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2
3   **UNITED STATES ENVIRONMENTAL PROTECTION AGENCY**
4   **WASHINGTON D.C. 20460**
5
6   **OFFICE OF THE ADMINISTRATOR**
7   **SCIENCE ADVISORY BOARD**
8
9
10   DATE
11
12
13   Honorable Stephen L. Johnson
14   Administrator
15   U.S. Environmental Protection Agency
16   1200 Pennsylvania Avenue, N.W.
17   Washington, DC 20460
18
19   Subject: Review of EPA's, "Toxicological Review of Acrylamide".
20
21   Dear Administrator Johnson:
22
23   In response to a request from EPA's Office of Research and Development (ORD), the Science
24   Advisory Board (SAB) convened an expert panel to conduct a peer review of EPA's draft
25   Integrated Risk Information System (IRIS) assessment entitled, "*Toxicologic Review of*
26   *Acrylamide*". This draft document updates EPA's current evaluation of the potential health
27   effects of acrylamide.
28
29   The SAB was asked to comment on the hazard characterization and dose-response assessment of
30   acrylamide, including the Agency's selection of the most sensitive health endpoint, the use of a
31   pharmacologically-based toxicokinetic (PBTK) model, the derivation of a proposed oral
32   reference dose (RfD), an inhalation reference concentration (RfC) for non-cancer endpoints, as
33   well as the cancer descriptor, oral slope factor, and inhalation unit risk for acrylamide. The SAB
34   Panel's report contains a number of recommendations that are aimed at making the assessment
35   more transparent and improve the scientific bases for the conclusions presented. The Panel's key
36   points and recommendations are highlighted below.
37
38   The Panel agreed with the EPA's conclusion that based on the existing toxicity data base for
39   acrylamide, neurotoxicity does appear to be the most sensitive endpoint, and therefore, the most
40   appropriate for developing the RfD and RfC for non-cancer health effects. The Panel believed
41   that the use of the benchmark dose methodology in this assessment was deemed scientifically
42   supportable given the nature and robustness of the data sets available on the endpoint of concern.
43   The Panel supported the Agency's conclusions that exposure to acrylamide in animals leads to
44   heritable gene mutations and that these results indicate that it may also pose a hazard to humans.
45   In addition, the Panel supported the Agency's conclusions that the available data on heritable
46   gene mutations are not adequate to conduct a robust assessment of this endpoint at this time. The

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1   Panel urges further research on acrylamide-induced heritable germ cell mutations, given the
2   serious nature of such effects.
3
4   The Panel concluded that the rationale and justification for acrylamide being a "*likely human*
5   *carcinogen*" via a mutagenic mechanism was well described and the conclusion was
6   scientifically supportable, however, it should be further enhanced and amplified. The Panel
7   encouraged the Agency to use the two main chronic bioassays in rats for deriving the oral cancer
8   slope factor and include an in depth discussion of the strengths and limitations of both studies.
9
10 The Panel commends EPA for using the PBTK model for developing the RfD, RfC and cancer
11 slope factor for acrylamide. The Panel notes that the use of internal dose metrics combined with
12 a fairly robust understanding of the mechanism of action may replace the use of the default
13 interspecies factor for toxicokinetic differences. The Panel agreed with the use of PBTK
14 modeling to conduct dose-route extrapolation and commended the EPA for using the PBTK
15 model to fill the gap resulting from the absence of robust animal toxicology studies investigating
16 neurotoxicity via the inhalation route that would support the development of an RfC. Finally, the
17 Panel agreed that the use of the age-dependent adjustment factors (ADAF) to adjust the unit risk
18 for early life exposure is well justified and transparently and objectively described.
19
20 The Panel appreciates the opportunity to provide EPA with advice on this important subject. A
21 more detailed description of the technical recommendations is contained in the body of the
22 report. We look forward to receiving the Agency's response.
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26 Sincerely,
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32 Dr. Deborah Cory-Slechta, Chair   Dr. Granger Morgan, Chair
33 SAB Acrylamide Review Panel   EPA Science Advisory Board
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2   # EXECUTIVE SUMMARY

3   This report was prepared by the Science Advisory Board (SAB) Acrylamide Review

4   Panel (the "Panel") in response to a request by EPA's Office of Research and Development

5   (ORD) to review the Draft IRIS Toxicological Review of Acrylamide (hereafter referred to as

6   the draft document). The Panel deliberated on the charge questions during a March 10-11, 2008

7   face-to-face meeting and discussed its draft report in a subsequent conference call on July 16,

8   2008. There were 26 charge questions that focused on the selection of the most sensitive health

9   endpoint, the use of a PBTK model, the derivation of a proposed oral reference dose (RfD), an

10   inhalation reference concentration (RfC) for non-cancer endpoints, as well as the cancer

11   descriptor, oral slope factor, and inhalation unit risk for acrylamide. This Executive Summary

12   highlights the Panel's major findings and recommendations. The responses that follow represent

13   the views of the Panel.

14

15   *Selection of Endpoint*

16   In the draft document, EPA identified neurotoxicity as the most sensitive non-cancer

17   effect from exposure to acrylamide. This endpoint was based on an extensive database of animal

18   and human studies. Other endpoints were also considered such as, reproductive toxicity and

19   heritable germ cell effects. The Panel agreed that based on the existing toxicity data base for

20   acrylamide, neurotoxicity does appear to be the most sensitive endpoint, and therefore, the most

21   appropriate for developing the RfD and RfC for non-cancer effects from exposure to

22   acrylamide.

23

24   *Mechanism of Action*

25   The Panel discussed two hypotheses regarding the mechanism of acrylamide

26   neurotoxicity; the Panel did not attempt to resolve the debate over a definitive or single MOA for

27   neurotoxicity; however, there was agreement that the discussion of MOA is important for

28   inclusion in the draft document. The Panel found the separation of the discussion of MOA(s) for

29   neurotoxicity in two different sections of the document confusing and recommended their

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1   incorporation into a single section. A more complete presentation by the Panel of these MOAs

2   has been appended to this report for EPA's consideration as they revise their draft document.

3

4   *Derivation of RfD*

5   EPA's proposed RfD (0.003 mg/kg-day) for acrylamide is based on a benchmark dose

6   analysis of the dose-response relationship for neurotoxicity in two chronic drinking water

7   exposure bioassays using Fischer 344 rats. Uncertainty factors and a PBPK model were used to

8   extrapolate the animal dose-response to a human equivalent dose-response in the derivation of

9   the RfD. The Panel afforded considerable discussion to the question of whether the Friedman et

10 al. (1995) and Johnson et al. (1986) studies were the best choices for derivation of the

11 quantitative RfD (and RfC). The main concerns with these studies are that they were primarily

12 designed as cancer bioassays and therefore did not include the most sensitive measures of

13 neurotoxicity. Nevertheless, the Panel agreed that the selected studies did have some important

14 strengths, including reasonable statistical power due to the relatively large number of animals,

15 chronic dosing, and the fact that the NOAELs for the endpoint in the two studies were similar,

16 implying some precision in the effect estimate measured. Several Panel members noted that the

17 lack of sensitive functional/behavioral assessments is a significant data gap that should be

18 considered in the context of setting a database uncertainty factor. Use of the benchmark dose

19 methodology in this assessment was deemed scientifically supported, given the nature and

20 robustness of the data sets available on the endpoint of interest. The calculations and choices

21 made were described clearly at an appropriate level of detail.

22

23 *Heritable Germ Mutations*

24   EPA's draft document concluded that data also exist that reveal acrylamide's capacity to

25 induce heritable germ cell effects at doses somewhat above those at which neurotoxicity has

26 been observed but that there are as yet no studies providing an in-depth examination of dose-

27 response or identification of credible no-effect levels. The Panel supports the Agency's

28 conclusions that exposure to acrylamide in animals leads to heritable gene mutations and that

29 these results indicate that it may also pose a hazard to humans. In addition, the Panel supports

30 the Agency's conclusions that the available data are not yet adequate to conduct a robust

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1   assessment of this endpoint at this time. There is still uncertainty about the mode of action of

2   acrylamide and it metabolite, glycidamide, in the induction of heritable genetic effects. The

3   potential for DNA adducts of glycidamide to play a role is an attractive hypothesis for the mode

4   of action. The Panel found the discussion in the document on heritable germ cell effects useful

5   and presented in a clear, transparent manner reflective of the current science. However, the Panel

6   suggested that, given the serious consequences of heritable germ cell effects, the considerable

7   deficiencies of the database should be identified and the significance of this endpoint

8   emphasized.

9

10 *Physiologically-Based Toxicokinetic (PBTK) modeling*

11   A physiologically-based toxicokinetic (PBTK) model originally developed by Kirman et

12 al. (2003), and recalibrated by EPA with more recent kinetic and hemoglobin binding data in

13 rats, mice, and humans was used in the derivation of the RfD to extrapolate from the animal

14 dose-response relationship to derive a human equivalent concentration. The Panel commends

15 EPA for their efforts to adapt the PBTK model of Kirman et al. (2003) for acrylamide and

16 glycidamide, recognizing that this was a complex and challenging task.   The Panel believes,

17 though, that the documentation is not adequate to determine whether the recalibrated Kirman

18 model is appropriate for its intended use. While the Panel considered that the model structure

19 was reasonable, the parameter estimates require greater justification. The Panel was concerned

20 about the ability of the model to adequately simulate the kinetics of acrylamide and glycidamide.

21 Several alternatives to the PBTK model exist for making the estimates of internal dose in rats

22 needed for both the non-cancer and cancer assessments and for calculating the Human

23 Equivalent Dose (HED).

24

25 *Uncertainty Factors*

26   EPA has proposed to use the default 10X UF to account for intraspecies (i.e., human)

27 differences. The Panel concurred with this choice, noting that there were insufficient data on

28 inter-individual differences, based upon lifestage, gender or genetic characteristics, to support

29 departing from the default. Consensus was not achieved on the issue of the inclusion of an UF to

30 account for deficiencies in the existing database.

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1   EPA has suggested that the acrylamide IRIS document include a Table that lists points of

2   departure for various endpoints to facilitate a Margin of Exposure (MOE) evaluation by EPA's

3   Regional or Program offices, or by other end users of the assessment. The Panel recommends

4   the inclusion of a table in IRIS documents which provides information that may be used to

5   conduct a variety of MOE analyses for specific endpoints of interest and/or for other than

6   lifetime durations of exposure, in addition to the traditional lifetime focus. Agency risk

7   assessments would benefit from the inclusion of transparently-developed, peer-reviewed

8   consensus hazard values.

9

10   *Carcinogenicity*

11   The Panel believes that the rationale and justification for acrylamide being a *"likely*

12   *human carcinogen"* has been well described and the conclusion is scientifically supportable

13   based on the fact that it produces tumors in both sexes, there are multiple tumor sites, and tumors

14   are induced via multiple routes of exposure. Acrylamide is also clearly and reproducibly

15   carcinogenic in both rats and mice. Nonetheless, the discussion should be further enhanced

16   The draft document can be improved by expanding the discussion of biological plausibility and

17   coherence beyond DNA adducts. Significant biological support and data on any putative

18   alternate MOAs are not sufficient to quantitate dose response relationships. More than one MOA

19   may operate for a given carcinogenic chemical, and the likelihood that more than a single MOA

20   is operative increases as levels of exposure increase.

21   EPA used two chronic drinking water exposure bioassays in Fischer 344 rats (Friedman

22   et al., 1995; Johnson et al., 1986) to derive the oral cancer slope factor, and to identify the tumors

23   of interest for the MOA discussion.   The Panel agrees that the two chronic bioassays in F344

24   rats are the main studies to consider in dose response analysis, but the rationale for using only the

25   Friedman et al. study for derivation of the oral cancer slope factor should be improved with the

26   strengths and limitations of both studies discussed in greater depth   The use of the Weibull-in-

27   time multistage-in-dose is a reasonable and scientifically justifiable way to take into account the

28   early mortality in the high dose group in the male study. The decision not to employ this

29   analysis in the case of the female because mortality across treatment and control groups did not

30   differ and the overall survival appears to be fairly good is also reasonable.

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1   The draft document used area under the curve (AUC) in the blood for the putative

2   genotoxic metabolite, glycidamide, as the dose metric for the PBTK model analysis to derive the

3   human equivalent concentration. The Panel agreed that the AUC for glycidamide is the best

4   choice for estimating the human equivalent concentration to derive the oral slope factor. One

5   consideration in using this as the dose metric, however, comes from some of the human studies

6   in which variability is not accounted for adequately. Consideration of additional human data can

7   provide an improved basis for adjustments for cross-species differences in pharmacokinetics, as

8   well as human variability in glycidamide formation from acrylamide.

9

10   *Derivation of the RfC*

11   As with the RfC, EPA concluded that there were insufficient cancer inhalation data to

12   derive an inhalation unit risk (IUR). The PBTK model was used in a route-to-route extrapolation

13   of the dose-response relationship from the oral data, and to estimate the human equivalent

14   concentration for inhalation exposure to acrylamide. The Panel agreed with the use of PBTK

15   modeling to conduct dose-route extrapolation and commended the EPA for using the PBTK

16   model to fill the gap resulting from the absence of robust animal toxicology studies investigating

17   neurotoxicity via the inhalation route that would support the development of an RfC. The Panel

18   agreed that the absence of evidence for route of entry specific effects would allow route-to-route

19   extrapolation for deriving an RfC based on using the PBTK model to calculate the human

20   equivalent concentration (HEC).

21   The Panel agreed that the recommendation to use the age-dependent adjustment factors is

22   well justified and transparently and objectively described.   Additionally the Panel believed that

23   the discussion of uncertainties is adequate, but human variability could be more completely

24   addressed. There is no characterization of sensitive populations, and this could be explored and

25   discussed to a much greater extent.

26

27   The Panel commends EPA for using the PBTK model for developing the RfD, RfC and

28   Cancer Slope Factors for acrylamide. The Panel notes that the use of internal dose metrics

29   combined with a fairly robust understanding of the mechanism of action may replace the use of

30   the default interspecies factor for toxicokinetic differences (i.e., $10^{1/2}$).

