<u>**Peer Reviewer #1**</u>

*Peer Review on*
**ATSDR Toxicological Profile for Acrylamide**
**Revised Minimal Risk Level Review**

**General Remarks**

The toxicological data on acrylamide have been monographed in the past by a number of official bodies. The present ATSDR Toxicological Profile for Acrylamide represents a very comprehensive, exhaustive and timely description of the data.

A pivotal element in the present Toxicological Profile is the introduction of the most recent PBPK model for acrylamide (Sweeney et al. 2010), which is used for the derivation of MRLs. The advantage of this type of modeling is that interspecies comparisons are based on intrinsic effective doses, which is more science-based than the plain use of an arbitrary toxicokinetic interspecies uncertainty factor. The present procedure uses a remaining interspecies uncertainty factor of 3 for possible variations in toxicodynamics, which appears acceptable. Because of the use of the refined procedure, the Revised MRLs derived now differ somewhat from previous values. The reviewer feels that this avenue of thinking within a regulatory context is a considerable step forward, which is probably paradigmatic for other compounds as well.

**Specific issues**

One point, which relates equally to derivation of all three MRLs, is to be made with regard to application of the factor 10 for human inter-individual variability. The value of 10 is just traditional, with no further scientific reasoning given. Yet, some more discussion on the underlying elements would be desirable. The key metabolic enzymes for acrylamide, as described in 3.4.3, are CYP2E1, GST and mEH. For all of these enzymes, human genetic polymorphisms are known. The most recent study addressing this specifically is that by Huang et al (Toxicol Lett 2011, marked "*" in the reference list of the Toxicological Profile). Also, for CYP2E1 remarkable

differences between human ethnicities in expression of the enzyme are known (review: Int Arch Occup Environ Health 76: 174-185, 2003). This could support the application of an individual difference factor of 10, eventually of an even higher factor. Regrettably, this is not discussed in the Toxicological Profile.

*Response:* The requested discussion of human differences in expression of key metabolic enzymes for acrylamide was added to the profile.

The toxicological key data underlying to this Toxicological Profile are practically identical to those evaluated by other institutions (e.g. the EU Risk Assessment Report, dated 2002). It can be stated that there is international agreement on the relevant toxicological endpoints, as well as on the respective LOAEL/NOAEL figures for acrylamide.

**Rationale, derivation, and clarity of presentation of MRLs**

The present Toxicological Profile does not derive MRLs for inhalation exposure. The justification is that no experimental data on inhalation exposures are available. This is true and must be accepted.

For "*acute-duration*" exposures, an oral MRL of 0.01 mg/kg daily is derived. Related to the incidence of unsuccessful impregnation in Long-Evans rats relevant blood concentrations of both acrylamide and glycidamide were modeled, and the $BMDL_{10}$ was obtained. After introduction of a total uncertainty factor of 30 (3 for interspecies variation, 10 for human variability) the finally resulting MRL was 0.01 mg/kg.

The PBPK procedure was used to model both the blood levels of acrylamide and glycidamide. The higher risk was found with the acrylamide model, which is plausible as there is no straightforward argumentation to connect glycidamide with the fertility effect.

In essence, both the rationale and the derivation are clear.

For derivation of an "*intermediate-duration*" MRL the key reference was the subchronic study of Burek et al (1980), showing the classical peripheral neurotoxic signs of acrylamide, which is well supported by other studies. There is common agreement that on this experimental basis the daily oral doses of 1 mg/kg and 0.2 mg/kg represent the LOAEL and NOAEL, respectively. The NOAEL/LOAEL approach was then used, together with PBPK modeling and application of a total uncertainty factor of 30. The resulting MRL was 0.001 mg/kg per day.

Thus, the longer (subacute *vs.* subchronic) duration of exposure leads to diminishment of the MRL by a factor of 10, which appears plausible.

In essence, both the rationale and the derivation are clearly described.

For derivation of a "*chronic-duration*" MRL the 2-year drinking water study of Friedman et al (1995) was chosen as the key study. Again, the relevant endpoint again was peripheral neuropathy (degenerative changes of the sciatic nerve). A benchmark approach was chosen, along with a total uncertainty factor of 30.

The resulting MRL was 0.001 mg/kg per day, the same as for "intermediate-duration". This coincidence appears plausible, as the endpoint is similar (peripheral neuropathy). The longer exposure time appears to have little effect on the degree of the changes.

In essence, both the rationale and the derivation are clearly described.

**Appropriateness of the application of the PBPK model**

Since the early 1980s, PBPK models for relevant toxicants have been developed and progressively refined. One of the driving forces behind was the necessity to base toxicological species extrapolations on more solid levels by replacing part of the uncertainty factors used in regulatory toxicology by science-based modeling. Initially, a weak point was that physiological descriptors for different species, which were taken from the literature, were not always correct. With the increasing use of PBPK models, such sources of error could be eradicated. The general procedure of

PBPK modeling is described in a very clear way in chapter 3.4.5 of the Toxicological Profile.

The development of PBPK models for acrylamide was much driven by the carcinogenicity of the compound, which led to requirements of a carcinogenic risk assessment. As the carcinogenic effect of acrylamide was clearly evident only in animals, with the situation in humans being unclear, species extrapolation was pivotal. A complicating issue was that the genotoxic species was the metabolite glycidamide, which had to be included in the models.

