences as was done for deriving the RfD. The use of the PBTK model however does not address cross-species differences in pharmacodynamics, which should be considered, following the Agency's 2005 Guidelines for Carcinogen Risk Assessment.

The Panel noted that the air concentration one would derive using the default approach (multiply the HED by body weight [70 kg] and dividing by daily inhalation rate [20 m<sup>3</sup>/day] yielding 0.266 µg/m<sup>3</sup>) is very similar to the HEC derived using the PBTK model (0.25 µg/m<sup>3</sup>). Therefore, if the EPA decides to also provide an extrapolation based on measured data (as described in the response to charge question 8), the default approach of extrapolating from an absorbed oral dose to an equivalent intake from the inhalation route (multiplying by 70 kg and dividing by 20 m<sup>3</sup>/day) can be used with confidence to calculate the RfC.

**Charge Question 25. *The recommendation to use the age-dependent adjustment factors (ADAFs) is based on the determination of a mutagenic MOA for carcinogenicity. Is this recommendation scientifically justifiable and transparently and objectively described?***

The recommendation to use the age-dependent adjustment factors is well justified and transparently and objectively described. The Panel's deliberations regarding quantitating age-dependent adjustment factors (Section 5.4.6) followed from discussions of a mutagenic mode of action for acrylamide and the typically enhanced sensitivity of fetal and neonatal animals from exposure to such agents in accordance with EPA's Supplemental Guidance for Assessing Susceptibility from Early Life Exposure to Carcinogens (2005b). The Panel also discussed the value of using the PBTK model to evaluate the effect of lifestage on CYP 2E1 and glutathione levels that could modify internal exposure to glycidamide. Such modeling results could be used to reduce the uncertainty associated with lifestage extrapolations and the derivation of age-dependent adjustment factors. Such efforts would be directed at pharmacokinetic aspects of the

1 age-dependent adjustment factors. Uncertainty regarding pharmacodynamics would remain to  
2 be addressed by the age-dependent adjustment factors.

3  
4 **Charge Question 26. *Please provide any other comments on the CSF or IUR, and on the***  
5 ***discussion of uncertainties in the cancer assessment.***

6  
7 The discussion of uncertainties is good, but human variability could be addressed in  
8 greater length. It is unclear why in Table 5-13 the consideration/approach is “Method used to  
9 protect sensitive populations.” There is no characterization of sensitive populations, and this  
10 could be explored and discussed to a much greater extent.

11 Specifically, not enough attention was paid to consequences of individual differences in  
12 metabolism and cancer risk. Both the CYP2E1 polymorphisms and glutathione transferase(s)  
13 (even though rodent data suggests no role for this pathway) polymorphisms could be looked at in  
14 human populations. The degree to which increased activity influences the risk should be  
15 considered, including whether this might be tumor site dependent. Also, much weight is put on  
16 the two chronic studies in the Fischer344 rat. The limitations of not having another rodent  
17 species should be discussed in more detail with respect to other carcinogens where 2 species  
18 were evaluated and similar or different results were found.

19 A factor to scale for toxicodynamic differences between humans and animals was not  
20 included in the derivation of the CSF and IUR. The 2005 EPA Carcinogenic Risk Assessment  
21 Guidelines (see e.g., Guidelines pp 1-13 and 3-7) discusses how toxicodynamics can be  
22 addressed by such a factor. The development of unit risk-based on HEC accounts for the  
23 toxicokinetic but not toxicodynamic interspecies differences.