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INTRODUCTION

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Background

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4   This report was prepared by the Science Advisory Board (SAB) Acrylamide Review

5   Panel (the "Panel") in response to a request by EPA's Office of Research and Development

6   (ORD) to review the Draft Toxicological Review of Acrylamide (hereafter referred to as the

7   "draft document"). The IRIS Toxicological Review(s) is a compilation and summary of the

8   available information on the potential for cancer and non-cancer hazardous effects in humans

9   from exposure to acrylamide.

10   The SAB was asked to comment on (1) whether the document is logical, clear and

11   concise, (2) if the discussion is objectively and transparently represented, and (3) if it presents an

12   accurate synthesis of the scientific evidence for non-cancer and cancer hazard. The SAB was

13   also asked to identify any additional relevant studies that should be included in the evaluation of

14   the non-cancer or cancer health effects of acrylamide, or in the derivation of toxicity values. In

15   addition, the SAB was asked to provide advice on 26 specific charge questions related to the

16   derivation of a proposed oral reference dose (RfD) and an inhalation reference concentration

17   (RfC) for non-cancer endpoints, as well as the cancer descriptor, oral slope factor, and inhalation

18   unit risk for acrylamide.

19   The Panel deliberated on the charge questions during a March 10-11, 2008, face-to-face

20   meeting and discussed their draft report in a subsequent conference call on July 16, 2008. The

21   responses that follow represent the views of the Panel.   The specific charge questions to the

22   Panel are available in Appendix A.

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RESPONSES TO THE CHARGE QUESTIONS

3

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.

7

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 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 effect on nerve terminals, producing damage in the peripheral and central nerve 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 qualitative experimental approach 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 as it does not correspond physiologically to any of the

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1   hypothesized MOAs. Animal studies indicate that nerve terminal degeneration can occur

2   prior to axonal degeneration at some doses. This would suggest that all of the cited studies,

3   including the subchronic Burek study and the 2 year bioassay studies of sciatic nerve

4   (Friedman et al, 1995) and tibial nerve (Johnson et al, 1986) axons, in looking at axonal

5   degeneration, may have missed a preceding terminal degeneration, particularly as no specific

6   mention of terminal degeneration is provided and functional/behavioral measures of

7   neurotoxicity were not included.

8   3) It should be noted that future studies may demonstrate effects of acrylamide exposure on

9   male reproductive function, as currently evidenced in animal studies by increased pre- and

10   post-implantation losses and decreased litter sizes, at even lower doses than those currently

11   associated with neurotoxicity after acrylamide dosing in animal studies. As noted in the draft

12   document, data also exist that reveal acrylamide's capacity to induce heritable germ cell

13   effects at doses somewhat above those at which neurotoxicity has been observed, but there

14   are as yet no studies providing an in-depth examination of dose response or identification of

15   credible no-effect levels. The draft document states that "associations between human

16   exposure to acrylamide and reproductive effects have not been reported" (p. 187 and p. 224);

17   rather, these associations *have not been studied*. The lack of human data is a major limitation

18   in this regard. The heritable germ cell effects are very worrisome and deserve even more

19   consideration, including perhaps the use of this endpoint to generate an independent RfD.

20   4) Although still controversial and recognizing that cigarette smoke is a complex mixtures made

21   up of hundreds of compounds, there is growing evidence that supports the association

22   between cigarette smoking, a known source of acrylamide exposure, and altered semen

23   parameters, including concentration, morphology, motility, and DNA fragmentation

24   (Richthoff et al., 2008; Sepaniak et al., 2006; Marinelli et al., 2004). The lack of data

25   regarding potential interactions between acrylamide and other exposures, including cigarette

26   smoke, alcohol use, and cosmetics (another source of acrylamide exposure) has been cited as

27   a major limitation in studies of human acrylamide exposure and adverse health effects (Rice

28   2005; draft document p.194; p. 224). The investigation of altered semen parameters among

29   occupationally exposed males, controlling for smoking and alcohol consumption, should be a

30   high priority.

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2 New References

3   Richthoff J, Elzanaty S, Rylander L, Hagmar L, Giwercman A. Association between

4 tobacco exposure and reproductive parameters in adolescent males. Int J Androl 2008;31:31-9.

5   Sepaniak S, Forges T, Foliguet B, Bene MC, Monnier-Barbarino P. The influence of

6 cigarette smoking on human sperm quality and DNA fragmentation. Toxicol 2006;223:54-60.

7   Marinelli D, Gaspari L, Pedotti P, Taioli E. Mini-review of studies on the effect of

8 smoking and drinking habits on semen parameters. Toxicol 2004;207:185-92.

9

10 **Charge Question 2 –** *Please comment on the discussion of mode of action for acrylamide-*

11 *induced neurotoxicity.*

12

13   The Panel found the separation of the discussion of MOA(s) for neurotoxicity in two

14 different sections of the document (Section 4.6.1, pages 123-124; and Section 4.7.3, pages 134-

15 136) confusing and recommends their incorporation into a single section.

16   Currently, there are two hypotheses regarding the mechanism of acrylamide

17 neurotoxicity: 1) Acrylamide/glycidamide inhibits fast axonal transport by forming adducts with

18 kinesin, the transport motor (reviewed in Sickles et al., 2002). 2) Acrylamide disrupts nerve

19 nitric oxide (NO) signaling at the nerve terminal (reviewed in LoPachin et al., 2006). The Panel

20 did not attempt to resolve the debate over the MOA of neurotoxicity; however, the Panel agreed

21 that its ultimate delineation will improve acrylamide risk assessment.

22   Both of the proposed MOAs suggest that visible axonal degeneration on light microscopy

23 is not likely to be the low-dose effect in the causal pathway. It is also possible that both MOAs

24 may be pertinent, and studies directly comparing the time course of the two proposed MOAs in a

25 single model have not been carried out. Regardless, it should also be evident that substantial,

26 detailed molecular information is available regarding mechanisms of acrylamide neurotoxicity

27 and that these data should be included.

28

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

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1   1) As drafted, the document's coverage of research findings is incomplete and does not

2   adequately reflect the current molecular understanding of the mechanisms of acrylamide

3   neurotoxicity. Moreover, information in the document regarding the hypothesized MOAs is

4   not presented in a sufficiently transparent manner consistent with the Agency's guidance on

5   identification of the key events leading to the effect of concern, i.e., use of the modified

6   Bradford Hill criteria with respect to dose-response concordance, temporal relationship(s),

7   strength, consistency, specificity of association and biological plausibility and coherence, as

8   is done for carcinogenicity.

9   2) There was insufficient discussion of acrylamide adduct chemistry and corresponding

10   neuronal targets pertinent to understanding the MOAs.

11   3) There was lack of a discussion of residual questions surrounding the respective roles of the

12   parent toxicant, acrylamide, and its epoxide metabolite, glycidamide, in the production of

13   neurotoxicity.

14

15   The committee recommends that the Agency expand its discussion of the two MOAs.

16 One committee member wrote specific text that more fully elaborates the two proposed MOAs,

17 and offers this text to EPA for consideration in revising the acrylamide assessment. The text is

18 given in Appendix B of this report.

19

20   *Charge Question 3. Please comment on the qualitative discussion of acrylamide's*

21 *heritable germ cell effects and whether the discussion is clear, transparently and objectively*

22 *described, and reflective of the current science.*

23

24   Discussion in the document on heritable germ cell effects, consisting of 5 heritable

25 translocation studies, the 2 specific locus studies, 2 studies on acrylamide transformation to

26 glycidamide and the importance of this metabolism to toxicity, is relevant and useful, and is

27 presented in a clear, transparent manner reflective of the current science. However, the

28 discussion is a linear description of germ cell toxicity with little synthesis, analysis and scrutiny.

29 While some SAB members considered the presentation objective, some expressed concerns over

30 the lack of inclusion of all potential MOAs. Given the serious consequences of heritable germ

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1   cell effects, the considerable deficiencies of the database should be identified and the

2   significance of this endpoint emphasized.

3   The entire section is prefaced and summarized with the perspective that DNA adduct

4   formation and mutagenicity is the only operative mechanism for heritable germ cell effects of

5   acrylamide. While adducts can certainly lead to the observations, there are alternative

6   mechanisms for discussion. Clastogenic mechanisms as well as mitotic spindle defects are

7   viable candidates for dominant lethal effects. There is a wealth of acrylamide studies reporting

8   these alternative mechanisms that should be included in this discussion as well. They were

9   briefly outlined in the carcinogenicity section, but should be identified here. In regards to spindle

10   defects, the effects of acrylamide on kinesin motors involved in cell division should be added to

11   the document. (Sickles et al, 2007).

12   Adequate response data are lacking in the existing heritable germ cell studies such that

13   the shape of the dose response relationship cannot be ascertained. All of the dominant lethal

14   studies were conducted at a dose of 50 mg/kg or higher and most with multiple exposures. The

15   specific locus studies were conducted at 50 mg/kg/d for 5 days (Russel et al, 1991) or with a

16   single 100-125 mg/kg exposure (Ehling and Neuhauser-Klaus, 1992). The discrepancy between

17   the negative results of Russel et al 1991 and the positive results of Ehling and Neuhauser-Klaus,

18   1992 may be dose-related or due to other factors. The fact that heritable translocations appeared

19   at high frequency at the lowest doses tested implies that even lower doses may produce such

20   effects.

21   However, in the absence of these data, the uncertainty should be identified. As a

22   consequence of these limitations in the database, there is some uncertainty related to the RfD.

23   The Panel unanimously agreed that this is an extremely serious data gap that should be a top

24   priority for further study. Additional studies to address the aforementioned database deficiencies

25   in mechanisms and dose-responses would be desirable.

26   The document requires correction in that the NTP/CERHR report was published in

27   February 2005, not 2004. Also, there appears to be a discrepancy in the text (Pg 117 indicates the

28   historical controls were 6%, yet on pg 116 in the discussion of the Adler et al 1994 study, the

29   historical controls are listed as 5/9890 which is 0.05%).

30

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1   *Charge Question 4. Please comment on whether the selection of the Friedman et al, 1995 and*

2   *Johnson et al, 1986 studies as co-principal studies has been scientifically justified. Although*

3   *EPA considers Friedman et al and Johnson et al to be co-principal studies, the final*

4   *quantitative RfD value is derived only from the Johnson study. Please comment on this aspect*

5   *of the EPA's approach. Please comment on whether this choice is transparently and*

6   *objectively described in the document. Please identify and provide the rationale for any other*

7   *studies that should be selected as the principal studies.*

8

9   The Panel afforded considerable discussion to the question of whether the Friedman et al,

10   1995 and Johnson et al, 1986 studies were the best choices for derivation of the quantitative RfD

11   (and RfC). The main concerns with these studies included the fact that they were primarily

12   designed as cancer bioassays and were not designed to evaluate neurotoxicity. Specifically, the

13   Panel contended that the endpoint of axonal degeneration visible under light microscopy is an

14   insensitive measure of neurotoxicity. Alterations visible under electron microscopy or

15   functional/behavioral alterations would have provided more sensitive endpoints.

16   Nevertheless, the Panel agreed that the selected studies did have some important

17   strengths, including reasonable statistical power due to the relatively large number of animals,

18   chronic dosing, and the fact that the NOAELs for the endpoint in the two studies were similar,

19   implying some precision in the effect estimate measured. The Panel also noted that there are no

20   studies yet available which include the sensitive functional/behavioral assessments that would be

21   most desirable. Several Panel members noted that this issue is a significant data gap that should

22   be considered in the context of setting a database uncertainty factor.

23   With respect to the Burek et al. (1980) study, the Panel notes that while the endpoint in

24   this study (axolemmal invaginations under electron microscopy) is a highly sensitive one for use

25   in risk assessment, the study was subchronic. One Panel member proposed that EPA consider

26   generating an RfD based on the data in Burek et al, but not use a subchronic-to-chronic

27   uncertainty factor given the existence of the two chronic studies, to compare the resulting RfD to

28   that based on the less sensitive endpoint of axonal degeneration. Such a comparison might begin

29   to quantify the degree of potential under-estimate of risk due to the less satisfactory choice of

30   endpoint in the Johnson and Friedman studies.

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1   There was a brief discussion of the report of foot splay at 0.5 mg/kg in F_0 males in the

2   Tyl et al. (2000a) two-generation reproductive toxicity/dominant lethal mutation study. The use

3   of this gross functional endpoint could also serve as a point of departure, although it was

4   considered questionable because it only was observed in the F_0 generation, was found in control

5   animals to some degree (raising questions about the methodology used in the lab), and did not

6   follow a clear dose-response relationship. Overall, the Panel decided that the Tyl study was not a

7   good choice for derivation of the RfD.

8   The Panel also considered the option of deriving an RfD based on human data. Both the

9   Calleman et al.(1994) and the Hagmar et al. (2001) studies contain sufficient data to allow the

10   Agency to calculate an RfC or potentially an RfD. In this regard, the Panel made the following

11   observations: (1) in general, it is preferable to use human data when available; (2) the Calleman

12   study included a measure of internal dose (adduct levels) and a fairly sensitive measure of effect,

13   thereby making it appealing for risk assessment; (3) PBTK modeling could allow dose

14   extrapolation based on adduct levels, such that an ingested or inhaled dose could be estimated for

15   purposes of setting either an RfC or an RfD from the data.

16   However, the Panel also cautioned that there are a number of drawbacks to using the

17   human studies, including the following: (1) the sample sizes are small; (2) the samples mostly

18   include young adult males; (3) the healthy worker effect would tend to bias these studies

19   (especially the Calleman study) toward the null, since workers with significant neurological

20   symptoms would leave the workplace, thus selecting for individuals with lower genetic

21   susceptibilities; (4) the workers in each study were exposed to other confounding neurotoxicants

22   (acrylonitrile and NMA), but this would tend to generate a more conservative risk estimate

23   because these other exposures would tend to result in an over-estimate of the effect; and (5) the

24   exposure duration was relatively short and variable (1 month to 11.5 years in the Calleman study

25   with an average of 3 years, and 55 days in the Hagmar study). In the end, the Panel suggested

26   that EPA undergo the exercise of generating an RfD from the Calleman study for purposes of

27   comparison with the RfD derived based on the animal data. The Panel stopped short of

28   recommending that the human RfD be used in place of the one in the draft document, but instead

29   saw this as a type of sensitivity analysis, to help determine whether the RfD based on the

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1   Johnson study appears to be adequately health-protective despite the insensitive endpoint used in

2   that study.