Calleman et al published the first PBPK applications for acrylamide in the early 1990s. This model included glycidamide, but was restricted to the rat species. More recently, the number of publications has substantially increased that provided metabolic/biomonitoring data on acrylamide in humans. Triggering forces for this research were occupational/environmental issues (e.g. the Norwegian airport tunnel near Oslo) and the detection of acrylamide in foodstuff and of baseline hemoglobin adduct levels derived from both acrylamide and glycidamide.

For PBPK model development/refinement related to acrylamide, a major breakthrough has been the work of Kirman et al. (2003). This model included a series of enzymatic parameters for detoxification of both acrylamide and glycidamide. It was a predecessor of the model of Sweeney (2010), which is used in the present Toxicological Profile. As it stands now (schematic sketch in Fig. 3-6) the model of Sweeney et al presents a very high degree of refinement and is to be regarded as state of the art. The details of the model are described in the Toxicological Profile in a very exhaustive and competent way.

I have one principal remark regarding nomenclature: When PBPK estimates acrylamide or glycidamide in blood are given in the Toxicological Profile (throughout the dossier, for example in Tab. A-2 / A-6), the term "dose" is used on the one hand for the administered/ingested dose (mg/kg per day), and on the other hand also for acrylamide/glycidamide blood concentrations (mM). The latter is confusing for toxicologists, although it is used this way by some PBPK modelers. Correctly, a dose

is always the mass of a compound that is applied; blood concentrations are concentrations and <u>not</u> doses! This should be considered throughout the document.

*Response:* The term "internal dose" is widely used in PBPK modeling and refers to the "dose" to a tissue or compartment that would result from a given "external dose". The term "internal dose" has been defined in the toxicological profile as TWA concentration or AUC. A definition of "internal dose" was added to the Glossary (Chapter 10) of the Toxicological Profile for Acrylamide.

**Study/endpoint selection in the derivation of chronic-duration MRL**

The endpoint selection for chronic duration MRL for acrylamide is not trivial, as chronic toxicity studies reveal both neurotoxicity and carcinogenicity. Neurotoxicity is a clear-cut threshold effect; for carcinogenicity this is presently being discussed, but at this time non-threshold mechanisms cannot be ruled out for acrylamide. The MRL is based on neurotoxicity. For the reviewer, this appears reasonable, because the plethora of human data from occupational settings very clearly confirms the neurotoxicity for humans, but provide no clear and direct proof for human carcinogenicity so far.

**Studies that would be more appropriate or impact the ATSDR proposed MRLs**

No comment.

**Concurrence with the proposed MRLs**

In essence, the present Toxicological Profile for Acrylamide provides an exhaustive and competent description and analysis of the database of this compound. The derivation of MRLs is straightforward. The inclusion of a timely PBPK model is an important element that adds both to the validity of derived MRLs and to the scientific value of the document.

I concur with the derivations of the proposed MRLs and with the values, which appear plausible to me.

**<u>Peer Reviewer #2</u>**


ATSDR Toxicological Profile for Acrylamide

Revised Minimal Risk Level Review


I have reviewed the MRL sections of the acrylamide document including the rationale, worksheets, and the discussion of the MRLs in Chapter 2. I have reviewed the pertinent literature provided and also reviewed the manuscript by Sweeney and coworkers on the physiologically based pharmacokinetic (PBPK) of acrylamide and glycidamide (2010). In my review, I paid particular attention to the rationale, derivation, and clarity of presentation of each of the MRLs, the appropriateness of the application of the PBPK model, the study/endpoint selection for derivation of the oral MRLs. I am not aware of any additional studies that would be more appropriate or have impact the proposed MRLs. I agree with the proposed MRLs for all three durations of oral exposure.

In summary:


<u>Acute-Duration Oral Exposure</u>


For the acute duration (Oral Exposure), the MRL of acrylamide is proposed as 0.01 mg/kg/day. The selection of the MRL of 0.01 mg/kg/day for the acute oral exposure is based on the study of Sublet et al., 1989, which showed a decrease in fertility in male rats. The rational and derivation of the presentation of this MRL is clear and concise. The physiologically based pharmacokinetic (PBPK) model developed by Sweeney and coworkers, for acrylamide and its metabolite glycidamide is appropriate for the estimation of the blood time weighted average for acrylamide and glycidamide in the rat. Using the reported acrylamide-induced reproductive toxicity in the male rat the selection of 0.31 mg acrylamide/kg/day as the point of departure is appropriate.

I am not aware of other studies that would impact on the the proposed MRL. The uncertainty factor of 30 (3 for interspecies extrapolation using a PBPK model and 10 for human variability) appears to be appropriate for the human equivalent dose of 0.31 mg/kg/day to derive an MRL of 0.01 mg/kg/day. I concur with the proposed MRl for the acute oral exposure for acrylamide of 0.01 mg/kg/day..

Intermediate-Duration Oral Exposure

For the intermediate oral exposure a MRL of 0.001 mg/kg/day for acrylamide is proposed. This MRL is based on a study by Burek and colleagues (1980) that showed degenerative nerve pathology  (by electron microscopy)  in rats treated with acrylamide.  Three of 10 rats examined should changes in Schwann cells including the axolemma modifications and degenerating myelin.  Using these data a NOAEL of 0.2 mg/kg/day was derived from the Burek et al study.  This dose was used , in concert with the PBPK modeling by Sweeney et al for acrylamide to produce a the human equivalent dose of 0.038 mg of acrylamide per kg body weight per day. These decisions and calculations appear appropriate and acceptable to this reviewer.  A MRL for the intermediate oral exposure of acrylamide (using an uncertainty factor of 30)(3 for interspecies extrapolation using a PBPK model and 10 for human variability)  is projected to be 0.001 mg/kg/day.   This appears to be appropriate and correctly derived and I concur with the MRL presented.  The rationale, of the MRL presentation and discussion is clear in the text.