## ABREVIATIONS

1		
2		
3	ADAF	age-dependent adjustment factor
4	AM-GSH	Acrylamide-Glutathione
5	AUC	area under the curve
6	BMD	benchmark dose
7	BMDL	benchmark dose level
8	BMR	benchmark response
9	CNS	Central Nervous System
10	CSAF	Chemical-specific Adjustment Factors
11	CSF	Cancer slope factor
12	DNA	Deoxyribonucleic Acid
13	EPA	Environmental Protection Agency
14	FQPA	Food Quality Protection Act
15	GA or Gly	Glycidamide
16	GA-GSH	Glycidamide-Glutathione
17	HEC	Human Equivalent Concentration
18	IARC	International Agency for Research on Cancer ()
19	IRIS	Integrated Risk Information System
20	IUR	inhalation unit risk
21	LOAEL	Lowest Adverse Effect Level
22	MMS	Methylmethanesulfonate
23	MOA	mode of action
24	MOE	Margin of Exposure
25	NMA	N-Methylolacrylamide
26	NO	Nitric Oxide
27	NOAEL	No Adverse Effect Level
28	NTP/CERHR	National Toxicology Program
29	PBPK	physiologically-based pharmacokinetic
30	PBTK	physiologically-based toxicokinetic
31	PK	Pharmacokinetic
32	POD	point of departure
33	RfC	reference concentration
34	RfD	reference dose
35	TP	tumor promoter
36	UF	uncertainty Factor
37	WHO	World Health Organization
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10/1/08 Draft

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This draft SAB panel report has been prepared for quality review  
and approval of the chartered SAB.

This report does not represent EPA policy

1 APPENDIX A

CHARGE QUESTIONS



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

OFFICE OF  
RESEARCH AND DEVELOPMENT

February 4, 2008

**MEMORANDUM**

**SUBJECT:** Request for SAB review of the Draft IRIS Assessment for Acrylamide

**FROM:** Ila Cote, Ph.D., Acting Director  
National Center for Environmental Assessment, Research Triangle Park (B243-01)  
Office of Research and Development

**TO:** Sue Shallal, Ph.D.  
Designated Federal Officer  
EPA Science Advisory Board Staff Office (1400F)

This is to request a review by the Science Advisory Board of the draft document entitled "Toxicological Review of Acrylamide (CAS No. 79-06-1)" in support of summary information on the Integrated Risk Information System (IRIS). This document is an assessment of the potential for cancer and noncancer effects following exposure to acrylamide. The Toxicological Review of Acrylamide was prepared by the National Center for Environmental Assessment (NCEA), which is the health risk assessment program in the Office of Research and Development. The document has been made available for public comment on the Agency's NCEA web site at the following URL:  
<http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=187729>

The Toxicological Review of Acrylamide broadly supports activities authorized in the 1990 Clean Air Act and is applicable to the information and regulatory needs of all program Offices and Regions in evaluating the cancer and noncancer effects following exposure to acrylamide. EPA last published an assessment of the potential hazardous effect of acrylamide in 1988. The current assessment reviews more recent data and applies more recent methodology for deriving toxicity values.

Attached are the charge questions to the Science Advisory Board that provide background information as well as the questions and issues that are to be the focus of the Science Advisory Board's consultation on this assessment.

Attachment: Charge for EPA's Science Advisory Board (SAB) - IRIS Toxicological Review of Acrylamide

## Charge Questions

### Selection of Studies and Endpoints for the Oral Reference Dose (RfD)

In the draft document, the proposed most sensitive noncancer effect from exposure to acrylamide is neurotoxicity. This endpoint is based on an extensive database of animal and human studies. The next most sensitive effect is reproductive toxicity, which was in the 3-5 fold higher exposure range for a no effect response in animal studies. No human data were identified for acrylamide related reproductive effects. Heritable germ cell effects, a potentially serious noncancer effect, have been observed in male mice, however, the lowest dose levels tested are considerably higher (two orders of magnitude) than the doses where neurotoxicity were observed, and there is uncertainty about the shape of the low-dose-response relationship.

1. Please comment on the selection of neurotoxicity as the most appropriate choice for the most sensitive endpoint (in contrast to reproductive toxicity, heritable germ cell effects, or other endpoint) based upon the available animal and human data.
2. Please comment on the discussion of mode of action for acrylamide-induced neurotoxicity. Is the discussion clear, transparently and objectively described, and accurately reflective of the current scientific understanding?
3. Please comment on the qualitative discussion of acrylamide's heritable germ cell effects and whether the discussion is clear, transparently and objectively described, and reflective of the current science.