3

4   *Charge Question 5. Please comment on the benchmark dose methods and the choice of*

5   *response level used in the derivation of the RfD, and whether this approach is accurately and*

6   *clearly presented. Do these choices represent the most scientifically justifiable approach for*

7   *modeling the slope of the dose-response for neurotoxicity? Are there other response levels or*

8   *methodologies that EPA should consider? Please provide a rationale for alternative*

9   *approaches that should be considered or preferred to the approach presented in the document.*

10

11   Use of the benchmark dose methodology has become the preferred approach and an

12   acknowledged improvement over the historically traditional NOAEL $\div$ UF procedure for the

13   derivation of RfDs. Its application in this instance is scientifically supported, given the nature

14   and robustness of the data sets available for the endpoint of interest. The calculations and

15   choices made were described clearly at an appropriate level of detail.

16   EPA's Benchmark Dose guidance provides default criteria to be used for selecting the

17   benchmark response (BMR). For quantal data, an excess risk of 10% is the default BMR, since

18   the 10% response is at or near the limit of sensitivity in most studies. In this case, even though

19   the BMR at 10% extra risk also was within the range of observation, the BMR_5 was selected for

20   the point of departure. The choice of a BMR_5 makes sense and is well-justified: (1) the 95%

21   lower bound of the benchmark dose (BMD), $BMDL_5$, remained near the range of observation;

22   (2) the 5% extra risk level is supportable given the relatively large number of animals used in the

23   critical studies; and (3) the use of $BMDL_5$ is consistent with the Agency's technical guidance for

24   BMD analysis which allows flexibility in making such a choice. One of the strengths of the

25   Johnson study is that it is sufficiently large (i.e., numbers of animals/group) to allow the lower

26   5% bound to be identified with sufficient stability that it is usable for risk assessment purposes.

27   Therefore, it is reasonable to use that strength in the underlying data set and choose this number.

28   Such a choice is appropriately conservative (i.e. public health protective).

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1   While alternative approaches such as averaging the BMDLs from each of the four data

2   sets (Friedman and Johnson, male and female) rather than using just the one for males in the

3   Johnson study were discussed, the Panel concluded that the steps described by the Agency in the

4   draft document represented the preferred approach.

5

6   ***Charge Question 6. Please comment on the selection of the uncertainty factors (other than the***

7   ***interspecies uncertainty factor) applied to the point of departure (POD) for the derivation of***

8   ***the RfD. For instance, are they scientifically justified and transparently and objectively***

9   ***described in the document? [Note: This question does not apply to the interspecies uncertainty***

10   ***factor which is addressed in the questions on the use of the PBTK model (see PBTK model***

11   ***questions below)]***

12

13   The Agency has proposed to use a composite uncertainty factor (UF) of 30: 10x to

14   represent human variability (10_H) and 3x to reflect the toxicodynamic component of the default

15   interspecies uncertainty factor (10_A). The other half of the 10x interspecies UF, i.e., the 3x that

16   would otherwise account for interspecies differences in toxicokinetics, is subsumed in the PBTK

17   modeling.

18   Two points were raised about the use of 3X as a default to account for interspecies

19   toxicodynamic differences. It was noted that the rat is less sensitive to the neurotoxic effects of

20   acrylamide than are other mammalian species, including humans. The Panel concluded that

21   while the application of a UF for interspecies toxicodynamics was directionally correct, there

22   was insufficient information available to define a chemical-specific factor. As a result, Panel

23   members did not reach consensus on the use of a 3X UF for interspecies. Secondly, it was noted

24   that recent International Programme for Chemical Safety guidelines divide the default 10_A into

25   2.5X for toxicodynamic differences and 4.0X for toxicokinetics differences, based upon a review

26   of the literature conducted -(WHO IPCS 2005. *Guidance Document for the Use of Data in*

27   *Development of Chemical-specific Adjustment Factors (CSAFs) for Interspecies Differences and*

28   *Human Variability in Dose/Concentration-Response Assessment*). Because EPA was

29   represented on the workgroup developing this approach, one Panel member questioned why it

30   had not been implemented in this case. Another Panel member questioned this, noting that the

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1   use of the factor of 3 (or $\sqrt{10}$) is consistent with current EPA practice: According to the recent

2   EPA (2004) Staff Paper "a default UF of 10 for interspecies variability that can now be reduced

3   to 3 when animal data are dosimetrically adjusted to account for toxicokinetics." The Staff paper

4   cites the EPA (2002) RfD/RfC methodology document. That document divides UFs "into

5   toxicokinetic and toxicodynamic components that have assigned default values of 3.16 ($10^{1/2}$)

6   each."

7   EPA has proposed to use the default 10X UF to account for intraspecies (i.e., human)

8   differences. The Panel concurred with this choice, noting that there were insufficient data on

9   interindividual differences, based upon lifestage, gender or genetic characteristics, to support

10   departing from the default.

11   Consensus was not achieved on the issue of the inclusion on an UF to account for

12   deficiencies in the existing database that would confound the derivation of the most

13   scientifically-defensible RfD. EPA concluded that an $UF_D > 1$ was not necessary, arguing that

14   the existing database is sufficiently robust, even though they acknowledge there are some

15   unresolved issues that warrant further research: describing the MOA(s) for neurotoxicity, the

16   potential for behavioral or functional adverse effects not detected in the assays to date, and the

17   uncertainty that heritable germ cell effects may occur at lower than previously reported doses.

18   Some Panel members agreed with EPA's position. One Panel member noted that additional UFs

19   were implicitly, if not explicitly, incorporated into the RfD derivation. Using the output of the

20   log-logistic model applied to the data set for the male rats in the Johnson study resulted in the

21   lowest set of BMDs/BMDLs. According to one Panel member, it was perhaps conferring an

22   extra UF of ~2X. In addition, using the $BMDL_5$ as the POD, rather than the default $BMDL_{10}$,

23   also could be seen as conferring an extra UF of ~2X.

24   Other Panel members, however, disagreed with the Agency's position regarding the

25   database UF, arguing that the remaining uncertainties have major implications that could result

26   in effects at significantly lower doses and thus a lower RfD. Database deficiencies include the

27   following:

28

29   1) EPA had to rely on the observation of axonal degeneration visible by light microscopy,

30   an endpoint which is not likely to be the most sensitive. EPA is using studies that were

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1   not designed to evaluate neurotoxicity robustly, e.g., histopathology coupled with

2   systematic evaluation of functional or behavioral parameters at multiple time points with

3   robust numbers of animals/treatment and robust number of treatment groups; these

4   studies should be done in adult animals and in a developmental neurotoxicity study in

5   order to determine whether or not critical lifestage differences exist;

6   2) Both existing chronic studies were done in the rat, creating some remaining uncertainty

7   about interspecies differences that is not addressed by the interspecies UF. Based upon

8   the comparison of results from the Tyl et al (2000) 2-generation study in rats and the

9   Chapin et al (1995) 2-generation study in mice, the NOAEL for (adult) neurotoxicity is

10   essentially the same (0.5 mg/kg/day in rats vs. 0.8 mg/kg/day in mice), the difference

11   potentially driven by dose spacing rather than a true difference in response. The

12   outcomes of long-term exposure in mice hold the possibility of yielding lower

13   NOAELs/LOAELs/BMDs than observed/calculated from the rat data. If this were to

14   occur, the RfD/RfC would be lower.

15   3) The germ cell effects have not been fully explored and have major intergenerational

16   implications if they do occur at dose levels lower than those for neurotoxicity. There is a

17   lack of adequate data to define the dose response for heritable germ cell effects. While

18   the existing data describe adverse effects at doses somewhat higher than those at which

19   neurotoxicity was observed, BMD modeling of robust dose-response data may yield

20   results competitive with/lower than the neurotoxicity BMDs/BMDLs.

21

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

23   *the discussion of uncertainties in the RfD.*

24

25   **Acrylamide and Cumulative Risk Assessment**

26   The Food Quality Protection Act (FQPA) of 1996 mandates EPA to consider the

27   "cumulative effects" of pesticides and other substances that have a "common mechanism of

28   toxicity" when setting, modifying or revoking tolerances for food use pesticides. Were

29   acrylamide registered as a food use pesticide, its activity as a type-2 alkene would support a

30   cumulative risk assessment of it and other chemicals in the class. From a scientific standpoint

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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.

10

11

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 $BMDL_5$). The model results were used in lieu of the default*

23   *interspecies uncertainty factor for toxicokinetics differences of $10^{1/2}$, which left a factor of*

24   *$10^{1/2}$ (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*

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1   *following an external oral exposure to acrylamide at the level of the proposed point of*

2   *departure (i.e., the BMDL$_5$).*

3

4   *Charge Question 8. Please comment on whether the documentation for the recalibrated*

5   *Kirman et al. (2003) PBTK model development, evaluation, and use in the assessment is*

6   *sufficient to determine if the model was adequately developed and adequate for its intended*

7   *use in the assessment. Please comment on the use of the PBTK model in the assessment, e.g.,*

8   *are the model structure and parameter estimates scientifically supportable? Is the dose metric*

9   *of area-under-the-curve (AUC) for acrylamide in the blood the best choice based upon what is*

10 *known about the mode of action for neurotoxicity and the available kinetic data? Please*

11 *provide a rationale for alternative approaches that should be considered or preferred to the*

12 *approach presented in the document.*

13

14   Acrylamide is a member of the type-2 alkene chemical class, which includes acrolein,

15 methylvinyl ketone and methyl acrylate.   The type-2 alkenes are a large group of chemicals that

16 share a conjugated α,β-unsaturated carbonyl structure (see LoPachin et al., 2007a). Because pi

17 electrons in a conjugated structure are mobile, these chemicals are soft electrophiles and will,

18 therefore, form adducts with soft nucleophiles, which in biological systems are sulfhydryl groups

19 of cysteine residues (reviewed in LoPachin and Barber, 2006). A weight of evidence evaluation

20 of the current body of data now suggests that the type-2 alkenes produce toxicity via a common

21 molecular mechanism; i.e., formation of adducts with essential sulfhydryl thiolate groups on

22 proteins that play regulatory roles in cellular processes (LoPachin et al., 2007a,b, 2008a;

23 reviewed in LoPachin and Barber, 2006; LoPachin et al., 2008b).

24   The Panel commends EPA for their efforts to adapt the PBTK model of Kirman et al.

25 (2003) for acrylamide and glycidamide, recognizing that this was a complex and challenging

26 task. The modified Kirman et al. model was produced by changing the model initially described

27 for the rat, and adapting it to fit updated data published since the original publication in 2003,

28 and to describe pharmacokinetics in humans.   Three major modifications were described to the

29 partition coefficients for glycidamide, the metabolic rate constants for oxidation and conjugation,

30 and the partition coefficients for acrylamide. The simulations of the modified Kirman model

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1   were presented as tables containing comparisons of AUC data, and the extent of metabolism of

2   acrylamide to glycidamide, and the extent of conjugation of each with glutathione.

3   However, the Panel believed that the documentation is not adequate to determine whether

4   the recalibrated Kirman model is appropriate for its intended use.   Among the items that the

5   Panel would like to see to justify the performance of the model are: the model code; graphical

6   presentation of the data for time course simulations; and graphical presentation of dose response

7   simulated by the model. Side by side comparisons of the model parameters for the rat and

8   human could be accomplished by combining Tables E-4 and E-6.

9   The Panel recognizes that the models were outdated and did not include important recent

10   publications for refinement of the PBTK model. This suggests that more flexible mechanisms

11   are required for modeling efforts that will be used in a regulatory context and subject to multiple

12   reviews. Flexible mechanisms will allow for updating the models based on current literature,

13   addressing reviewer comments, and running simulations that were not initially accomplished.

14   The Panel noted that the model with some changes has been described in a manuscript published

15   in 2007 by Walker et al. If life stage considerations are planned for subsequent work, PBTK

16   modeling is the recommended tool for dosimetry estimates across life stages. The Panel would

17   like to see the model used to simulate or show the degree of consistency with data published

18   since 2005, for example:

19

20   Boettcher MI, Bolt HM, Drexler H, Angerer J (2006). Excretion of mercapturic acids of

21   acrylamide and glycidamide in human urine after single oral administration of deuterium-

22   labelled acrylamide. Arch Toxicol. 80(2):55-61

23
24   Fennell, T. R., Sumner, S. C., Snyder, R. W., Burgess, J. and Friedman, M. A. (2006).

25   Kinetics of elimination of urinary metabolites of acrylamide in humans. Toxicol Sci 93:256-67.

26

27   Tareke, E., Twaddle, N. C., McDaniel, L. P., Churchwell, M. I., Young, J. F., and

28   Doerge, D. R. 2006. Relationships between biomarkers of exposure and toxicokinetics in

29   Fischer 344 rats and B6C3F1 mice administered single doses of acrylamide and glycidamide and

30   multiple doses of acrylamide. Toxicol Appl Pharmacol 217, 63-75.

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1

2   Doerge, D. R., Young, J. F., McDaniel, L. P., Twaddle, N. C., and Churchwell, M. I.

3   2005. Toxicokinetics of acrylamide and glycidamide in Fischer 344 rats. Toxicol Appl

4   Pharmacol 208, 199-209.

5

6   Vesper, H.W., Ospina, M., Meyers, T., Ingham, L., Smith, A., Gray, J.G., Myers, G.L.

7   2006. Automated method for measuring globin adducts of acrylamide and glycidamide at

8   optimized Edman reaction conditions. Rapid Commun Mass Spectrom 20, 959-964.

9

10   Bjellaas, T., Janak, K., Lundanes, E., Kronberg, L., Becher, G. 2005. Determination and

11   quantification of urinary metabolites after dietary exposure to acrylamide. Xenobiotica. 35(10-

12   11):1003-18.

13

14   Fuhr U., Boettcher, M.I., Kinzig-Schippers, M., Weyer, A., Jetter A., Lazar, A., Taubert,

15   D.,Tomalik-Scharte, D., Pournara, P., Jakob V., Harlfinger, S., Klaassen T., Berkessel, A.,

16   Angerer, J., Sorgel, F., Schomig, E. 2006. Toxicokinetics of acrylamide in humans after

17   ingestion of a defined dose in a test meal to improve risk assessment for acrylamide

18   carcinogenicity. Cancer Epidemiol Biomarkers Prev. 15(2):266-71.