Chronic Duration Oral Exposure

For the chronic oral exposure MRL of acrylamide, a MRL of 0.001 mg/kg/day has been proposed. This is based on the studies of Friedman et al. (1995) where a degeneration of the sciatic nerve was reported in rats exposed to acrylamide. Using the predicted human PBPK modeling of acrylamide by Sweeney and coworkers (2010), a human equivalent dose of 0.042 mg acrylamide/kg/day is proposed for the chronic oral exposure to acrylamide. Incorporating an uncertainty factor of 30 (3 for interspecies extrapolation and 10 for human variability), the human equivalent dose of 0.042 mg/kg/day produces a MRL of 0.001 mg/kg/day. Overall this reviewer agrees with the proposed MRL. The presentation and discussion of the derivation of the MRL based on the Friedman study appears logical and reasonable.   The utilization of the EPA Benchmark Dose in this calculation may warrant some additional explanation and discussion for readability in the text of the report.  However, overall the presentation is very well done.

*Response:* The level of detail is considered appropriate for the purpose of the ATSDR toxicological profile.

**ATSDR Draft Toxicological Profile for Acrylamide, MRL**
# Reviewer #3

Review comments on the ATSDR Draft Toxicological Profile for Acrylamide, MRL sections, the Rationale Statement, MRL Worksheets, the MRL discussion in Chapter 2.3., and the Chapter 2.

The documents provided for review represent an evaluation of the toxicology of acrylamide, and selection of appropriate studies for use in estimating the Minimal Risk Levels (MRLs) from exposure to acrylamide under a variety of scenarios, including acute duration oral exposure, intermediate-duration oral exposure, and chronic-duration oral exposure. The endpoints selected were decreased male fertility (acute), ultrastuctural evaluation of degenerative nerve changes (intermediate) and degenerative sciatic nerve changes (chronic). A physiologically based pharmacokinetic model was used with each scenario to produce internal dose measures that were used to calculate human equivalent exposures. For the selection of the neurotoxic endpoints, the studies selected may be the best that are available, but may not be ideally suited to use in risk assessment, and represent a deficiency of the database available for this review. In general there are commendable aspects to the approach used, with the PBPK model giving an improved understanding of the relationship between exposure and internal dose, and in turn the generation of effects. Faced with ambiguity about potential mode of action (with acrylamide and its metabolite glycidamide being reactive), ATSDR has selected the most conservative value. A mixed mode of action has not been considered. Specific aspects of each MRL and responses to the charge questions are provided below.

**Acute-Duration Oral Exposure**

*The rationale, derivation, and clarity of presentation of each of the MRLs*

ATSDR has derived an MRL of **0.01 mg/kg/day** for acute-duration oral exposure (14 days or less) to acrylamide. The MRL is based on decreased male fertility in rats (Sublet et al., 1989). The rational for selection was clear, and has used an endpoint that is generally overlooked in the toxicity of acrylamide. However, the presentation of the data used in deriving the MRL is not clear. Page A-4 last sentence indicates that "The MRL is based on a $BMDL_{10}$ ......for decreased fertility in rats (as assessed by number of nonpregnant rats/number of sperm-positive females) ..." The data is not presented in this way in Table A-1 that presents the data from Sublet et al. In Table A-2, there are columns presented for the number of sperm positive females and the number of non-pregnant females, but there is no calculation of a ratio. It is not clear what time period the values presented in Table A-2 correspond to, and whether these are actual observations or modeled values. Similarly in Table A-3 and A-4, what is meant in the title by "Incidences of Unsuccessful Impregnation", also in Figures A-1, A-2. In Figure A-1, what is plotted as % affected? In the absence of a clear justification for using acrylamide vs glycidamide based on mode of action information, the most conservative measure was used. The application of uncertainty factors appears appropriate.

*Response:* Information was added to text, tables, and figures to clarify that the fertility data refer to fraction of females that were not pregnant (i.e., number of nonpregnant females/ number of sperm-positive females).

*Appropriateness of the application of the PBPK model*

Whether the PBPK model has been appropriately applied is difficult for a reviewer to assess in this instance. There is insufficient information presented about the model implementation for this analysis. The model has not been developed for the Long Evans rats. The age of the rats in the Sublet study is older than those generally used in toxicokinetic studies. The TWA for acrylamide and glycidamide were used as the dose measures in the calculations. There is no indication or evidence that in this case as to whether the mode of action involves acrylamide or glycidamide. There is also no indication as to whether the concentration in blood (of either acrylamide or glycidamide) is an improved dose metric. The use of TWA presented as the model endpoint makes it difficult for the reader to relate the reported TWA concentration to the alternative (and more frequently presented) Area under the Curve (AUC). Peak concentration could also be relevant. Given the endpoint, a more relevant dose metric would be acrylamide (or glycidamide) in the testis. The main difficulties in assessing the appropriate application of the PBPK model is a lack of any graphical representation of the simulations produced to derive the values presented in Table A-2, or the simulations conducted to derive the human equivalent concentrations.

What is the justification for dividing the human exposure into 12 equivalent hourly doses? This would most likely not represent an exposure scenario for oral ingestion.

*Response:* The model has not been developed for the Long Evans rats.