### Derivation of the Reference Dose (RfD)

The proposed RfD (0.003 mg/kg-day) for acrylamide is based on a benchmark dose analysis of the dose-response relationship for neurotoxicity in two chronic drinking water exposure bioassays using Fischer 344 rats. Uncertainty factors and a PBPK model are used to extrapolate the animal dose-response to a human equivalent dose-response in the derivation of the RfD.



4. Please comment on whether the selection of the Friedman et al., (1995) and Johnson et al., (1986) studies as co-principal studies has been scientifically justified. Although EPA considers Friedman et al. and Johnson et al. to be co-principal studies, the final quantitative RfD value is derived only from the Johnson study. Please comment on this aspect of EPA's approach. Please also comment on whether this choice is transparently and objectively described in the document. Please identify and provide the rationale for any other studies that should be selected as the principal study(s).
5. Please comment on the benchmark dose methods and the choice of response level used in the derivation of the RfD, and whether this approach is accurately and clearly presented. Do these choices represent the most scientifically justifiable approach for modeling the slope of the dose-response for neurotoxicity? Are there other response levels or methodologies that EPA should consider? Please provide a rationale for alternative approaches that should be considered or preferred to the approach presented in the document.
6. Please comment on the selection of the uncertainty factors (other than the interspecies uncertainty factor) applied to the point of departure (POD) for the derivation of the RfD. For instance, are they scientifically justified and transparently and objectively described in the document? [Note: This question does not apply to the interspecies uncertainty factor which is addressed in the questions on the use of the PBPK model (see PBPK model questions below)]
7. Please provide any other comments on the derivation of the RfD and on the discussion of uncertainties in the RfD.

#### **Use of a PBPK Model in the Derivation of the RfD and the Inhalation Reference Concentration (RfC)**

A physiologically-based toxicokinetic (PBTK) model originally developed by Kirman et al. (2003), and recalibrated by EPA with more recent kinetic and hemoglobin binding data in rats, mice, and humans (Boettcher et al., 2005; Doerge et al., 2005a,b; Fennell et al., 2005) was used in the derivation of the RfD to extrapolate from the animal dose-response relationship (observed in the co-principal oral exposure studies for neurotoxicity) to derive a human equivalent

1 concentration (HEC). The HEC is the external acrylamide exposure level that would produce the  
2 same internal level of parent acrylamide (in this case the area under the curve [AUC] of  
3 acrylamide in the blood) that was estimated to occur in the rat following an external exposure to  
4 acrylamide at the level of the proposed point of departure, and related to a response level of 5%  
5 (i.e., the BMDL<sub>5</sub>). The model results were used in lieu of the default interspecies uncertainty  
6 factor for toxicokinetics differences of 10<sup>1/2</sup>, which left a factor of 10<sup>1/2</sup> (which is rounded to 3)  
7 for interspecies differences in toxicodynamics.

8  
9 With respect to the RfC, there are presently insufficient human or animal data to directly derive  
10 an RfC for acrylamide. The PBPK model was thus used to conduct a route-to-route extrapolation  
11 (oral-to-inhalation) to derive an RfC based on the dose-response relationship observed in the co-  
12 principal oral exposure studies for neurotoxicity. In this case, the HEC was based on a  
13 continuous inhalation exposure to acrylamide in the air that would yield the same AUC for the  
14 parent acrylamide in the blood as that estimated for the rat following an external oral exposure to  
15 acrylamide at the level of the proposed point of departure (i.e., the BMDL<sub>5</sub>).