19

20   In addition, it was noted that the limited number of human subjects examined in the

21   Fennell et al. study may result in an under-characterization of cross species (e.g., mean human

22   vs. mean rat) and human interindividual pharmacokinetic variability. Also the ratio of valine

23   glycidimide and acrylamide adduct levels among individuals, as reported by Vesper et al. (2006)

24   may help inform this variability.

25   The Panel noted discrepancies between the PBTK predicted and measured critical dose

26   metrics for the non-cancer (acrylamide AUC) or cancer (glycidamide AUC) PODs following

27   drinking water exposures in rats (see table below).

28

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

1

2

3   The draft document notes that the data of Doerge et al. (2005 a,b,c) were available (page

4   E-5), but it is not clear if the data were actually considered in updating the model.

5   While the Panel concluded that the model structure was reasonable, the parameter

6   estimates require greater justification. The review notes (Page E-18 last paragraph) that: "In

7   comparing different versions of the model, it was also noted that the model parameters were

8   underdetermined, that is, there is just not enough basic pharmacokinetic data to derive a unique

9   set of optimal parameter values, given the number of "adjustable" parameters in the current

10   model."

11   The Panel was concerned about the ability of the model to adequately simulate the

12   kinetics of acrylamide and glycidamide. There is little justification presented for the adjustment

13   of parameters from the original Kirman model. The method of optimization was not well

14   described. The comparisons provided between observed data and model simulations are largely

15   for AUC in tables. Thus it is difficult to determine how the model would perform under the kind

16   of tests usually applied to a model, including the ability to fit kinetic data. Table E-4 indicates

17   that while AUC for acrylamide and glycidamide can be simulated reasonably well with the

18   revised rat model, and AM-GSH is reasonably close, the extent of metabolism to GA-GSH is

19   overestimated by 3 fold by the model. Approximately 40% of the urinary metabolites were

20   reported as GA-GSH (Fennell et al., 2005), but the model simulates that 70% would be derived

21   from GA-GSH.

22   Table E-9 indicates that almost 50% of acrylamide is converted to glycidamide in

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1   humans. The Fennell et al. data suggests this may be a high rate of conversion in humans,

2   although the ratio of adduct levels in Vesper et al. suggest a wide range and high conversion for

3   some individuals. The data reported in Fennell et al. (2005) indicate approximately 13.5 % of

4   the urinary metabolites were derived from glycidamide; the Vesper data suggest a considerably

5   higher conversion of acrylamide to glycidamide for some individuals. The model simulations

6   are based on the assumption that all of the acrylamide not accounted for by excretion in urine by

7   24 hours is converted to glycidamide. As noted above, there are data not modeled that could

8   greatly improve the model parameter estimates, using human urine kinetic data for acrylamide,

9   glycidamide and urinary metabolites published by Fennell et al. (2006).   Table E-7 cites the

10   Ratio of GA-GSH to AA-GSH metabolite excretion at low doses reported by Boetcher et al. as

11   0.206 as a data point used for calibration. Yet the model simulation reports a value of 0.733

12   (Table E-9). Still inter-individual differences in pharmacokinetics are expected, given that the

13   levels and activities of enzymes that activate and detoxify acrylamide differ across the

14   population, and it is unclear the degree to which the few healthy human subjects studied in the

15   acrylamide experiments are representative of the general population. For example, recent data

16   (Heudorf et al. 2008; Int J Hyg Environ Health [Epub ahead of print]) suggest that the rate of

17   formation of glycidamide from acryamide may be greater in children. The polymorphisms in

18   CYP2E1 enzyme involved in the activation of acrylamide as well as its variability due to

19   conditions such as diabetes, alcohol consumption and obesity also need to be considered in

20   selecting representative values. The several fold increase in the level of hemoglobin adducts in

21   females compared to male rats suggests gender differences are potentially important; age

22   dependencies were also observed in rats (Sanchez et al. 2008; J Agric Food Chem. 2008 [Epub

23   ahead of print]).

24   The half-life estimated for acrylamide in the model is approximately 5.8 hours and the

25   half-life estimated for glycidamide is approximately 6.1 hours. The half life calculated from

26   urinary excretion rate for acrylamide in humans by Fennell et al. (2006), who studied small

27   groups of healthy infertile adult men, was approximately half this, ranging from 3.13-3.49 hours.

28   The half-life from other studies indicates inter-human differences. For two male subjects, Sorgel

29   et al. (2002) showed half lives of 2 and 7 hours, data from Boettcher et al. is consistent with a

30   half life of 10 hours. One problem in comparisons is the degree of sensitivity for certain urinary

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1   metabolites in the human subject experiments.

2   The issue of adjusting the parameters for partition coefficients and the rates of

3   glutathione conjugation and oxidation is a serious one. It is possible to simulate the same AUC

4   in blood with different model parameters, but with wildly different extents of metabolism and

5   dose to the tissues for acrylamide or glycidamide, by adjusting partition coefficients, and

6   metabolic rate constants. In other words, there may not be unique solutions unless the full body

7   of reported data can be used in model verification. It is exceedingly important to carefully

8   consider the extent of metabolism as a key piece of information in making parameter selections.

9   The description of the parameters and calibration for the human Kirman model are

10   generally presented clearly on pages E-17 and E-18. A possible exception is the very general

11   description of the "iterative process" that was used to evaluate physiologically feasible options to

12   best fit the Fennell et al. (2005) and Boettcher (2005) human data on adult adduct levels and

13   urinary metabolites. A rough comparison of the final rat and human values suggests increased

14   values for a number of tissue binding and metabolic parameters in the human model. Many of

15   these parameters that changed from rat to human increased roughly by a factor of 2 with the

16   exception of the Cytochrome P-450 oxidation rate that decreased by a factor of almost 2.1. It is

17   not clear from the description of the iterative process used to calibrate these values whether the

18   process was designed to force these parameters to move as groups or exactly what logic was

19   employed to adjust these multiple parameters. The general logic behind the iterative testing of

20   permutations of values could be clarified here without going into extreme detail.

21   An alternative approach that should be considered is a re-evaluation of the revised PB-PK

22   model of Kirman. Determining how well it simulates the more recent data, and adjusting the

23   metabolic parameters as necessary is one approach.   Another approach is to apply

24   pharmacokinetic principles to interpret measurements of hemoglobin adducts of acrylamide and

25   glycidamide, to evaluate the degree of formation of acrylamide from glycidamide, and how this

26   may vary across the population.

27   The Panel had an extensive discussion as to whether the dose metric of area-under-the-

28   curve (AUC) for acrylamide in the blood was the best choice based upon what is known about

29   the mode of action for neurotoxicity and the available kinetic data.   A variety of opinions were

30   expressed, ranging from the assertion that AUC for acrylamide in blood was a suitable dose

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1   metric, to the fact that it may not the best choice, but may be expedient. The best choice would

2   be to have compartments for the tissues of interest, and to model the amount of acrylamide

3   and/or glycidamide reaching the tissues. The Kirman model and the modified Kirman model are

4   both limited by the tissue descriptions: liver, lung, blood and a single compartment for

5   remaining tissues.

6   There was extensive discussion among the Panel members about whether the

7   neurotoxicity of acrylamide could clearly be attributed to acrylamide alone, to glycidamide, or to

8   a mixed mode of action.   This question was raised in the review document (Page 136, last full

9   paragraph). Therefore the choice of acrylamide in blood as the dose metric may need to be

10 revisited as this question is clarified.

11   Several alternatives to the PBTK model exist for making the estimates of internal dose in

12 rats needed for both the non-cancer and cancer assessments and for calculating the Human

13 Equivalent Dose (HED). The data available in Doerge et al. (2005) and Tareke et al. (2006)

14 provide measured serum acrylamide and glycidamide AUCs in rats exposed at drinking water

15 concentrations and resulting doses near the PODs. Simple linear extrapolation could be used to

16 calculate the critical internal dose metrics. The data available in Fennell et al. 2005 provides a

17 robust means of converting serum acrylamide or glycidamide AUCs into HEDs. Also,

18 hemoglobin adducts may provide stable indicators of AUC for acrylamide and glycidamide and

19 the fraction of acrylamide transformed to glycidamide, and therefore may provide a robust

20 approach for evaluating internal dose and pharmacokinetic differences across and within species,

21 especially for cases like this where assay exposures are in the range of linear kinetics.

22

23

24 *Charge Question 9. Is the Young et al model adequately discussed relative to structure,*

25 *parameter values and data sets used in the model?*

26

27 The Young et al. paper does not provide citations or values for many of its physiological model

28 parameters. This is an unusual situation for a PBTK modeling paper. For chemical specific

29 model parameter values, the authors fitted the chemical specific model parameter values for each

30 administered dose, creating a model that is calibrated for each dose. This results in an unwieldy

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1   model for use in risk assessment. The preferred approach is to use all the administered dose

2   groups and create a model with one set of chemical specific model parameters that describes all

3   the pharmacokinetic data sets. The model was based on the use of linear terms to describe

4   chemical specific reactions (e.g., binding, DNA adducts, and metabolism). This approach may

5   not hold (and non-linear terms will be needed) when developing one set of chemical specific

6   model parameters to describe the kinetics over a range of doses.

7

8   ***Do you agree with the conclusion that the recalibrated Kirman et al. 2003 model is the best for***

9   ***deriving toxicity values?***

10

11   In the opinion of the Panel, the recalibrated Kirman model was superior to the Young et

12   al. PBTK model. However, the Panel noted that the recalibrated model requires updating to

13   include new data sets in the rat and human. The concerns described in Charge Question 8 need

14   to be addressed to use the recalibrated Kirman et al 2003 model. The Panel also noted that an

15   approach to calculating internal doses at the non-cancer and cancer PODs is available that relies

16   on measured data (and minimal linear extrapolation in a dose range that has been shown to be

17   linear) instead of the PBTK model. This approach also affords the ability to calculate the HED

18   corresponding with the critical internal dose metrics associated with the PODs (see response to

19   question 8). If life stages are considered, the PBTK modeling is the preferred approach for

20   determining a HED or HEC.

21

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1 *Charge Question 10. According to US EPA's RfC Methodology (1994), the use of PBTK*

2 *models is assumed to account for uncertainty associated with the toxicokinetic component of*

3 *the interspecies uncertainty factor across routes of administration. Does the use of the PBTK*

4 *model for acrylamide objectively predict internal dose differences between the F344 rat and*

5 *humans, is the use of the model scientifically justified, and does the use of the PBTK reduce*

6 *the overall uncertainty in this estimate compared to the use of the default factor? Are there*

7 *sufficient scientific data and support for use of this PBTK model to estimate interspecies*

8 *toxicokinetic differences and to replace the default interspecies factor for toxicokinetic*

9 *differences (i.e., $10^{1/2}$)? Is the remaining uncertainty factor for toxicodynamic differences*

10 *scientifically justified, appropriate and correctly used?*

11

12   The Panel commends EPA for using the PBTK model for developing the RfD, RfC and

13 Cancer Slope Factors for acrylamide. The kinetics of acrylamide are well characterized and thus

14 the use of internal dose metrics that are thought to represent the critical dose metrics for non-

15 cancer (neurotoxicity) and cancer (various tumor types) is a preferred approach for extrapolating

16 across species. The Panel agrees that the use of internal dose metrics (calculated using the

17 PBTK model or other pharmacokinetic approaches alluded to above) combined with a fairly

18 robust understanding of the mechanism of action and thus the critical dose metric replaces the

19 use of the default interspecies factor for toxicokinetic differences (i.e., $10^{1/2}$).

20   The Panel agreed with the use of the remaining UFs representing interspecies differences

21 in pharmacodynamics and intraspecies variability in both pharmacokinetics and

22 pharmacodynamics.

23

24 *Charge Question 11. Please comment on whether the PBTK model is adequate for use to*

25 *conduct a route-to-route extrapolation for acrylamide to derive an RfC in the absence of*

26 *adequate inhalation animal or human dose-response data to derive the RfC directly. Was the*

27 *extrapolation correctly performed and sufficiently well documented?*

28

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1   The Panel discussed the lack of inhalation toxicology and PK studies. One Panel

2   member who has conducted inhalation PK exposure studies noted the difficulty with conducting

3   controlled rodent exposure studies and the difficulty in maintaining stable exposure

4   concentrations because of the low volatility of acrylamide and its propensity to sublime.

5   The Panel agreed with the use of PBTK modeling to conduct dose-route extrapolation.

6   Additionally, the Panel commends the EPA for using the PBTK model to fill the gap resulting

7   from the absence of robust animal toxicology studies investigating neurotoxicity via the

8   inhalation route that would support the development of an RfC. The Panel agreed that the

9   absence of evidence for route of entry specific effects would allow route-to-route extrapolation

10   for deriving an RfC by using the PBTK model to calculate the human equivalent concentration

11   (HEC) that would yield an equivalent internal dose (Acrylamide AUC) associated with those

12   achieved at the POD from the oral sentinel (Johnson et al.) studies. The Panel noted that few

13   inhalation PK studies exist to allow a robust parameterization of the inhalation component of the

14   PBTK model for either rats or humans. Despite this, the Panel noted that acrylamide is very

15   water soluble and non-volatile, and the compound has a relatively long half-life. Therefore, the

16   absorption of acrylmide via inhalation should be nearly complete, and first pass effects are

17   negligible, thereby making the pharmacokinetics of acrylamide via inhalation easy to extrapolate

18   from the oral case, using simple principles of pharmacokinetics. The Panel agreed that the

19   application of pharmacokinetic approaches (e.g,. the use of the PBTK model) reduces

20   uncertainty associated with animal to human extrapolation and thus warrants replacing the

21   default UF associated with interspecies extrapolation for pharmacokinetic differences as was

22   done for deriving the RfD.

23   The Panel noted that the air concentration one would derive using the default approach

24   (multiply the HED by body weight [70 kg] and dividing by daily inhalation rate [20 m^3/day]

25   yielding 0.266 μg/m^3) is very similar to the HEC derived using the PBTK model (0.25 μg/m^3).