Although the Sweeney et al. (2010) model was parameterized primarily to simulate toxicokinetics in F334 rats, data from studies of Long Evans rats were also used to develop the model (e.g., acute exposures). Toxicokinetics of acrylamide have been studied in a variety of rat strains, including F344, Sprague-Dawley, and Long Evans (see Sweeney et al. 2010 for review) and there is no evidence to suggest that toxicokinetics varies appreciably across rat strains, although this has not been rigorously examined. Variation in toxicokinetics between rat strains (e.g., F344 vs Long Evans) is likely to be less than variation across species (e.g., rat to human). If we had a model for the Long Evans rat, this would not reduce the uncertainty in the pharmacokinetics extrapolation to humans.

The age of the rats in the Sublet study is older than those generally used in toxicokinetic studies.

*Response:* The PBPK model does not simulate age. It is not clear from the comment if the reviewer is concerned about the age of the animals in the Sublet et al. (1989) study, or that the model does not simulate age. The Sublet et al. (1989) study utilized rats that were 90– 100 days of age. It is not clear why this age range would be an issue for evaluating male reproductive effects.

There is no indication or evidence that in this case as to whether the mode of action involves acrylamide or glycidamide.

*Response:* The extent to which acrylamide and glycidamide contribute to toxicity observed following ingestion exposures to acrylamide is not known with certainty. There is evidence to suggest that epoxide metabolites of acrylamide contribute to germ cell mutations in male mice (e.g., Ghanayem et al. 2005a). Given the uncertainty in the relative importance of acrylamide and glycidamide in producing toxicity, the conservative assumption was made that toxic response would be related to a time-integrated function of blood acrylamide concentration (e.g., TWA). This assumption resulted in lower human equivalent doses and MRLs than those that would result from an assumption that toxic response is related to blood glycidamide concentrations.

Peak concentration could also be relevant.

*Response:* Although it is possible that peak concentrations contribute to male reproductive effects, there is no convincing evidence for this. Furthermore, TWA blood concentration is a more health-protective measure than peak blood concentration for either acrylamide or glycidamide for an acute-duration exposure scenario as demonstrated in the following table:

**PBPK Model-predicted Human Equivalent Doses (HEDs) Based on Peak Blood Concentration (PBC) and Time-weighted Average (TWA) for Acrylamide and Glycidamide for a 1 mg/kg/day Rat Dose Administered for 5 Days**

| Parameter | Acrylamide | Glycidamide |
| --- | --- | --- |
| PBC | 0.47 | 2.89 |
| TWA | 0.23 | 1.39 |

Given the endpoint, a more relevant dose metric would be acrylamide (or glycidamide) in the testis.

*Response:* Mechanisms of acrylamide-induced male reproductive effects are not understood and it is possible that toxicity may be related to acrylamide or glycidamide levels in testis. Unfortunately, the model does not simulate the distribution of either to testis; therefore, testis doses could not be predicted with the model. However, concentrations of acrylamide or glycidamide in testis would be expected to be related to concentrations in the blood that perfuse the testis (the model predicts this for other tissues). If so, TWA blood concentrations of acrylamide or glycidamide would be a dose surrogates for dose to the testis.

The use of TWA presented as the model endpoint makes it difficult for the reader to relate the reported TWA concentration to the alternative (and more frequently presented) Area under the Curve (AUC).

*Response:* TWA concentrations of acrylamide and glycidamide in mixed venous blood were calculated as follows:

$$TWA = \left( \int_{t=0}^{t=i} C_{Blood} \right) \div t_i$$

where $C_{Blood}$ is the mixed venous blood concentration of acrylamide or glycidamide (mM) and $t_i$ is the exposure time (hours). This explanation is provided in the revised Appendix A. Note, TWA as calculated above is a time-integrated blood concentration, is conceptually

equivalent to the AUC, and can be converted to units of AUC by multiplying the TWA by the time integration interval of interest. For example, if the TWA is multiplied by 24 hours, the resulting value is the AUC for 24 hours (mM 24hr).

The main difficulties in assessing the appropriate application of the PBPK model is a lack of any graphical representation of the simulations produced to derive the values presented in Table A-2, or the simulations conducted to derive the human equivalent concentrations.

*Response:* Typically, graphical presentations of PBPK model simulations would not be included in the MRL Rationale. Nor is it clear why such presentations would explain or clarify the MRL derivation. The elimination kinetics of acrylamide and glycidamide are relatively fast, resulting in a steady state within 1–2 days of repeated exposure. The various simulations used to estimate human equivalent doses differ only in the steady-state concentration achieved in blood. Examples of this are provided below (Figures 1 and 2):