- 16  
17 8. Please comment on whether the documentation for the recalibrated Kirman et al. (2003)  
18 PBTK model development, evaluation, and use in the assessment is sufficient to determine if  
19 the model was adequately developed and adequate for its intended use in the assessment.  
20 Please comment on the use of the PBTK model in the assessment, e.g., are the model  
21 structure and parameter estimates scientifically supportable? Is the dose metric of area-  
22 under-the-curve (AUC) for acrylamide in the blood the best choice based upon what is  
23 known about the mode of action for neurotoxicity and the available kinetic data? Please  
24 provide a rationale for alternative approaches that should be considered or preferred to the  
25 approach presented in the document.
- 26 9. Is the Young et al. (2007) PBTK model adequately discussed in the assessment with respect  
27 to model structure, parameter values, and data sets used to develop the model? Do you agree  
28 with the conclusion (and supporting rationale) that the recalibrated Kirman et al. (2003)  
29 model (model structure and parameter values presented in the Toxicological Review)  
30 currently represents the best model to use in the derivation of the toxicity values?

- 1 10. According to US EPA's RfC Methodology (1994), the use of PBTK models is assumed to  
2 account for uncertainty associated with the toxicokinetic component of the interspecies  
3 uncertainty factor across routes of administration. Does the use of the PBTK model for  
4 acrylamide objectively predict internal dose differences between the F344 rat and humans, is  
5 the use of the model scientifically justified, and does the use of the PBTK reduce the overall  
6 uncertainty in this estimate compared to the use of the default factor? Are there sufficient  
7 scientific data and support for use of this PBTK model to estimate interspecies toxicokinetic  
8 differences and to replace the default interspecies factor for toxicokinetic differences (i.e.,  
9  $10^{1/2}$ )? Is the remaining uncertainty factor for toxicodynamic differences scientifically  
10 justified, appropriate and correctly used?
- 11 11. Please comment on whether the PBTK model is adequate for use to conduct a route-to-route  
12 extrapolation for acrylamide to derive an RfC in the absence of adequate inhalation animal or  
13 human dose-response data to derive the RfC directly. Was the extrapolation correctly  
14 performed and sufficiently well documented?
- 15 12. Please provide any other comments on the derivation of the RfC and on the discussion of  
16 uncertainties in the RfC.

17  
18 **Margin of Exposure (MOE) Analysis**  
19

20 IRIS documents do not include exposure assessments, which precludes the ability to conduct a  
21 Margin of Exposure (MOE) analysis. It has been suggested, however, that the acrylamide  
22 assessment include a Table that lists points of departure for various endpoints to facilitate a MOE  
23 evaluation by EPA's Regional or Program offices, or by other end users of the assessment.

- 24
- 25 13. Would you suggest that EPA include a Table that lists points of departure (e.g., NOAELs,  
26 BMDs, etc.) for various endpoints that could be used, in conjunction with exposure  
27 assessments, to conduct a MOE analysis?

28  
29 **Quantitating Heritable Germ Cell Effects**

The Toxicological Review includes a discussion of methods to quantitate the risk for heritable germ cell effects (Section 5.4). The questions below address the uncertainty and utility of the quantitative results.

14. Please comment on the discussion of methods to quantitate the dose-response for heritable germ cell effects as to whether it is appropriate, clear and objective, and reflective of the current science. Has the uncertainty in the quantitative characterization of the heritable germ cell effects been accurately and objectively described?
15. Please comment on the scientific support for the hypothesis that heritable germ cell effects are likely to occur at doses lower than those seen for neurotoxicity? What on-going or future research might help resolve this issue?
16. The risks of heritable germ cell effects (i.e., number of induced genetic diseases per million offspring) for some estimated exposure in workers and the population are presented in Table 5-11, and are based on the quantitative methods and parameter estimates discussed in Section 5.4 of the Toxicological Review. Please comment on whether or not the quantitation of heritable germ effects should be conducted, the level of uncertainty in the results, if Table 5-11 is useful for risk assessment purposes, and if the RfD should be included in the Table as one of the exposure levels.
17. Do you know of any additional data or analyses that would improve the quantitative characterization of the dose-response for acrylamide-induced heritable germ cell effects? Would these data also support the quantitative characterization of “total” male-mediated reproduction risks to offspring (i.e., lethality + heritable defect)? If data are not available, do you have any recommendations for specific needed studies?