26   Therefore, if the EPA also decides to provide an extrapolation based on measured data (as

27   described in the response to charge question 8), the default approach of extrapolating from an

28   absorbed oral dose to an equivalent intake from the inhalation route (multiplying by 70 kg and

29   dividing by 20 m^3/day) can be used with confidence to calculate the RfC.

30

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1   *Charge Question 13. Would you suggest that EPA include a Table that lists points of*

2   *departure (e.g., NOAELs, BMDs, etc.) for various endpoints that could be used, in*

3   *conjunction with exposure assessments, to conduct a MOE analysis?*

4

5   To the extent permitted by the available data, the Panel supports the concept of the

6   inclusion of a table in the IRIS acrylamide document which provides information that could be

7   used to conduct a variety of MOE analyses for specific endpoints of interest and/or for other than

8   lifetime durations of exposure, in addition to the traditional lifetime focus. In doing so the

9   magnitude of the MOE that represents a negligible risk should be reported for each point of

10 departure tabulated.

11   Currently, for those environmental agents for which sufficient data exist, IRIS documents

12 will present the derivation of a Reference Dose (RfD) and a Reference Concentration (RfC), as

13 traditionally defined, to be used in the assessment of scenarios which assume that long-term or

14 lifetime exposures are occurring to non-carcinogenic hazards. Additionally, in those cases where

15 the agent of interest has been shown to have carcinogenic potential, an oral cancer slope factor

16 (CSF) and/or an inhalation unit risk (IUR) may be derived, in order to estimate lifetime cancer

17 risks. Whether or not this step is included is determined by a weight-of-evidence evaluation of

18 the body of evidence supporting carcinogenic potential and an understanding, or lack thereof, of

19 the mode(s) of action by which the carcinogenic responses are mediated. These four values (the

20 RfD, RfC, CSF and IUR) are applicable in situations where the assessment is focused on the

21 general population exposed over a lifetime, and may have more limited utility in the assessment

22 of specific subpopulations and/or less-than-lifetime exposure durations.

23   EPA Program and Regional offices and other end-users of IRIS documents often must

24 develop risk assessments for specific populations and/or less-than-lifetime exposure scenarios in

25 order to carry out their respective legislative and regulatory mandates. These risk assessments

26 would benefit from the inclusion of transparently-developed, peer-reviewed consensus hazard

27 values.

28   A comprehensive table would, for example, include NOAELs, LOAELs, BMDs and

29 BMDLs at the 1%, 5% and 10% risk levels (as the default) for those studies deemed the most

30 appropriate for the assessment of specific endpoints and for acute, intermediate and long-term

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1   exposure scenarios, data permitting. It is recognized that it will typically not be possible to fill in

2   every cell for every endpoint and all exposure durations of interest and that a different

3    $BMD_R/BMDL_R$ may better reflect the study's results. Some EPA program offices have extensive

4   experience in the selection of study types and durations that best lend themselves to the

5   assessment of specific endpoints, exposure durations and subpopulations.

6   For this draft acrylamide assessment such a table would display the relevant outcomes of

7   a review of the reliable and well-performed studies which evaluated the potential for

8   neurotoxicity in the adult and developing organism, reproductive toxicity including heritable

9   germ effects, developmental toxicity, and general systemic toxicity following acute, intermediate

10   and long-term exposure, as appropriate.

11

12   ***Charge Question 14. Please comment on the discussion of methods to quantitate the dose-***

13   ***response for heritable germ cell effects as to whether it is appropriate, clear and objective, and***

14   ***reflective of the current science. Has the uncertainty in the quantitative characterization of***

15   ***the heritable germ cell effects been accurately and objectively described?***

16   *[It should be noted that the section under review is 5.5 rather than 5.4. In addition, page 215*

17   *which includes figures 5-2 and 5-2a, was inadvertently omitted in the draft EPA report and thus*

18   *not available for review by the Panel. Correction of this error, however, is not expected to*

19   *impact the recommendations of the Panel on this question as outlined below.]*

20

21   Although reservations were expressed about the lack of data to quantify dose-response, it

22   was the consensus of the Panel that the discussion of the methods should be retained in the

23   report. The report adequately characterizes the current science, reflects historical attempts to

24   estimate these risks and notes that the quantitation methods are based only on the Dearfield et al.

25   (1995) publication. Concerns about the validity of the data and methods are given throughout

26   the section and it is appropriately noted on page 217, " these uncertainties in the assumptions and

27   data gaps warrant further research to improve the usefulness of the following quantitative

28   estimates of risk of acrylamide-induced heritable effects."

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

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1 • The parallelogram models were clearly described and the rationale for the decision to use

2 the modified direct and doubling dose approach appears appropriate.

3 • Clearly, there is considerable uncertainty regarding the validity of the underlying

4 assumptions for these methods and these methods may underestimate risk since they do not take

5 into account all elements that may contribute to the risk.

6 • The extrapolation of exposure is based on animal studies using relatively high dosages

7 (50 to 100 mg/kg or even higher)

8 • The risk extrapolation factors (REFs; pg. 217) should be explained in more detail and

9 information included on how each number is derived (range, etc).

10 • In agreement with the report, given the differences in glycidamide production in different

11 species, an REF of 1 for the metabolic and dose rate variability is likely incorrect. There

12 appears to be significant dose-rate and species-dependent variations in acrylamide metabolism to

13 glycidamide (e.g., see Barber et al., 2001; Fennell and Friedman, 2005).

14 • An REF for uncertainty in the mode of action was recommended since the doubling dose

15 is dramatically higher when generated using specific locus studies which are clearly point

16 mutations (53.1 mg/kg using Ehling and Neuhauser-Klaus, 1992) versus using heritable

17 translocation data that could be based on clastogenic mechanisms (1.8, 3.3, 0.39 mg/kg for

18 Shelby et al 1987, Adler et al 1994 and Adler 1990).

19 • The implementation of the modified direct approach was difficult to understand when, in

20 the absence of the number of human loci capable of mutating to dominantly expressed disease

21 alleles, it was assumed to be 1000. Clarification of how this number was derived would be

22 helpful (i.e. how do we know the number of mutable genes?).

23 • In the doubling dose approach it was not clear how the four data sets, each of which used

24 high acrylamide dosing rates without significant dose ranges, could accurately predict the

25 number of new diseases in the offspring at low doses.

26 Lack of current research in this area is a major concern and little has been done to update the

27 research and data collection based on the Dearfield et al. (1995) methods. The Panel is in

28 agreement with the report that recommends further research and data to fill the critical data gaps

29 and reduce uncertainties including gaps in interspecies extrapolation factors, the quantitative

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1   relationship between genetic alterations in germ cells and heritable disease, and the shape of the

2   low-dose response relationship. Research might include multiple dose studies, including dose

3   selection comparable to that employed in the repeated dose studies which identified

4   neurotoxicity as a critical effect. It is also recommended that impacts on different cell types be

5   determined and that biomonitoring data be utilized in any models developed.

6

7   ***Charge Question15. Please comment on the scientific support for the hypothesis that heritable***

8   ***germ cell effects are likely to occur at doses lower than those for neurotoxicity? What on-***

9   ***going or future research might help resolve this issue?***

10

11   The Panel unanimously agreed that germ cell-induced effects should be taken very

12   seriously, as their implications are highly significant from a public health perspective. There is

13   an absence of data on these effects in lower dose ranges, making it very difficult to speculate

14   about the relevance of this endpoint at or below the dose levels that cause neurotoxicity.

15   Panelists did point out that heritable translocations appeared with very high frequency at the

16   lowest doses tested (i.e., 5 x 40 mg/kg resulted in 24% translocation carriers, Shelby et al, 1987).

17   The high frequency of germ cell effects at these doses implies that these studies were far from

18   identifying a LOAEL or NOAEL, and that there would likely be germ cell effects at much lower

19   doses. However, the combination of lack of testing at lower doses, and the narrow dose range in

20   which testing has been done, makes it very difficult to extrapolate down to a low dose range. The

21   Panel agreed that it is a high priority to extend the heritable translocation studies down into lower

22   dose ranges, and that this information would be very useful for risk assessment once it is

23   completed.

24

25   ***Charge Question 16. The risks of heritable germ cell effects (i.e., number of induced genetic***

26   ***diseases per million offspring) for some estimated exposure in workers and the population are***

27   ***presented in Table 5-11, and are based on the quantitative methods and parameter estimates***

28   ***discussed in Section 5.4 of the Toxicological Review. Please comment on whether or not the***

29   ***quantitation of heritable germ effects should be conducted, the level of uncertainty in the***

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1   *results, if Table 5-11 is useful for risk assessment purposes, and if the RfD should be included*

2   *in the Table as one of the exposure levels.*

3

4   The Panel supports the Agency's conclusions that exposure to acrylamide in animals

5   leads to heritable gene mutations and that these results indicate that it may also pose a hazard to

6   humans. In addition, the Panel supports the Agency's conclusions that the available data are not

7   adequate to conduct a robust assessment of this endpoint at this time.

8   The Panel's deliberations regarding quantifying heritable germ cell mutations centered on

9   the importance of including data such as those presented in Table 5-14 (not Table 5-11, as noted

10 in the final question), the potential significance of these endpoints to human risk assessment, and

11 the paucity of new data developed since the Dearfield et al. (1995) review upon which this

12 section relied heavily (including Table 5-14). A majority of Panel members were supportive of

13 the inclusion of this table in the document and for including the RfD and RfC among the

14 concentrations in the table as this would facilitate comparison with the neurological endpoints.

15 Suggestions also included adding more information into the review regarding the role of CYP

16 2E1 in the dominant lethal effects of acrylamide, which indicated a requirement for metabolism

17 to glycidamide. While the caveats from the Dearfield et al. (1995) review were recapitulated in

18 the document, the Panel discussed the need to further elaborate the limitations in the underlying

19 data and to include reference to the new relevant studies that pertain to uncertainty and dose-

20 response.

21

22 *Charge Question 17. Do you know of any additional data or analyses that would improve the*

23 *quantitative characterization of the dose-response for acrylamide-induced heritable germ cell*

24 *effects? Would these data also support the quantitative characterization of "total" male-*

25 *mediated reproduction risks to offspring (i.e., lethality + heritable defect)? If data are not*

26 *available, do you have any recommendations for specific needed studies?*

27

28 A concern raised by the Panel was that there is a lack of a suitable data set for dose

29 response assessment for acrylamide-induced heritable germ cell effects. The majority of the

30 studies reported have been conducted in mice, using relatively high doses.

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1   Using wild type and Cyp 2E1 knockout mice, it has been demonstrated that oxidation of

2   acrylamide to glycidamide is required for the dominant lethal effect (Ghanayem et al. 2005a) and

3   for the induction of erythrocyte micronuclei and DNA strand breaks in lymphocytes, liver and

4   lung using the Comet assay (Ghanayem et al. 2005b). The greater incidence of heritable

5   translocation carriers in mice administered glycidamide (Generoso et al. 1996) compared with

6   acrylamide (Adler et al. 1994) suggests that glycidamide plays a key role in the mode of action

7   for heritable genetic effects.

8   The risk equivalent factors (REFs, page 217) need to be updated. There are profound

9   differences between rats, mice and humans in the extent of metabolism of acrylamide to

10   glycidamide, and the relative internal dose of acrylamide and glycidamide differs markedly

11   between mice, rats and humans. The extension of the physiologically-based pharmacokinetic

12   modeling approach to include the mouse should be a priority. The blood-testis barrier is thought

13   to contribute to the reduction of internal dose in the testis compared with other tissues for

14   ethylene oxide (Fennell et al., 2001). Testis should be included as a compartment in the model.

15   Data permitting, including the testis as a compartment in the model could potentially improve the

16   dose response characterization for this endpoint.

17   In reviewing data needs (page 220), it is noted that "The estimates do not take into

18   account other potential genotoxic mechanisms such as effects in spermatogonia stem cells,

19   effects in female germ cells, or induction of recessive mutations that would not appear in the first

20   generation, but could lead to additional adverse effects in subsequent generations." Studies to

21   examine the dose response for heritable genetic effects, and the effect of long-term exposure to

22   acrylamide are needed.

23   There is still uncertainty about the mode of action of acrylamide and glycidamide in the

24   induction of heritable genetic effects. The potential for DNA adducts of glycidamide to play a

25   role is an attractive hypothesis for the mode of action. With respect to the possible role for

26   protamine modification in the generation of effects, there was extensive Panel discussion

27   concerning the potential of glycidamide to form adducts with cysteine in proteins and peptides.

28   Adducts to protamine from acrylamide have been identified in late stage spermatids and

29   suggested to mediate the dominant lethal effects (Sega et al. 1989). Whether glycidamide will

30   form similar protamine adducts has not been determined. Surveying populations occupationally

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1   exposed to acrylamide in manufacturing plants was suggested as an approach for evaluation in

2   humans.

3

4   *Charge Question 18. Have the rationale and justification for the cancer designation for*

5   *acrylamide been clearly described? Is the conclusion that acrylamide is a likely human*

6   *carcinogen scientifically supportable?*

7

8   Yes, the rationale and justification has been clearly described, although it should be

9   further expanded (see below), and the conclusion is scientifically supportable. Acrylamide is

10   clearly and reproducibly carcinogenic in both rats and mice. As outlined in the draft document, it

11   produced tumors at multiple sites in the rat in multiple chronic studies, and was a skin tumor

12   initiator in mice by multiple routes. To paraphrase the International Agency for Research on

13   Cancer (IARC) Monographs Preamble, in the absence of tumor data in humans it is both

14   reasonable and prudent to regard evidence of carcinogenicity in experimental animals as

15   evidence for a probable cancer hazard to humans. This conclusion is consistent with both

16   national and international guidelines for carcinogenic hazard identification. The U.S. National

17   Toxicology Program (NTP) has long emphasized that chemicals that cause tumors at multiple

18   sites or in more than a single species are reasonably anticipated to be human carcinogens. Both

19   the NTP and IARC have placed acrylamide in cancer classifications similar to that of EPA's

20   "likely human carcinogen" (This could be noted in the Toxicological Review).