**Figure 1. Simulation of Blood Acrylamide and Glycidamide Concentrations in Humans Exposed to 0.31 mg Acrylamide/kg/day, the Human Equivalent Dose Based on the $BMDL_{10}$ for Blood Acrylamide TWA from Sublet et al. (1989)**
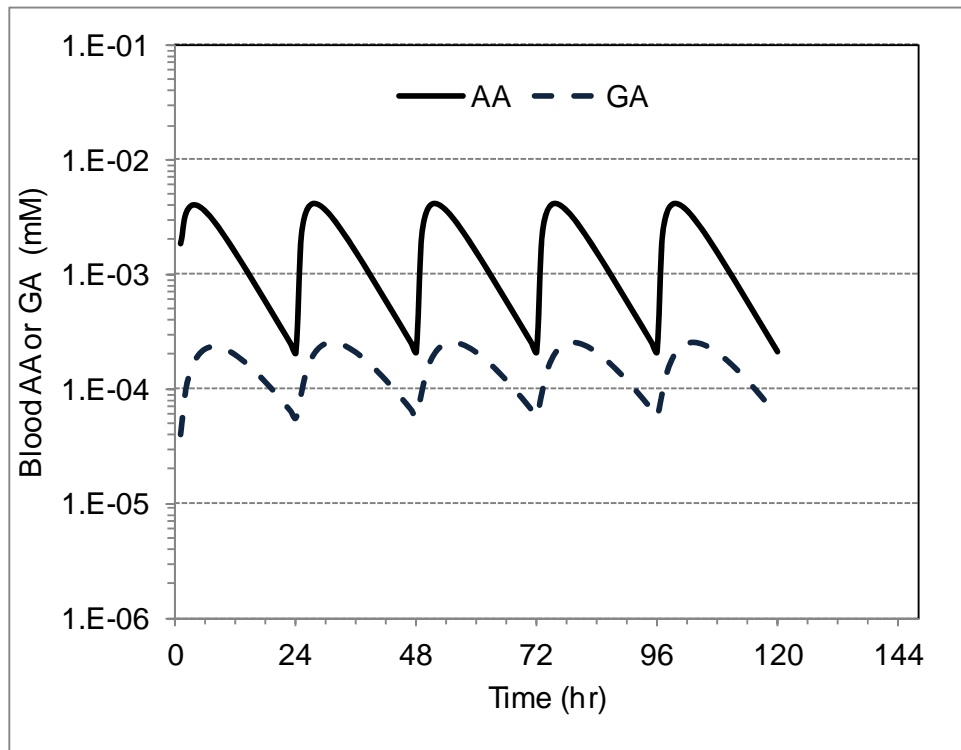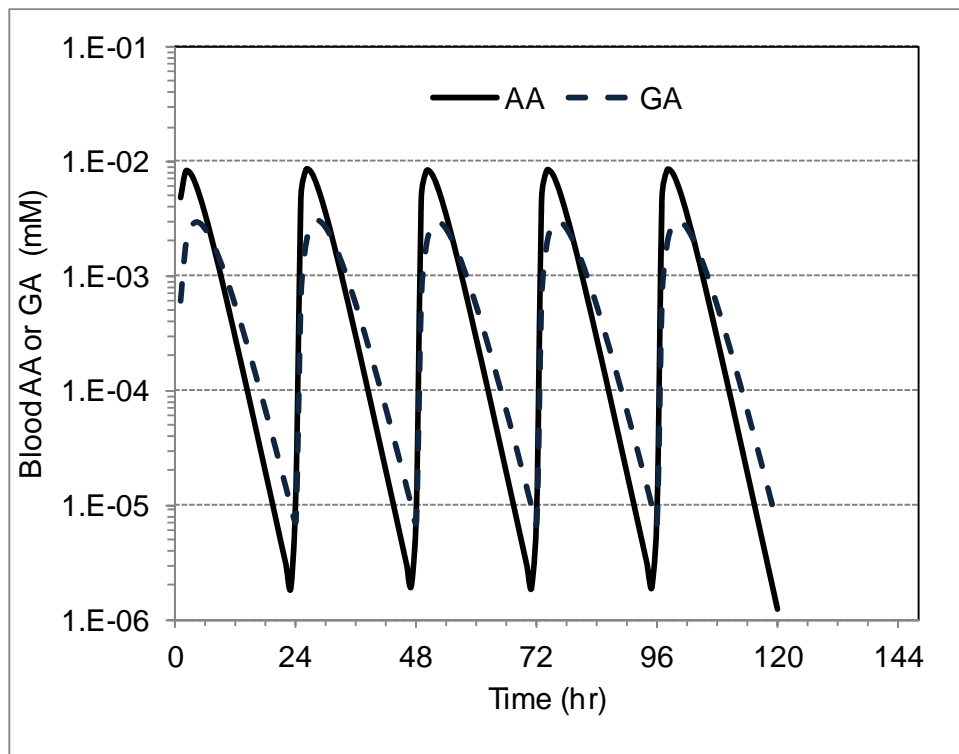
**Figure 2. Simulation of Blood Acrylamide and Glycidamide Concentrations in Rats at the PBPK Model-Predicted Rat External Dose Corresponding to the BMDL$_{10}$ from the Results of Sublet et al. (1989)**



What is the justification for dividing the human exposure into 12 equivalent hourly doses? This would most likely not represent an exposure scenario for oral ingestion.

*Response:* The rationale for the 12-hour dosing period was the expectation that ingestion would occur only during a 12-hour (food and water) consumption period of the day. Other ingestion profiles could apply to specific individuals and populations. However, at the dose range of interest (<1 mg/kg/day), the model is essentially linear, which means that blood AUC has a very low dependence on dosing interval at a constant daily dose. For example, for an external dose of 1 mg/kg/day, decreasing dosing interval from 12 hours (daily dose administered over a 12-hour period) to 24 hours results in a change in the TWA for acrylamide or glycidamide of <1%.