### **Carcinogenicity of Acrylamide**

In accordance with EPA’s 2005 *Guidelines for Carcinogen Risk Assessment* ([www.epa.gov/iris/backgr-d.htm](http://www.epa.gov/iris/backgr-d.htm)), acrylamide is described as *likely to be carcinogenic to humans* based on: (1) significant increased incidences of thyroid tumors in male and female rats, scrotal sac mesotheliomas in male rats, and mammary gland tumors in female rats in two drinking water

1 bioassays; (2) initiation of skin tumors following oral, intraperitoneal, or dermal exposure to  
2 acrylamide and the tumor promoter, TPA, in two strains of mice; and (3) increased incidence of  
3 lung adenomas in another mouse strain following intraperitoneal injection of acrylamide.  
4 Evidence from available human studies is judged to be limited to inadequate.

5  
6 The mechanisms by which acrylamide may cause cancer are poorly understood, but EPA has  
7 determined that the weight of the available evidence supports a mutagenic mode of carcinogenic  
8 action, primarily for the acrylamide epoxide metabolite, glycidamide (GA). Other mode(s) of  
9 action (MOA) have been proposed for the carcinogenicity of acrylamide, but there is less  
10 support.

11  
12 18. Have the rationale and justification for the cancer designation for acrylamide been clearly  
13 described? Is the conclusion that acrylamide is a likely human carcinogen scientifically  
14 supportable?

15 19. Do you agree that weight of the available evidence supports a mutagenic mode of  
16 carcinogenic action, primarily for the acrylamide epoxide metabolite, glycidamide (GA)?  
17 Has the rationale for this MOA been clearly and objectively presented, and is it reflective of  
18 the current science?

19 20. Are there other MOAs that should be considered? Is there significant biological support for  
20 alternative MOAs for tumor formation, or for alternative MOAs to be considered to occur in  
21 conjunction with a mutagenic MOA? Please specifically comment on the support for  
22 hormonal pathway disruption. Are data available on alternate MOAs sufficient to quantitate a  
23 dose-response relationship?

24 21. Two chronic drinking water exposure bioassays in Fischer 344 rats (Friedman et al., 1995;  
25 Johnson et al., 1986) were used to derive the oral slope factor, and to identify the tumors of  
26 interest for the MOA discussion. Are the choices for the studies, tumors, and methods to  
27 quantify risk transparent, objective, and reflective of the current science? Do you have any  
28 suggestions that would improve the presentation or further reduce the uncertainty in the  
29 derived values?

- 1 22. The cancer slope factor (CSF) derivation includes an adjustment for early mortality (i.e.,  
2 time-to-tumor analysis). Is this adjustment scientifically supported in estimating the risk from  
3 the 2-year bioassay data for increased incidence of tumors in the rats?
- 4 23. The dose metric used in the PBTK model analysis to derive the human equivalent  
5 concentration was area under the curve (AUC) in the blood for the putative genotoxic  
6 metabolite, glycidamide. Please comment on whether AUC for glycidamide is the best  
7 choice of the dose metric in estimating the human equivalent concentration to derive the oral  
8 slope factor. If other dose metrics are preferable, please provide the scientific rationale for  
9 their selection.
- 10 24. As with the RfC, there were insufficient cancer inhalation data to derive an inhalation unit  
11 risk (IUR). The PBTK model was used in a route-to-route extrapolation of the dose-response  
12 relationship from the oral data, and to estimate the human equivalent concentration for  
13 inhalation exposure to acrylamide. Please comment on whether this extrapolation to derive  
14 the inhalation unit risk was correctly performed and sufficiently well documented.
- 15 25. The recommendation to use the age-dependent adjustment factors (ADAFs) is based on the  
16 determination of a mutagenic MOA for carcinogenicity. Is this recommendation scientifically  
17 justifiable and transparently and objectively described
- 18 26. Please provide any other comments on the CSF or IUR, and on the discussion of  
19 uncertainties in the cancer assessment.