21   Some facts about tumor endpoints in experimental animals need to be emphasized, in

22   light of some comments from the public about the draft document that may reflect

23   misunderstandings. First, when experimental exposure of rats or mice to known human

24   carcinogens is via diet or drinking water, tumor sites observed in those species do not necessarily

25   correspond to tumor sites in humans. Exposure to chemicals that cause tumors of the mammary

26   gland or the liver in mice or rats, for example, does not necessarily correspond to increased

27   cancer risk specifically for female breast or liver in humans. The essential point to be considered

28   is that in any given case a tumor at these or any other site(s) results from an MOA known to

29   operate in humans, such as somatic cell mutagenicity.

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1   Primary CNS tumors as a group, which are discussed at considerable length in the draft

2   document, should be restored to the list of experimental tumors produced by acrylamide and that

3   are of interest for the MOA discussion. It must be emphasized that the diagnostic subcategory of

4   such tumors that was eliminated from the analysis of tumor data in the Friedman et al 1995

5   bioassay, the so-called "malignant reticuloses," are identified only on the basis of their histologic

6   pattern and not by definitive histogenetic criteria that identify their cell of origin. In fact, this

7   pattern overlaps with the histologic pattern of malignant astrocytomas, and the argument that

8   only CNS tumors of unequivocally glial origin should be counted is unjustified. Primary CNS

9   tumors are among the categories of experimental tumors that are only known to be induced by

10   ionizing radiation and by DNA-reactive chemical carcinogens, and their inclusion in the list of

11   acrylamide-induced neoplasms strengthens the weight of evidence for a DNA-reactive MOA for

12   carcinogenicity of acrylamide. The Panel cautions that the viruses that can cause primary CNS

13   tumors in hamsters and other non-human species are not relevant to this discussion.

14   It should be emphasized that the spectrum of tumors consistently seen in acrylamide-

15   exposed rats is completely consistent with a DNA-reactive MOA, based on published data about

16   other substances that induce or initiate the same kinds of neoplasms. The only agents known

17   conclusively to induce tumors of the brain and peritesticular mesothelium in rats are all DNA-

18   reactive, and in fact a single exposure to a direct-acting mutagenic carcinogen has been observed

19   to suffice for tumor induction at either site. The concept that acrylamide acts by a mutagenic

20   MOA is thus supported by the spectrum of acrylamide-associated tumors that occur in exposed

21   rats and mice, as well as by the biotransformation pathway of acrylamide *in vivo*.

22   Tumor initiation – promotion data for mouse skin are perhaps not sufficiently emphasized

23   in the draft document. First, only DNA-reactive chemicals or chemicals biotransformed to

24   DNA-reactive metabolites are established tumor initiators. As acrylamide is an initiator, and by

25   multiple routes of administration, it is a permissible inference that acrylamide is also acting by a

26   DNA-reactive MOA in mouse skin, as do other initiators. It is most striking that, in mice,

27   systemic exposure to acrylamide is more effective for skin tumor initiation than direct

28   application to the skin. The order of efficiency, oral > ip > dermal application, for initiation of

29   TPA-promotable squamous cell papillomas and carcinomas on mouse skin strongly supports the

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1   importance of systemic exposure and post-hepatic distribution of a reactive metabolite in the

2   MOA for carcinogenicity at this site.

3

4   *Charge Question 19. Do you agree that weight of the available evidence supports a mutagenic*

5   *mode of carcinogenic action, primarily for the acrylamide epoxide metabolite, glycidamide*

6   *(GA)? Has the rationale for this MOA been clearly and objectively presented, and is it*

7   *reflective of the current science?*

8

9   A sound rationale and justification already supports the mutagenic MOA, and this

10   evidence is further supported by additional new data as described below. The weight of evidence

11   supports a mutagenic mode of action, and overall the rationale for this mode of action has been

12   clearly and objectively presented. Some improvements to the presentation are as follows. The

13   discussion of biological plausibility and coherence could be expanded beyond DNA adducts and

14   the human relevance section could be somewhat more expansive without being repetitive. The

15   argument on page 145 regarding the lack of relationship of cytogenetic damage to a mutagenic

16   MOA should be carefully re-considered, as the literature is full of these correlations. Evidence

17   for and against the arguments set out should be carefully evaluated, and much better referencing

18   included. Reports from Bonassi and Hagmar are cited as supportive, yet contradictory findings

19   from the same authors supporting an alternative argument could just as easily have been cited.

20   The discussion includes strong generalizations that may not hold up to close scrutiny.

21   A more supportive human post-menopausal breast cancer study was reported in 2007

22   (reference?).   After adjusting for smoking, acrylamide hemoglobin adducts were found to be

23   correlated with tumors (hemoglobin adducts indicating high probability that there is genotoxic

24   damage as well). Although the correlations were not strong, they nevertheless demonstrated a

25   positive association of acrylamide adducts concentration with estrogen receptor positive breast

26   cancer risk in this nested case control study within a prospective cohort study. This study further

27   supports the "likely human carcinogen" status for acrylamide discussed in response to charge

28   question 18.

29   For very high levels of acrylamide exposure, the animal and other experimental data do

30   support a mutagenic effect of acrylamide. It has been questioned whether such a mechanism

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1 might also apply to lower doses (and indeed, at the lowest doses to which humans are exposed),

2 because of uncertainty about whether the compensatory mechanisms are in place to detoxify

3 acrylamide. But data clearly indicate that glycidamide is formed. There are the consistent

4 observations in humans of glycidamide-hemoglobin adducts (Bjellaas et a. 2007; Chevolleau et

5 al. 2007; Vesper et al. 2006, 2007) or glycidamide urinary metabolites (Urban et al. 2006) ,

6 including children (Heudorf et al. 2008), thus demonstrating the widespread internal exposure to

7 the putative mutagenic metabolite of acrylamide at ongoing low levels of exposure in the general

8 population.

9   The Panel did not consider the carcinogenicity to be hormonally-related. The existing

10 short-term mouse studies in SENCAR, ICR (skin) and A/J (lung) show no such selectivity of

11 carcinogenicity for hormonally regulated tissues. Also, the Panel discussed the fact

12 acrylamide/glycidamide is not unique among DNA-reactive epoxides for carcinogenic action in

13 thyroid, peritesticular mesothelium, and mammary tissue (e.g., glycidol, ethylene oxide). In

14 addition, this argument does not consider the CNS tumors observed in both chronic acrylamide

15 cancer bioassays, a site that was discussed by the Panel as representing strong evidence for a

16 DNA-damaging mechanism (cf. Rice, 2005). Finally, a recent publication considered by the

17 Panel of short-term exposures to high doses of acrylamide in male F344 rats found essentially no

18 evidence for hormonal dysregulation in the hypothalamus-pituitary-thyroid axis based on

19 measurements of gene expression, neurotransmitters, hormones, and histopathology (Bowyer et

20 al., 2008)]. Some studies of chronic low dose exposure, such as the cohort study of acrylamide

21 and ovarian/endometrial cancers (Hogervorst et al. 2007) and others (Khan et al. 1999) have

22 shown positive associations with hormones.

23

24 *Charge Question 20. Are there other MOAs that should be considered? Is there significant*

25 *biological support for alternative MOAs for tumor formation, or for alternative MOAs to be*

26 *considered to occur in conjunction with a mutagenic MOA? Please specifically comment on*

27 *the support for hormonal pathway disruption. Are data available on alternate MOAs sufficient*

28 *to quantitate a dose-response relationship?*

29

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1   No, there is not significant biological support for MOA alternatives to the mutagenic

2   MOA, and data on any putative alternate MOAs are not sufficient to quantitate dose response

3   relationships. It must be emphasized that more than one MOA may operate for a given

4   carcinogenic chemical, and the likelihood that more than a single MOA is operative increases as

5   levels of exposure increase. Some well-documented non-DNA reactive MOAs appear to be

6   high-dose phenomena. These are often important for understanding bioassay results in

7   experimental animals, and sometimes for high-exposure situations in human experience, but they

8   are usually less important because they represent negligible risks when cumulative human

9   exposures to these and similarly acting compounds fall considerably below bioassay dosage

10   levels. MOAs that can occur both in experimental rodents and in humans and that operate both

11   at bioassay dosage levels in experimental animals and at lower levels as well, into the human

12   exposure range, are most significant for humans. In general, for chemicals such as acrylamide

13   where there is a compelling body of data to support a DNA-reactive MOA via biotransformation

14   to glycidamide, the evidence for alternative or additional high-dose MOAs would have to be

15   convincing to explore alternative approaches to dose response and risk assessment.

16   Occasionally high-dose or "unique rodent-specific" MOAs may be invoked or postulated

17   to discredit bioassay results as irrelevant to humans, especially when such putative MOAs are

18   observed uniquely in non-human species. Such a postulated MOA needs to be very precisely

19   defined and its relevance thoroughly investigated and critically tested before the postulated MOA

20   is accepted by the biomedical and risk assessment communities. Any MOA developed for a

21   single substance is at best speculative until a general pattern can be rigorously demonstrated for a

22   family of substances that operate via the same MOA. The hormonal disruption MOAs proposed

23   for acrylamide as tissue-specific alternatives to a DNA-reactive MOA are highly speculative, are

24   supported by at most limited evidence, and do not meet this standard as noted in response to

25   charge question 19. The data are insufficient for characterizing dose-response relationships for

26   any of these proposed alternatives.

27

28   *Charge Question 21. Two chronic drinking water exposure bioassays in Fischer 344 rats*

29   *(Friedman et al., 1995; Johnson et al., 1986) were used to derive the oral slope factor, and to*

30   *identify the tumors of interest for the MOA discussion. Are the choices for the studies,*

41

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1   *tumors, and methods to quantify risk transparent, objective, and reflective of the current*

2   *science? Do you have any suggestions that would improve the presentation or further reduce*

3   *the uncertainty in the derived values?*

4

5   The two chronic studies bioassays in F344 rats are the main studies to consider in dose

6   response analysis. Overall the document does a good job discussing these studies, but the

7   rationale for using only the Friedman et al. study for derivation of the oral slope factor is

8   problematic, and the strengths and limitations of both studies should be discussed in greater

9   depth. The text describes the Friedman et al. study as "superior" and "larger and better

10   designed" but the Panel does not agree that this is the case, and recommends that both studies

11   should be subjected to modeling for the purposes of deriving oral slope factors. The two studies

12   may have fairly similar oral slope factors. At a minimum, estimates for the second study should

13   also be presented to clarify the impact of study selection in the uncertainty discussion.

14   The methods to quantify risk are transparently presented and reflective of current science,

15   with the exception that a factor to scale for pharmacodynamic differences in potency between

16   humans and animals has not been applied. The development of unit risk based on HEC accounts

17   for the pharmacokinetic but not pharmacodynamic differences, and in such situations EPA's

18   2005 *Guidelines for Carcinogen Risk Assessment* (p. 3-7) indicates inclusion of a

19   pharmacodynamic factor be considered. The potential human variability in cancer response

20   attributable to human pharmacokinetic variability in handling acrylamide should be discussed

21   qualitatively and analyzed quantitatively. Hemoglobin adduct data could provide the basis for

22   such an analysis. The assumption underlying the modeling is that each and every individual of

23   the same age exposed to the same external dose faces the same risk of cancer is inconsistent with

24   these data.

25   With respect to study selection, one of the reasons for not using the Johnson study had to

26   do with the rates of CNS tumors in this study, particularly in the controls. The Friedman et al.

27   study was designed "to investigate whether glial tumors in the Johnson et al. study were

28   significant." But, as Rice (2005) points out, the histopathological examination for glial tumors

29   was incomplete. Only one-fifth of the 1.0 mg/kg-day dose females' spinal cords were subjected

30   to histopathological examination, even though one-third of the glial tumors in the Johnson et al.

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1 study were seen in the spinal cord. Also excluding malignant reticulosis, given the common

2 features with glial neoplasms, appears odd and raises concerns regarding underreporting in this

3 study. The approach to the evaluation of CNS tumors in Friedman et al. was seen by the Panel

4 as a significant study limitation.

5   Another improvement over the Johnson study noted in the document for the Friedman et

6 al. study was different, presumably better dose spacing. The doses for males in the Friedman et

7 al. and Johnson et al. studies were the same, except Johnson et al. had one additional lower dose

8 group. The doses in Friedman for females were 1.0 and 3.0 mg/kg-day compared to 0.01, 0.1,

9 0.5 and 2.0 mg/kg-day for the Johnson study. The Friedman study did extend the high end of the

10 dose response range for females and did offer a more complete dose response function for

11 thyroid tumors, employed somewhat larger dose groups (100 per group and two control groups).

12 But Johnson et al. did have 60 animals per dose group, did provide a complete histopathological

13 evaluation, and had more dose groups than a standard bioassay.

14   Another limitation of the Friedman et al. study is that the degree of histopathological

15 examination of oral tissue is unclear. The Friedman study does not tabulate findings for certain

16 tumor sites seen in the Johnson study, so quantitative comparisons are not possible and the reader

17 is not able to consider these sites or perform independent evaluations regarding the significance

18 of the findings. It appears EPA may have the data needed to do the analysis since it was able to

19 do a time-dependent analysis for slope estimation using the Tegeris Lab report. EPA could then

20 look at the data and analyze as appropriate the data for these sites.

21   A criticism about the possible impact of a sialodacryoadenitis virus on tumor findings

22 had been raised and was another reason given for using the Friedman study. On the other hand,

23 US FDA had raised some issues in auditing the Friedman et al. study regarding environmental

24 controls at the lab facility and the possibility of some under-dosing of animals. Ultimately both

25 studies have strengths and weaknesses and on balance neither seems clearly superior. Both are

26 reasonably strong studies, and thus oral slope estimates should be presented for both studies.

27   Some comments regarding details on tumor data presentation and analysis in the EPA

28 draft document follow:

29   Tests for dose-related trends should be conducted and presented for the all tabulated sites. By

30 Fisher's exact test, the mammary tumors in the 0.5 mg/kg-d group in the Friedman et al. study

43

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1 are significant (p<0.05). The statistics used in the draft document that correct for intercurrent

2 mortality should be re-checked. It appears this group has a treatment related finding and this

3 should be noted and the discussion that this group is devoid of treatment related tumors (page 75)

4 changed. The clitoral gland findings in the Johnson et al. study stand out because histology was

5 done only on clitoral tissues observed with gross masses. This is worth an explanatory footnote.