**Intermediate-Duration Oral Exposure**

*The rationale, derivation, and clarity of presentation of each of the MRLs*

ATSDR has derived an MRL of **0.001 mg/kg/day** for intermediate-duration oral exposure (15–364 days) to acrylamide. The MRL is based on degenerative nerve change (Burek et al. (1980). The rationale for the selection of this study was clear, and represents a reasonable selection. "This was selected as the principal study because it identified the lowest lowest-observed-adverse-effect-level (LOAEL) for the critical effect. A NOAEL/LOAEL approach was selected because results of the ultrastructural evaluations included only 3 of 10 rats/group and were reported only as the total numbers of fields (per group) with ultrastructural changes as axolemma invaginations or Schwann cells without axons and/or

with degenerating myelin. The distribution of fields exhibiting ultrastructural changes among the three rats within a particular dose group was not included in the study report." The quantitative deficiencies of the data and the rationale for not using the BMD approach were also clearly explained.

*Appropriateness of the application of the PBPK model*

As described above with the acute-duration MRL, I have questions about the application of the PBPK model. Again, there is insufficient information presented about the model implementation for this analysis. The TWA for acrylamide and glycidamide were used as the dose measures in the calculations. There is no indication or evidence that in this case as to whether the mode of action involves acrylamide or glycidamide. There is also no indication as to whether the concentration in blood (of either acrylamide or glycidamide) is an improved dose metric. The use of TWA presented as the model endpoint makes it difficult for the reader to relate the reported TWA concentration to the alternative (and more frequently presented) Area under the Curve (AUC). Peak concentration could also be relevant. The text (page A-15) indicates that the exposure for 90 days was modeled to produce TWA values for acrylamide and glycidamide that are not presented. This was then used to determine the dose necessary to produce the same concentration in humans. How the comparison of the 90-day exposure in rats with the 365-day exposure in humans was conducted is not clear.

*Response:* The main difficulties in assessing the appropriate application of the PBPK model is a lack of any graphical representation of the simulations of the acrylamide exposures in rats, and the simulations conducted to derive the human equivalent concentrations. In this case, there is little useful information presented to evaluate the application of the model.

There is no indication or evidence that in this case as to whether the mode of action involves acrylamide or glycidamide.

*Response:* There is evidence to suggest that some of the neurological effects observed in animals can be elicited by administration of acrylamide or its epoxide metabolite (glycidamide). However, in one study of male rats administered acrylamide (25 or 50 mg/kg/day) or glycidamide (50 or 100 mg/kg/day) via intraperitoneal injection for 8 days, only acrylamide elicited poor performance on the hindlimb splay test (Costa et al. 1995). Given the uncertainty in the relative importance of acrylamide and glycidamide in producing toxicity, the conservative assumption was made that toxic response would be related to a time-integrated function of blood acrylamide concentration (e.g., TWA). This assumption resulted in lower human equivalent doses and MRLs based on an assumption that toxic response ias related to blood glycidamide concentration.

The use of TWA presented as the model endpoint makes it difficult for the reader to relate the reported TWA concentration to the alternative (and more frequently presented) Area under the Curve (AUC).

*Response:* See previous response to same comment related to acute MRL.

Peak concentration could also be relevant.

*Response:* Although it is possible that peak concentrations contribute to toxic response, there is no convincing evidence for this. Neurological effects occur only after repeated exposures

of >30 days in duration (reference). This suggests that toxic response is related to time integrated dose or to steady state rather than to peak concentrations.

How the comparison of the 90-day exposure in rats with the 365-day exposure in humans was conducted is not clear.

*Response:* The Burek et al (1980) study exposed rats for a period of 90 days; therefore, blood TWA for acrylamide or glycidamide in the rat was simulated for the same exposure period. However, the intermediate duration MRL is intended to be protective of human exposures up to 365 days; therefore, the human equivalent dose was simulated as a 365-day exposure.

The main difficulties in assessing the appropriate application of the PBPK model is a lack of any graphical representation of the simulations of the acrylamide exposures in rats, and the simulations conducted to derive the human equivalent concentrations.

*Response:* Examples of this are provided below (Figures 3 and 4).