## **APPENDIX B      Proposed MOAs for Acrylamide Neurotoxicity**

The following text on the two proposed MOAs for acrylamide neurotoxicity was written by one panel member. It is offered for the Agency's consideration in writing the revised version of the acrylamide IRIS document:

### **1. Disruption of Nitric Oxide (NO) Signaling at the Nerve Terminal Hypothesis**

Acrylamide is a conjugated  $\alpha,\beta$ -unsaturated carbonyl derivative in the type-2 alkene chemical class. Because electrons in pi orbitals of a conjugated system are mobile, the  $\alpha,\beta$ -unsaturated carbonyl structure of acrylamide is characterized as a soft electrophile according to the hard-soft, acid-base principle (reviewed in Pearson, 1967). Characteristically, soft electrophiles will preferentially form Michael-type adducts with soft nucleophiles, which in biological systems are primarily sulfhydryl groups on cysteine residues (Hinson and Roberts, 1992; LoPachin and DeCaprio, 2005). Free sulfhydryl groups can exist in the reduced thiol-state or in the anionic thiolate-state and recent research indicates that the highly nucleophilic thiolate is the preferential adduct target for acrylamide (LoPachin et al., 2007b; see also Friedman et al., 1995). Based on the pKa of cysteine (pH 8.5), at physiological pH (7.4) the thiolate state exists only in unique protein motifs called catalytic triads, where proton shuttling through an acid-base pairing of proximal amino acids (e.g., aspartic acid and lysine) regulates the protonation and deprotonation of the cysteine sulfhydryl group. Indeed, both mass spectrometric and kinetic data have demonstrated the selective adduction of cysteine residues on many neuronal proteins (Barber and LoPachin, 2004; Barber et al., 2007). Furthermore, it is now recognized that the redox state or nucleophilicity of cysteine sulfhydryl groups within catalytic triads can determine the functionality of these proteins (reviewed in LoPachin and Barber, 2006; Stamler et al., 2001). In contrast to acrylamide, the epoxide metabolite glycidamide (Gly), is a hard electrophile that preferentially forms adducts with hard nucleophiles such as nitrogen, carbon and oxygen. Nucleotide residues of DNA contain abundant hard nucleophilic targets, which is consistent with the formation of glycidamide adducts on adenine and guanine bases in acrylamide-intoxicated animals (Doerge et al., 2005; reviewed in Besaratinia and Pfeifer, 2007).

Based on the observation that the processes affected (e.g., neurotransmitter release and storage) and corresponding kinetics ( $K_m$ ,  $V_{max}$ ) were similar in synaptosomes exposed in vitro to acrylamide and those isolated from acrylamide-intoxicated rats (Barber and LoPachin, 2004; LoPachin et al., 2004, 2006), LoPachin and colleagues have reasoned that the parent compound, acrylamide, is responsible for neurotoxicity. Moreover, cysteine thiolate groups have clear regulatory functions in many critical neuronal processes (LoPachin and Barber, 2006), whereas protein valine, lysine and histidine residues, which are the likely hard nucleophilic targets for a hard electrophile such as Gly, have unclear functional and therefore toxicological relevance. Quantitative morphometric and silver stain analyses of PNS and CNS of acrylamide-intoxicated animals have shown that axon degeneration was an epiphenomenon related to dose-rate; i.e., degeneration occurred at lower but not higher dose-rates. In contrast, nerve terminal degeneration occurred regardless of dose-rate and in correspondence with the onset and development of neurological deficits (Crofton et al., 1996; Lehning et al., 1998, 2002a,b, 2003; reviewed in LoPachin et al., 1994, 2002, 2003), suggesting the nerve terminals as a primary site of action. Subsequent neurochemical studies showed that both in vitro and in vivo acrylamide exposure produced early disruptions of neurotransmitter release, reuptake and vesicular storage (Barber and LoPachin, 2004; LoPachin et al., 2004, 2006, 2007a). Further, proteomic analyses indicated that the inhibition of presynaptic function was due to the formation of cysteine adducts on proteins that regulate neurotransmitter handling; e.g., Cys 264 of *N*-ethylmaleimide sensitive factor, Cys 254 of v-ATPase (see Barber and LoPachin, 2004; Barber et al., 2007; Feng and Forgac, 1992; LoPachin et al., 2007a,b, 2008b; reviewed in LoPachin and Barber, 2006). The anionic sulfhydryl state, which is only found in the catalytic triads of regulatory proteins, is an acceptor for nitric oxide (NO) and, therefore, has lead to the proposal that acrylamide-induced neurotoxicity results from disruption of neuronal NO signaling (LoPachin and Barber, 2006; LoPachin et al., 2008a).