6 Also given the approach taken to collecting this tissue, the clitoral tumors in the 0.5 mg/kg dose

7 group also appear worthy of note. All four masses analyzed indicated tumor compared to none in

8 controls (p<0.1). In the Friedman et al. study, CNS tumors of glial origin should be combined for

9 analysis (as was done by WHO 2005. What reference is this? It is not the same as WHO 2005

10 noted under Question # 6). Considering the findings of glial tumors in females in the Johnson

11 study, the dose related trend for both sexes in the Friedman study, although falling a hair short of

12 statistical significance at the $p \leq 0.05$ level, provide some evidence of a CNS glial cell effect in

13 the Friedman study. This should be discussed. Also, the extent of examination of oral tissue in

14 the Friedman study is unclear. Finally, the Friedman study employed two control groups for the

15 male rats that do not differ from one another. For the statistical treatments, there is no apparent

16 reason why these groups should not be combined. The Toxicological Review did this for the

17 dose response analysis but may not have done the same for the pairwise comparisons.

18   The data choice for modeling to address the discrepancy between the Friedman et al. and

19 the Tegeris laboratory reporting of thyroid tumors for the male noted in Appendix D was

20 appropriate. A final minor point, in the discussion of the confidence in dose response analysis in

21 chapter 6 (page 229), issues are raised that seem better placed in the discussion of the hazard

22 characterization.

23

24 *Charge Question 22. The cancer slope factor (CSF) derivation includes an adjustment for*

25 *early mortality (i.e., time-to-tumor analysis). Is this adjustment scientifically supported in*

26 *estimating the risk from the 2-year bioassay data for increased incidence of tumors in the*

27 *rats?*

28

29   The use of the Weibull-in-time multistage-in-dose is a reasonable and scientifically

30 justifiable way to take into account the early mortality in the high dose group in the male study.

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1   The decision not to employ this analysis in the case of the females is also reasonable since

2   mortality across treatment and control groups did not differ and the overall survival appears to be

3   fairly good.

4

5   *Charge Question 23. Please comment on whether AUC for glycidamide is the best choice of*

6   *the dose metric in estimating human equivalent concentration to derive the oral slope factor.*

7

8   The Panel agreed that using the AUC for glycidamide is the best choice for estimating the

9   human equivalent concentration to derive the oral slope factor. This decision was based on the

10   strong evidence from experimental results that the AUC was linearly correlated with adduct

11   levels in single/repeat dosing studies. There was agreement that glycidamide is the more

12   mutagenic metabolite based on experimental studies. The Panel felt there was good

13   documentation in the draft document regarding the correlation between levels of DNA adducts

14   and extent of mutations *in vivo*. Moreover, the metabolic conversion of acrylamide to

15   glycidamide supports the MOA.

16   One consideration in using this as the dose metric, however, comes from some of the

17   human studies in which variability is not accounted for adequately, specifically, inter-individual

18   variation is not assessed. Also, the value used for cross-species comparisons is based on small

19   numbers of healthy adult male humans. This is discussed at greater length in the response to

20   Question 8. Consideration of additional human data (e.g., Vesper et al., 2006) to evaluate the

21   degree humans form glycidamide from acrylamide is clearly warranted. Such data may provide

22   the basis for comparing human acrylamide and glycidamide AUCs, using methodology of

23   Callaman, Bergmark and colleagues. This in turn can provide an improved basis for adjustments

24   for cross-species differences in pharmacokinetics, as well as, human variability in glycidamide

25   formation from acrylamide.

26

27   *Charge Question 24. As with the RfC, there were insufficient cancer inhalation data to derive*

28   *an inhalation unit risk (IUR). The PBTK model was used in a route-to-route extrapolation of*

29   *the dose-response relationship from the oral data, and to estimate the human equivalent*

30   *concentration for inhalation exposure to acrylamide. Please comment on whether this*

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1   *extrapolation to derive the inhalation unit risk was correctly performed and sufficiently well*

2   *documented.*

3

4   The response to this question is nearly identical to the response to charge question #11.

5   The Panel agreed with the use of PBTK modeling to conduct dose-route extrapolation and

6   commended the EPA for using the PBTK model to fill the gap resulting from the absence of

7   robust animal toxicology studies investigating neurotoxicity via the inhalation route that would

8   support the development of an RfC. The Panel agreed that the absence of evidence for route of

9   entry specific effects would allow route-to-route extrapolation for deriving an RfC based on

10 using the PBTK model to calculate the human equivalent concentration (HEC). This would yield

11 an equivalent internal dose (Glycidamide- AUC) associated with those achieved at the point of

12 departure from the oral sentinel (Johnson et al.) studies. The Panel noted that few inhalation PK

13 studies exist to allow a robust parameterization of the inhalation component of the PBTK model

14 for either rats or humans. Despite this, the Panel noted that acrylamide is very water soluble and

15 non-volatile, and the compound has a relatively and first pass effects are negligible, thereby

16 making the pharmacokinetics of acrylamide via long half-life. Therefore, the absorption of

17 acryalmide via inhalation should be nearly complete,inhalation easy to extrapolate from simple

18 principles of phramacokinetics. The Panel agreed that the application of pharmacokinetic

19 approaches (e.g,, the use of the PBTK model) reduces uncertainty associated with animal to

20 human extrapolation and thus warrants replacing the default uncertainty factor associated with

21 interspecies extrapolation for pharmacokinetic differences as was done for deriving the RfD.

22 The use of the PBTK model however does not address cross-species differences in

23 pharmacodynamics, which should be considered, following the Agency's 2005 Guidelines for

24 Carcinogen Risk Assessment.

25   The Panel noted that the air concentration one would derive using the default approach

26 (multiply the HED by body weight [70 kg] and dividing by daily inhalation rate [20 m^3/day]

27 yielding 0.266 $\mu g/m^3$) is very similar to the HEC derived using the PBTK model (0.25 $\mu g/m^3$).

28 Therefore, if the EPA decides to also provide an extrapolation based on measured data (as

29 described in the response to charge question 8), the default approach of extrapolating from an

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1 absorbed oral dose to an equivalent intake from the inhalation route (multiplying by 70 kg and

2 dividing by 20 m^3/day) can be used with confidence to calculate the RfC.

3

4 *Charge Question 25. The recommendation to use the age-dependent adjustment factors*

5 *(ADAFs) is based on the determination of a mutagenic MOA for carcinogenicity. Is this*

6 *recommendation scientifically justifiable and transparently and objectively described?*

7

8   The recommendation to use the age-dependent adjustment factors is well justified and

9 transparently and objectively described. The Panel's deliberations regarding quantifying age-

10 dependent adjustment factors (Section 5.4.6) followed from discussions of a mutagenic mode of

11 action for acrylamide and the typically enhanced sensitivity of fetal and neonatal animals from

12 exposure to such agents in accordance with EPA's Supplemental Guidance for Assessing

13 Susceptibility from Early Life Exposure to Carcinogens (2005b). The Panel also discussed the

14 value of using the PBTK model to evaluate the effect of lifestage on CYP 2E1 and glutathione

15 levels that could modify internal exposure to glycidamide. Such modeling results could be used

16 to reduce the uncertainty associated with lifestage extrapolations and the derivation of age-

17 dependent adjustment factors. Such efforts would be directed at pharmacokinetic aspects of the

18 age-dependent adjustment factors. Uncertainty regarding pharmacodynamics would remain to

19 be addressed by the age-dependent adjustment factors.

20

21 *Charge Question 26. Please provide any other comments on the CSF or IUR, and on the*

22 *discussion of uncertainties in the cancer assessment.*

23

24   The discussion of uncertainties is good, but human variability could be addressed in

25 greater length. It is unclear why in Table 5-13 the consideration/approach is "Method used to

26 protect sensitive populations." There is no characterization of sensitive populations, and this

27 could be explored and discussed to a much greater extent.

28   Specifically, not enough attention was paid to consequences of individual differences in

29 metabolism and cancer risk. Both the CYP2E1 polymorphisms and glutathione transferase(s)

30 (even though rodent data suggests no role for this pathway) polymorphisms could be looked at in

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1   human populations. The degree to which increased activity influences the risk should be

2   considered, including whether this might be tumor site dependent. Also, much weight is put on

3   the two chronic studies in the Fischer 344 rat. The limitations of not having another rodent

4   species should be discussed in more detail with respect to other carcinogens where 2 species

5   were evaluated and similar or different results were found.

6   A factor to scale for toxicodynamic differences between humans and animals was not

7   included in the derivation of the CSF and IUR. The 2005 Carcinogen Risk Assessment

8   Guidelines (see e.g., Guidelines pp 1-13 and 3-7) discusses how toxicodynamics can be

9   addressed by such a factor. The development of unit risk-based on HEC accounts for the

10   toxicokinetic but not toxicodynamic interspecies differences.

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1 **ABREVIATIONS**

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	MOA	mode of action
23	MOE	Margin of Exposure
24	NMA	N-Methylolacrylamide
25	NO	Nitric Oxide
26	NOAEL	No Adverse Effect Level
27	NTP/CERHR	National Toxicology Program
28	PBPK	physiologically-based pharmacokinetic
29	PBTK	physiologically-based toxicokinetic
30	PK	Pharmacokinetic
31	POD	point of departure
32	RfC	reference concentration
33	RfD	reference dose
34	TP	tumor promoter
35	UF	uncertainty Factor
36	WHO	World Health Organization

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APPENDIX A **CHARGE QUESTIONS**

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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

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Charge Questions

3

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

5

6 In the draft document, the proposed most sensitive noncancer effect from exposure to acrylamide

7 is neurotoxicity. This endpoint is based on an extensive database of animal and human studies.

8 The next most sensitive effect is reproductive toxicity, which was in the 3-5 fold higher exposure

9 range for a no effect response in animal studies. No human data were identified for acrylamide

10 related reproductive effects. Heritable germ cell effects, a potentially serious noncancer effect,

11 have been observed in male mice, however, the lowest dose levels tested are considerably higher

12 (two orders of magnitude) than the doses where neurotoxicity were observed, and there is

13 uncertainty about the shape of the low-dose-response relationship.

14

15 1. Please comment on the selection of neurotoxicity as the most appropriate choice for the most

16   sensitive endpoint (in contrast to reproductive toxicity, heritable germ cell effects, or other

17   endpoint) based upon the available animal and human data.

18 2. Please comment on the discussion of mode of action for acrylamide-induced neurotoxicity.

19   Is the discussion clear, transparently and objectively described, and accurately reflective of

20   the current scientific understanding?

21 3. Please comment on the qualitative discussion of acrylamide's heritable germ cell effects and

22   whether the discussion is clear, transparently and objectively described, and reflective of the

23   current science.

24

25 **Derivation of the Reference Dose (RfD)**

26

27 The proposed RfD (0.003 mg/kg-day) for acrylamide is based on a benchmark dose analysis of

28 the dose-response relationship for neurotoxicity in two chronic drinking water exposure

29 bioassays using Fischer 344 rats. Uncertainty factors and a PBPK model are used to extrapolate

30 the animal dose-response to a human equivalent dose-response in the derivation of the RfD.

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2   4. Please comment on whether the selection of the Friedman et al., (1995) and Johnson et al.,

3   (1986) studies as co-principal studies has been scientifically justified. Although EPA

4   considers Friedman et al. and Johnson et al. to be co-principal studies, the final quantitative

5   RfD value is derived only from the Johnson study. Please comment on this aspect of EPA's

6   approach. Please also comment on whether this choice is transparently and objectively

7   described in the document. Please identify and provide the rationale for any other studies

8   that should be selected as the principal study(s).

9   5. Please comment on the benchmark dose methods and the choice of response level used in the

10   derivation of the RfD, and whether this approach is accurately and clearly presented. Do

11   these choices represent the most scientifically justifiable approach for modeling the slope of

12   the dose-response for neurotoxicity? Are there other response levels or methodologies that

13   EPA should consider? Please provide a rationale for alternative approaches that should be

14   considered or preferred to the approach presented in the document.

15 6. Please comment on the selection of the uncertainty factors (other than the interspecies

16   uncertainty factor) applied to the point of departure (POD) for the derivation of the RfD. For

17   instance, are they scientifically justified and transparently and objectively described in the

18   document? [Note: This question does not apply to the interspecies uncertainty factor which is

19   addressed in the questions on the use of the PBPK model (see PBPK model questions

20   below)]

21 7. Please provide any other comments on the derivation of the RfD and on the discussion of

22   uncertainties in the RfD.

23

24 **Use of a PBPK Model in the Derivation of the RfD and the Inhalation Reference**

25 **Concentration (RfC)**

26

27 A physiologically-based toxicokinetic (PBTK) model originally developed by Kirman et al.

28 (2003), and recalibrated by EPA with more recent kinetic and hemoglobin binding data in rats,

29 mice, and humans (Boettcher et al., 2005; Doerge et al., 2005a,b; Fennell et al., 2005) was used

30 in the derivation of the RfD to extrapolate from the animal dose-response relationship (observed

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1 in the co-principal oral exposure studies for neurotoxicity) to derive a human equivalent

2 concentration (HEC). The HEC is the external acrylamide exposure level that would produce the

3 same internal level of parent acrylamide (in this case the area under the curve [AUC] of

4 acrylamide in the blood) that was estimated to occur in the rat following an external exposure to

5 acrylamide at the level of the proposed point of departure, and related to a response level of 5%

6 (i.e., the $BMDL_5$). The model results were used in lieu of the default interspecies uncertainty

7 factor for toxicokinetics differences of $10^{1/2}$, which left a factor of $10^{1/2}$ (which is rounded to 3)

8 for interspecies differences in toxicodynamics.

9

10 With respect to the RfC, there are presently insufficient human or animal data to directly derive

11 an RfC for acrylamide. The PBPK model was thus used to conduct a route-to-route extrapolation

12 (oral-to-inhalation) to derive an RfC based on the dose-response relationship observed in the co-

13 principal oral exposure studies for neurotoxicity. In this case, the HEC was based on a

14 continuous inhalation exposure to acrylamide in the air that would yield the same AUC for the

15 parent acrylamide in the blood as that estimated for the rat following an external oral exposure to

16 acrylamide at the level of the proposed point of departure (i.e., the $BMDL_5$).