**Figure 3. Simulation of Blood Acrylamide and Glycidamide Concentrations in Humans Exposed to 0.042 mg Acrylamide/kg/day, the Human Equivalent Dose Based on the NOAEL for Blood Acrylamide TWA from Burek et al. (1980)**
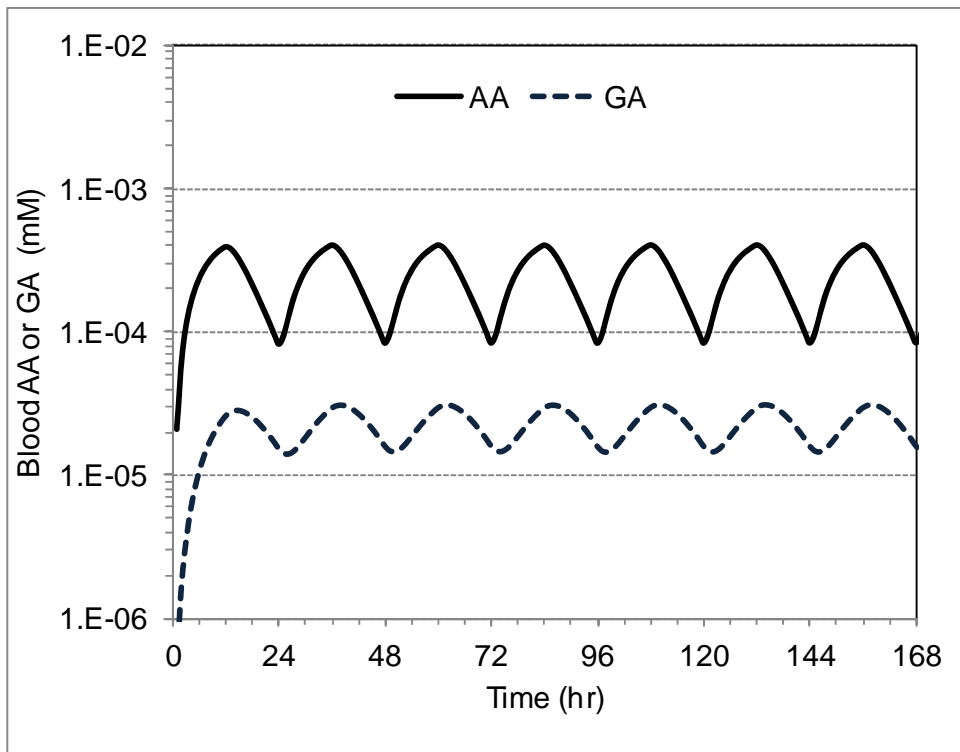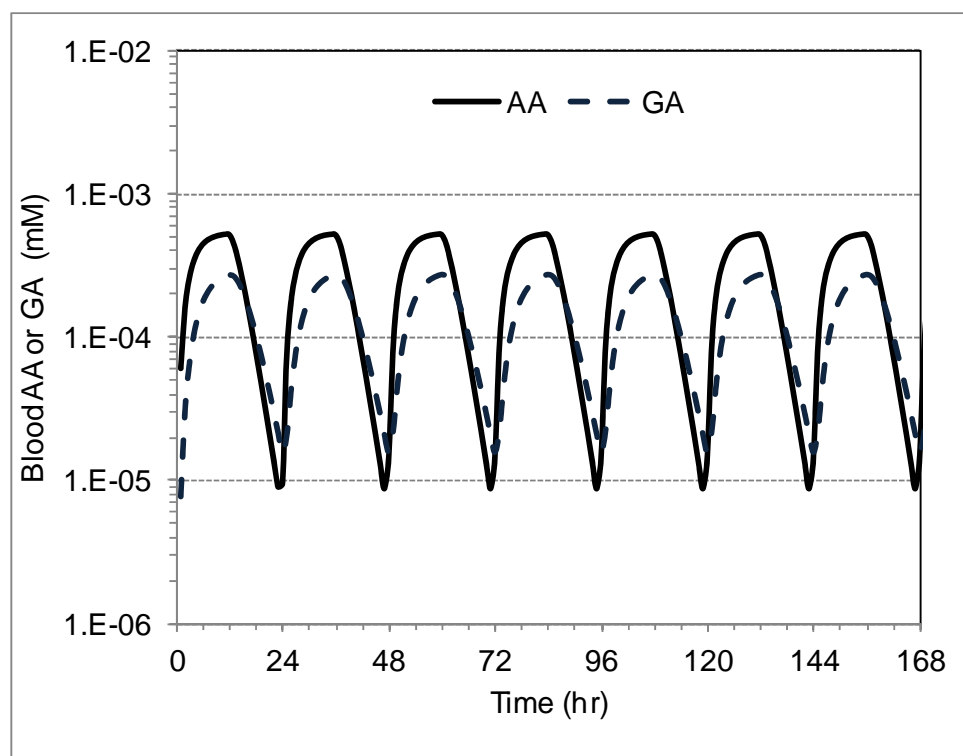
**Figure 4. Simulation of Blood Acrylamide and Glycidamide Concentrations in Rats Exposed to 0.2 mg Acrylamide/kg/day, the NOAEL Based On Burek et al. (1980)**



## Chronic-Duration Oral Exposure

### *The rationale, derivation, and clarity of presentation of each of the MRLs*

ATSDR has derived an MRL of **0.001 mg/kg/day** for chronic-duration oral exposure (365 days or more) to acrylamide. The MRL is based on degenerative sciatic nerve changes (Friedman et al. 1995).

The general approach outlining the use of the rat PBPK model (Sweeney et al. 2010) to estimate blood TWA acrylamide and glycidamide dose metric for each of the administered acrylamide doses for male and female rats from chronic studies (Friedman et al. 1995; Johnson et al. 1986; NTP 2011b) and the human PBPK model (Sweeney et al. 2010) to predict the HED corresponding to the $BMDL_{10}$ and $BMDL_{05}$ values for rat blood TWA acrylamide and glycidamide from the best-fitting models for each of the three chronic studies appears reasonable. The uncertainty factor of 30 (3 for interspecies extrapolation using a PBPK model and 10 for human variability) applied to the HED of 0.042 mg/kg/day also appears reasonable.

The description of the implementation of the model again raises some questions (pages A-18 and A-19). It appears that the rats are modeled as a single time weighted average body weight. The dose in the rat was modeled as delivered daily over a 12-hour period, for 2 years, whereas the human model had exposure for 12 hourly doses, 7 days per week over 366 days.

***Response:*** The chronic studies exposed rats for a period of 2 years days; therefore, blood TWA for acrylamide or glycidamide in the rat was simulated for the same exposure period.

However, the chronic duration MRL is intended to be protective of human exposures ≥365 days; therefore, the human equivalent dose was simulated as a 365-day exposure. Note, steady state is achieved in a few days; therefore, the TWA is essentially constant for exposures ≥365 days.