## 2. Fast Axonal Transport Disruption Hypothesis

Another proposed MOA is that both acrylamide and Gly inhibit the movement of materials in fast axonal transport (Sickles et al., 2002). According to the “kinesin/axonal transport” hypothesis, toxicant inhibition of kinesin could lead to reductions in the axonal



1 delivery of macromolecules that would eventually produce a deficiency of essential proteins  
2 required to maintain axon structure and/or function. Distal axons and nerve terminals are  
3 particularly vulnerable to transport defects based upon an exceptionally large axonal volume (as  
4 much as 1000 times the volume of the neuron cell body) and the dependence of these distal  
5 regions on long distance transport (100 fold longer length than diameter of the cell body). This  
6 regional sensitivity is consistent with the previously identified distal spatial distribution of  
7 toxicant-induced damage (Cavanagh, 1964).

8         Microtubule motility assays using purified kinesin from bovine brain identified a dose-  
9 dependent inhibition of kinesin as well as a less sensitive effect on microtubules (Sickles *et al.*,  
10 1996). Preincubation of either kinesin or taxol-stabilized microtubules produced a reduction in  
11 the affinity between kinesin and microtubules, recognized as a reduced number of microtubules  
12 bound or locomoting on an adsorbed bed of kinesin. Microtubules that were locomoting did so in  
13 a less directed or staggering type of progression. The inhibitions were due to covalent adduction,  
14 presumably through sulfhydryl alkylation, although adduction of other amino acid residues such  
15 as valine was possible. The non-neurotoxic analogue, propionamide had no effect. Other  
16 investigators have identified kinesin inhibition by sulfhydryl reagents such as N-ethylmaleimide  
17 and ethacrynic acid (Walker *et al.*, 1997). As with acrylamide, inhibition by these sulfhydryl  
18 reagents produced the characteristic staggering movement of microtubules. The reaction was  
19 slow and temperature dependent suggesting a sterically hindered cysteine residue as an important  
20 adduct target. Additional studies have demonstrated a comparable effect of glycidamide on  
21 kinesin (Sickles, unpublished data). The predicted outcome of such an effect would be reduced  
22 quantity of flow, precisely the outcome from several experiments where rate of transport versus  
23 quantity could be discriminated (Sickles, 1989a; Sickles, 1989b; Stone et al., 1999).

24         Fast axonal transport has been studied in a variety of model systems using diverse  
25 techniques. A comprehensive survey of acrylamide effects on fast anterograde and retrograde  
26 axonal transport (Sickles et al., 2002) revealed that all studies measuring fast transport within 24  
27 hours of acrylamide exposure demonstrated significant reductions, whereas longer postexposure  
28 delay was not associated with changes in transport. Furthermore, a reduction in transport  
29 quantity (but not rate) has been reported within 20 minutes of exposure. The duration of this  
30 effect was 16 hours, with full recovery at 24 hours (Sickles, 1991). Quantitation of transport