17

18 8. Please comment on whether the documentation for the recalibrated Kirman et al. (2003)

19   PBTK model development, evaluation, and use in the assessment is sufficient to determine if

20   the model was adequately developed and adequate for its intended use in the assessment.

21   Please comment on the use of the PBTK model in the assessment, e.g., are the model

22   structure and parameter estimates scientifically supportable? Is the dose metric of area-

23   under-the-curve (AUC) for acrylamide in the blood the best choice based upon what is

24   known about the mode of action for neurotoxicity and the available kinetic data? Please

25   provide a rationale for alternative approaches that should be considered or preferred to the

26   approach presented in the document.

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1   9. Is the Young et al. (2007) PBTK model adequately discussed in the assessment with respect

2   to model structure, parameter values, and data sets used to develop the model? Do you agree

3   with the conclusion (and supporting rationale) that the recalibrated Kirman et al. (2003)

4   model (model structure and parameter values presented in the Toxicological Review)

5   currently represents the best model to use in the derivation of the toxicity values?

6   10. According to US EPA's RfC Methodology (1994), the use of PBTK models is assumed to

7   account for uncertainty associated with the toxicokinetic component of the interspecies

8   uncertainty factor across routes of administration. Does the use of the PBTK model for

9   acrylamide objectively predict internal dose differences between the F344 rat and humans, is

10   the use of the model scientifically justified, and does the use of the PBTK reduce the overall

11   uncertainty in this estimate compared to the use of the default factor? Are there sufficient

12   scientific data and support for use of this PBTK model to estimate interspecies toxicokinetic

13   differences and to replace the default interspecies factor for toxicokinetic differences (i.e.,

14      $10^{1/2}$)? Is the remaining uncertainty factor for toxicodynamic differences scientifically

15   justified, appropriate and correctly used?

16 11. Please comment on whether the PBTK model is adequate for use to conduct a route-to-route

17   extrapolation for acrylamide to derive an RfC in the absence of adequate inhalation animal or

18   human dose-response data to derive the RfC directly. Was the extrapolation correctly

19   performed and sufficiently well documented?

20 12. Please provide any other comments on the derivation of the RfC and on the discussion of

21   uncertainties in the RfC.

22

23 **Margin of Exposure (MOE) Analysis**

24

25 IRIS documents do not include exposure assessments, which precludes the ability to conduct a

26 Margin of Exposure (MOE) analysis. It has been suggested, however, that the acrylamide

27 assessment include a Table that lists points of departure for various endpoints to facilitate a MOE

28 evaluation by EPA's Regional or Program offices, or by other end users of the assessment.

29

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1   13. Would you suggest that EPA include a Table that lists points of departure (e.g., NOAELs,

2   BMDs, etc.) for various endpoints that could be used, in conjunction with exposure

3   assessments, to conduct a MOE analysis?

4

5   **Quantitating Heritable Germ Cell Effects**

6   The Toxicological Review includes a discussion of methods to quantitate the risk for heritable

7   germ cell effects (Section 5.4). The questions below address the uncertainty and utility of the

8   quantitative results.

9

10   14. Please comment on the discussion of methods to quantitate the dose-response for heritable

11   germ cell effects as to whether it is appropriate, clear and objective, and reflective of the

12   current science. Has the uncertainty in the quantitative characterization of the heritable germ

13   cell effects been accurately and objectively described?

14   15. Please comment on the scientific support for the hypothesis that heritable germ cell effects

15   are likely to occur at doses lower than those seen for neurotoxicity? What on-going or future

16   research might help resolve this issue?

17   16. The risks of heritable germ cell effects (i.e., number of induced genetic diseases per million

18   offspring) for some estimated exposure in workers and the population are presented in Table

19   5-11, and are based on the quantitative methods and parameter estimates discussed in Section

20   5.4 of the Toxicological Review. Please comment on whether or not the quantitation of

21   heritable germ effects should be conducted, the level of uncertainty in the results, if Table 5-

22   11 is useful for risk assessment purposes, and if the RfD should be included in the Table as

23   one of the exposure levels.

24   17. Do you know of any additional data or analyses that would improve the quantitative

25   characterization of the dose-response for acrylamide-induced heritable germ cell effects?

26   Would these data also support the quantitative characterization of "total" male-mediated

27   reproduction risks to offspring (i.e., lethality + heritable defect)? If data are not available, do

28   you have any recommendations for specific needed studies?

29

30   **Carcinogenicity of Acrylamide**

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2   In accordance with EPA's 2005 *Guidelines for Carcinogen Risk Assessment*

3   (www.epa.gov/iris/backgr-d.htm), acrylamide is described as *likely to be carcinogenic to humans*

4   based on: (1) significant increased incidences of thyroid tumors in male and female rats, scrotal

5   sac mesotheliomas in male rats, and mammary gland tumors in female rats in two drinking water

6   bioassays; (2) initiation of skin tumors following oral, intraperitoneal, or dermal exposure to

7   acrylamide and the tumor promoter, TPA, in two strains of mice; and (3) increased incidence of

8   lung adenomas in another mouse strain following intraperitoneal injection of acrylamide.

9   Evidence from available human studies is judged to be limited to inadequate.

10

11 The mechanisms by which acrylamide may cause cancer are poorly understood, but EPA has

12 determined that the weight of the available evidence supports a mutagenic mode of carcinogenic

13 action, primarily for the acrylamide epoxide metabolite, glycidamide (GA). Other mode(s) of

14 action (MOA) have been proposed for the carcinogenicity of acrylamide, but there is less

15 support.

16

17 18. Have the rationale and justification for the cancer designation for acrylamide been clearly

18   described? Is the conclusion that acrylamide is a likely human carcinogen scientifically

19   supportable?

20 19. Do you agree that weight of the available evidence supports a mutagenic mode of

21   carcinogenic action, primarily for the acrylamide epoxide metabolite, glycidamide (GA)?

22   Has the rationale for this MOA been clearly and objectively presented, and is it reflective of

23   the current science?

24 20. Are there other MOAs that should be considered? Is there significant biological support for

25   alternative MOAs for tumor formation, or for alternative MOAs to be considered to occur in

26   conjunction with a mutagenic MOA? Please specifically comment on the support for

27   hormonal pathway disruption. Are data available on alternate MOAs sufficient to quantitate a

28   dose-response relationship?

29 21. Two chronic drinking water exposure bioassays in Fischer 344 rats (Friedman et al., 1995;

30   Johnson et al., 1986) were used to derive the oral slope factor, and to identify the tumors of

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1   interest for the MOA discussion. Are the choices for the studies, tumors, and methods to

2   quantify risk transparent, objective, and reflective of the current science? Do you have any

3   suggestions that would improve the presentation or further reduce the uncertainty in the

4   derived values?

5   22. The cancer slope factor (CSF) derivation includes an adjustment for early mortality (i.e.,

6   time-to-tumor analysis). Is this adjustment scientifically supported in estimating the risk from

7   the 2-year bioassay data for increased incidence of tumors in the rats?

8   23. The dose metric used in the PBTK model analysis to derive the human equivalent

9   concentration was area under the curve (AUC) in the blood for the putative genotoxic

10   metabolite, glycidamide. Please comment on whether AUC for glycidamide is the best

11   choice of the dose metric in estimating the human equivalent concentration to derive the oral

12   slope factor. If other dose metrics are preferable, please provide the scientific rationale for

13   their selection.

14   24. As with the RfC, there were insufficient cancer inhalation data to derive an inhalation unit

15   risk (IUR). The PBTK model was used in a route-to-route extrapolation of the dose-response

16   relationship from the oral data, and to estimate the human equivalent concentration for

17   inhalation exposure to acrylamide. Please comment on whether this extrapolation to derive

18   the inhalation unit risk was correctly performed and sufficiently well documented.

19   25. The recommendation to use the age-dependent adjustment factors (ADAFs) is based on the

20   determination of a mutagenic MOA for carcinogenicity. Is this recommendation scientifically

21   justifiable and transparently and objectively described

22   26. Please provide any other comments on the CSF or IUR, and on the discussion of

23   uncertainties in the cancer assessment.

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1   **APPENDIX B   PROPOSED MOAs for Acrylamide Neurotoxicity**

2

3   The text on the two proposed MOAs for acrylamide neurotoxicity was written by one committee

4   member. It is offered for the Agency's consideration in writing the revised version of the

5   acrylamide IRIS document:

6

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

8   Acrylamide is a conjugated α,β-unsaturated carbonyl derivative in the type-2 alkene

9   chemical class. Because electrons in pi orbitals of a conjugated system are mobile, the α,β-

10   unsaturated carbonyl structure of acrylamide is characterized as a soft electrophile according to

11   the hard-soft, acid-base principle (reviewed in Pearson, 1990). Characteristically, soft

12   electrophiles will preferentially form Michael-type adducts with soft nucleophiles, which in

13   biological systems are primarily sulfhydryl groups on cysteine residues (Hinson and Roberts,

14   1992; LoPachin and DeCaprio, 2005). Free sulfhydryl groups can exist in the reduced thiol-state

15   or in the anionic thiolate-state and recent research indicates that the highly nucleophilic thiolate

16   is the preferential adduct target for acrylamide (LoPachin et al., 2007b; see also Friedman et al.,

17   1965; Esterbauer et al., 1991). Based on the pKa of cysteine (pH 8.5), at physiological pH (7.4)

18   the thiolate state exists only in unique protein motifs called catalytic triads, where proton

19   shuttling through an acid-base pairing of proximal amino acids (e.g., aspartic acid and lysine)

20   regulates the protonation and deprotonation of the cysteine sulfhydryl group. Indeed, both mass

21   spectrometric and kinetic data have demonstrated the selective adduction of cysteine residues on

22   many neuronal proteins (Barber and LoPachin, 2004; Barber et al., 2007). Furthermore, it is now

23   recognized that the redox state or nucleophilicity of cysteine sulfhydryl groups within catalytic

24   triads can determine the functionality of these proteins (reviewed in LoPachin and Barber, 2006;

25   Stamler et al., 2001). In contrast to acrylamide, the epoxide metabolite glycidamide (Gly), is a

26   hard electrophile that preferentially forms adducts with hard nucleophiles such as nitrogen,

27   carbon and oxygen. Nucleotide residues of DNA contain abundant hard nucleophilic targets,

28   which is consistent with the formation of glycidamide adducts on adenine and guanine bases in

29   acrylamide-intoxicated animals (Doerge et al., 2005; reviewed in Besaratinia and Pfeifer, 2007).

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1   Based on the observation that the processes affected (e.g., neurotransmitter release and

2   storage) and corresponding kinetics (K_m, V_{max}) were similar in synaptosomes exposed in vitro to

3   acrylamide and those isolated from acrylamide-intoxicated rats (Barber and LoPachin, 2004;

4   LoPachin et al., 2004, 2006), LoPachin and colleagues have reasoned that the parent compound,

5   acrylamide, is responsible for neurotoxicity. Moreover, cysteine thiolate groups have clear

6   regulatory functions in many critical neuronal processes (LoPachin and Barber, 2006), whereas

7   protein valine, lysine and histidine residues, which are the likely hard nucleophilic targets for a

8   hard electrophile such as Gly, have unclear functional and therefore toxicological relevance.

9   Quantitative morphometric and silver stain analyses of PNS and CNS of acrylamide-intoxicated

10   animals have shown that axon degeneration was an epiphenomenon related to dose-rate; i.e.,

11   degeneration occurred at lower but not higher dose-rates. In contrast, nerve terminal

12   degeneration occurred regardless of dose-rate and in correspondence with the onset and

13   development of neurological deficits (Crofton et al., 1996; Lehning et al., 1998, 2002a,b, 2003;

14   reviewed in LoPachin et al., 2002, 2003), suggesting the nerve terminals as a primary site of

15   action. Subsequent neurochemical studies showed that both in vitro and in vivo acrylamide

16   exposure produced early disruptions of neurotransmitter release, reuptake and vesicular storage

17   (Barber and LoPachin, 2004; LoPachin et al, 2004, 2006, 2007a). Further, proteomic analyses

18   indicated that the inhibition of presynaptic function was due to the formation of cysteine adducts

19   on proteins that regulate neurotransmitter handling; e.g., Cys 264 of *N*-ethylmaleimide sensitive

20   factor, Cys 254 of v-ATPase (see Barber and LoPachin, 2004; Barber et al., 2007; Feng and

21   Forgac, 1992; LoPachin et al., 2007a,b, 2008b; reviewed in LoPachin and Barber, 2006). The

22   anionic sulfhydryl state, which is only found in the catalytic triads of regulatory proteins, is an

23   acceptor for nitric oxide (NO) and, therefore, has lead to the proposal that acrylamide-induced

24   neurotoxicity results from disruption of neuronal NO signaling (LoPachin and Barber, 2006;

25   LoPachin et al., 2008a).

26

27   2. Fast Axonal Transport Disruption Hypothesis

28   Another proposed MOA is that both acrylamide and Gly inhibit the movement of

29   materials in fast axonal transport (Sickles et al., 2002). According to the "kinesin/axonal

30   transport" hypothesis, toxicant inhibition of kinesin could lead to reductions in the axonal

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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 absorbed 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

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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

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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 Gly are well understood and could

8   have fundamental implications for neurotoxicity regardless of the proposed mechanism; i.e.,

9   kinesin inhibition (Sickles et al., 2002) or blockade of NO signaling (LoPachin and Barber,

10   2006; LoPachin et al., 2008). Accordingly, an obvious data gap in the current mechanistic

11   understanding of acrylamide neurotoxicity, is the relative roles of the parent compound and Gly.

12   Thus, although early research suggested that Gly produced neurotoxicity both in whole animal

13   (Abou-Donia et al., 1993) and in vitro (Harris et al., 1994) model systems, other studies using

14   similar models failed to find neurotoxic effects associated with this metabolite (Brat and

15   Brimijoin, 1993; Costa et al., 1992, 1995). Clearly, resolving the relative roles of acrylamide vs.

16   Gly is an important issue that will require more research. Although the adduct chemistry of

17   these toxicants has been reasonably defined, the precise molecular mechanisms and sites of

18   neurotoxicity are unknown.

19

20