***The study/endpoint selection for derivation of the chronic-duration oral MRL***

We know that acrylamide is a peripheral neurotoxin in people. The selection of a study that evaluates neurotoxicity is appropriate. However, the studies by Friedman and Johnson were designed as cancer bioassays, and the evaluation of neurotoxic effects did not evaluate functional endpoints. Concern has been expressed that evaluation in a neurotoxicity study appropriately designed may produce effects at lower exposures. However, the studies evaluated are currently the most appropriate.

***Awareness of studies that would be more appropriate or impact the ATSDR proposed MRLs***

I am not aware of any studies that would be more appropriate for the derivation of MRLs.

***Additional Comments on the ATSDR DRAFT TOXICOLOGICAL PROFILE FOR ACRYLAMIDE***

### 3.4.2.2  Oral Exposure

Page 125. "Following 13 daily oral doses of $[1,3\text{-}^{14}C]$-labeled acrylamide at 0.05 or 30 mg/kg/day, tissue concentrations of acrylamide in male F344 rats were similar among tissues with the exception of red blood cells, which showed higher concentrations, presumably due to the formation of acrylamideVal and/or glycidamideVal hemoglobin adducts (Ramsey et al. 1984)." Need to modify statement – binding to hemoglobin accounts for this, and not just formation of valine adducts in hemoglobin. Cysteine is much more reactive than valine in rat hemoglobin and is most likely contributing to much of the binding.

*Response:* Information regarding hemoglobin binding of acrylamide to cysteine was added.

### 3.4.3    Metabolism

Page 126 "Figure 3-3 depicts a metabolic scheme for acrylamide adapted from reports of Calleman (1996), IARC (1994), and Sumner et al. (1992, 1999)." The sulfoxide depicted in Figure 3-3 was originally reported in Fennell et al., 2006, which is not among the cited references, which date from 1992- 1999.

*Response:* The requested citation is included in Figure 3-3.

Page 128, "Levels of hemoglobin adducts of acrylamide were approximately 2-fold higher in the CYP2E1-null mice compared to the wild-type mice." What about the level of GAVal reported in this study?

*Response:* A Statement regarding GAVal hemoglobin adducts was added.

Page 129 last paragraph. This paragraph is disorganized, and does not review appropriately the work that was done on species differences and sex differences. Sumner et al. reported differences between rats and mice in 1992. There is a difference between metabolism and pharmacokinetics that should be clarified.

*Response:* The findings of Sumner et al. (1992) were added to the text.

### 3.4.4.2 Oral Exposure

Page 133 on. There is uncertainty about the extent of GAMA formation in those studies that do not report investigating the sulfoxide of NACP. GAMA and the AMA sulfoxide metabolites are isomeric, and have the same mass. They have to be resolved chromatographically to avoid interference in mass spectroscopic assays. It is possible that many of these studies overestimate the formation of GAMA by a great extent, since the sulfoxide can account for a major portion of the metabolism of acrylamide.

*Response:* A cautionary statement was added to the text.

### Sweeney et al. (2010) Model

Page 139 on. While the Sweeney et al. model is an substantial improvement over the previous models, it has some limitations. The Sweeney model does not appropriately encode the formation of hemoglobin adducts, or their removal. Removal should be a zero order process, rather than first order as indicated in Table 3-12.

*Response:* The basis for the reviewer's conclusion that adduct removal rates should be zero order is not provided. The assumption of first-order removal made in the Sweeney et al. (2010) model is based on observations reported in Tareke al. (2006). In rats observed following cessation of a steady-state exposure to acrylamide, adduct loss from hemoglobin DNA was approximately first order (half-times were 9 days in mice and 11–13 days in rats) and was clearly not zero order.

### 3.8.1 Biomarkers Used to Identify or Quantify Exposure to Acrylamide

Page 163. "It should be noted that hemoglobin adducts of N-methylolacrylamide are indistinguishable from hemoglobin adducts of acrylamide." Reference?

*Response:* Citations for the statement in question were added.

## 8. REGULATIONS, ADVISORIES, AND GUIDELINES

Page 221. Section needs to be updated with new analysis. See my underlines.

"ATSDR has derived an acute-duration oral MRL of 0.02 mg/kg/day for acrylamide based on BMD analysis of results of fertility testing of male F344 rats administered acrylamide by gavage for 5 days prior to 1-week mating sessions with untreated female rats (Sublet et al. 1989). The resulting $BMDL_{10}$ of 1.78 mg/kg/day was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability). See Appendix A for details regarding BMD analysis.

ATSDR has derived an intermediate-duration oral MRL of 0.002 mg/kg/day for acrylamide based on a NOAEL of 0.2 mg/kg/day and a LOAEL of 1 mg/kg/day for ultrastructural changes in peripheral nerve fibers in male F344 rats receiving acrylamide from the drinking water for up to 93 days (Burek et al. 1980). The NOAEL of 0.2 was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

ATSDR has derived a chronic-duration oral MRL of 0.002 mg/kg/day for acrylamide based on a BMDL$_{05}$ of 0.18 mg/kg/day for degenerative changes in sciatic nerves from male F344 rats receiving acrylamide from the drinking water for up to 2 years, as detected by light microscopy (Friedman et al. 1995). The BMDL$_{05}$ of 0.18 mg/kg/day was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability)."

*Response:* The inadvertent omission of updated MRL information to this section was corrected as suggested.

## 9. REFERENCES

Some of the citations are not complete, e.g. Takahashi et al., 2009, Takami et al., 2011.

*Response:* The reference for Takahashi et al. (2009) was revised to include the full published citation. The study of Takami et al. (2011) remains as an epublication ahead of print as of April 20, 2012.