1 after multiple dosings (i.e. 4, 7 or 10 doses) had a similar effect on transport in the proximal  
2 sciatic nerve (Sickles, 1991). The changes in transport were not due to an effect on protein  
3 synthesis and exposure of only the axons confirmed that the target was axonal (Sickles, 1989a;  
4 Sickles, 1992). Collectively, these results suggested action on a target that is replaced via the fast  
5 transport system, consistent with kinesin. The actions of acrylamide on fast axonal transport  
6 were independent of effects on axonal neurofilaments, as similar reductions were observed in  
7 wild-type and transgenic mice lacking axonal neurofilaments (Stone *et al.*, 1999; Stone *et al.*,  
8 2000). The same results were observed using radiolabelling of proteins in mouse optic nerves  
9 and differential interference microscopy of isolated sciatic nerve axons. Other recent studies  
10 have identified a parallel inhibition of retrograde axonal transport by acrylamide (Sabri and  
11 Spencer, 1990), although it is unclear whether this effect is due to inhibition of cytoplasmic  
12 dynein, the retrograde axonal transport motor, or whether this is a result of indirect effects of  
13 kinesin motor inhibition (Brady *et al.*, 1990).

14 The predicted outcome from axonal transport compromise is a reduction in vital  
15 macromolecules in the distal axons and an accumulation of transported material within the axon.  
16 Morphological studies have consistently identified accumulations of tubulovesicular profiles and  
17 neurofilaments in axons of acrylamide-intoxicated animals (Spencer and Schaumburg, 1991),  
18 which are morphological elements transported via kinesin along microtubules. Other studies  
19 have identified reduced synaptic vesicles in neuromuscular junctions (DeGrandchamp and  
20 Lowndes, 1990; DeGrandchamp *et al.*, 1990). A reduction in GAP-43 in the terminal neurites of  
21 cultured primary spinal cord neurons following acrylamide exposure has been observed (Clarke  
22 and Sickles, 1996). Future studies are required to quantitate reductions in specific axonal  
23 compartments using a variety of neurotoxic and non-neurotoxic dosing regimens in vivo to  
24 confirm the loss of physiologically or structurally important macromolecules.

25 Additional supportive data for the axonal transport hypothesis comes from several studies  
26 of kinesin knockouts as well as similarity to human diseases. While most knockouts are lethal,  
27 low level mutations of kinesin motors in *Drosophila* have identified an identical spatial pattern of  
28 dysfunction and morphological similarity in axonal pathology (Gho *et al.*, 1992; Hurd and  
29 Saxton, 1996) as with acrylamide intoxication. The group of neurological disorders classified as  
30 hereditary spastic paraplegias has a spatial pattern of ataxia, spasticity and muscle weakness as

1 observed with acrylamide intoxication. Some of these types have been associated with mutations  
2 in kinesin motors (Reid *et al.*, 2002), while others are the result of either axonal or glial protein  
3 mutations. However, the common theme is alteration in axonal transport (Reid, 2003; Gould and  
4 Brady, 2004).

#### 5 6 Role of Acrylamide vs. Glycidamide

7       The respective adduct chemistries of acrylamide and glycidamide are well understood  
8 and could have fundamental implications for neurotoxicity regardless of the proposed  
9 mechanism; i.e., kinesin inhibition (Sickles et al., 2002) or blockade of NO signaling (LoPachin  
10 and Barber, 2006; LoPachin et al., 2008). Accordingly, an obvious data gap in the current  
11 mechanistic understanding of acrylamide neurotoxicity, is the relative roles of the parent  
12 compound and Gly. Thus, although early research suggested that Gly produced neurotoxicity  
13 both in whole animal (Abou-Donia et al., 1993) and in vitro (Harris et al., 1994) model systems,  
14 other studies using similar models failed to find neurotoxic effects associated with this  
15 metabolite (Brat and Brimijoin, 1993; Costa et al., 1992, 1995; Moser et al., 1992). Clearly,  
16 resolving the relative roles of acrylamide vs. glycidamide is an important issue that will require  
17 more research. Although the adduct chemistry of these toxicants has been reasonably defined,  
18 the precise molecular mechanisms and sites of neurotoxicity are unknown.