Draft Detailed Comments on

"USEPA Toxicological Review of Acrylamide" In Support of Summary Information on the Integrated Risk Information System (IRIS) December 2007"

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INTRODUCTION AND SUMMARY —

On behalf of GMA, *The Sapphire Group, Inc.*, is pleased to offer these comments on USEPA's draft review of acrylamide. Because of its inadvertent formation in cooked foods, acrylamide is of major public health importance not only in the United States but also around the world; in other circumstances, it is of commercial importance worldwide. We recognize that USEPA's regulatory purview extends to environmental exposures such as drinking water and not to exposures in the workplace or to foods and some consumer products. However, because acrylamide has been recently reported as being relatively pervasive in the human diet for example, USEPA's judgments about human safety and risks may well have impacts in our society and globally that greatly transcend its legislated mandates. Therefore, the Agency should proceed with particular caution in finalizing its Toxicological Review and in the formulation of its IRIS documentation for acrylamide.

Our comments are organized into two categories: Generic and Specific. Our Specific Comments are subdivided into three subcategories that correspond to broad and far-reaching topics in EPA's draft Toxicological Review: (1) Cancer mode(s)-of-action considerations [2 specific comments]; (2) Internal dosimetry (toxicokinetic) modeling considerations [5 specific comments]; and (3) Carcinogenic potency (dose-response) considerations [7 specific comments]. Note that the absence of our comments about other parts of the draft toxicological review should not be considered as agreement with those Agency's analyses or conclusions.

We conclude that the cancer potency factor for acrylamide proposed in the draft IRIS document has been <u>overestimated</u> based in part on (1) differences between linear and non-linear extrapolation to doses below the range of experimental doses administered to rats, (2) overestimates of the amount and types of hemoglobin adducts for rats in EPA's PBTK model, (3) overstatements of tissue volumes and blood flow in the PBTK model, (4) overestimates of the administered dose for thyroid tumor data, and (5) the needless application of the time-to-response model. Such an overestimation of the cancer potency factor would has the consequence of overstating cancer risk-specific doses for humans by comparable similar orders of magnitude by which the cancer potency factor was overstated.

Although some evidence suggests that acrylamide may cause cancer through a genotoxic mode-of-action, we believe that the scientific weight-of-evidence does not support a genotoxic mode-of-action as the sole or even primary mode-of-action, as detailed in this submission and that of others (for instance, Dourson *et al.*, 2008). Based on the entirety of the data related to mode-of-action of tumor development in laboratory rats administered doses of acrylamide quite high in comparison to human exposures, the dose-response curve is non-linear, and consequently its shape in the dose range encountered by humans produces a lower cancer potency than that estimated in the dose range used in the rat studies. As such, we conclude that it is scientifically inappropriate to apply a linear extrapolation method.

In acknowledging the other mode(s) of cancer-action in rats, a non-linear method of extrapolation (*e.g.*, Benchmark Dose with Uncertainty Factors) should be employed in this document, and logically should indicate that potential cancer risks from acrylamide exposure would be virtually zero for lifetime daily doses below 4 µg/kg. The non-linear approach (using an UF of 30) will result in safe doses that are approximately 300-fold higher that the $1x10^{-5}$ risk-specific dose predicted by the linear extrapolation. We note that Shipp *et al.* (2006) has demonstrated a scientific rationale for using a non-linear approach to acrylamide's cancer data leading a much lower cancer potency and risk-specific dose for acrylamide for humans that estimated in the Agency's draft.

If the non-linear extrapolation were performed with the considerations we recommend, the estimated safe lifetime daily doses would be 4 µg/kg and less. Were the full measure of acrylamide detoxification resulting from rapid, irreversible binding and other detoxification mechanisms to be considered, that safe level would conceivably be even higher.

Even if acrylamide were assumed to be causing cancer through a genotoxic mode-of-action, it is important to present quantitatively the impact of both possibilities: genotoxic and non-genotoxic modes-of-action. In other words, the IRIS document should say that, depending on whether acrylamide is assumed to be acting through a genotoxic or non-genotoxic mode-of-action, the cancer potency factor would be either "X" or "Y." Furthermore, when using the linear assumption, the IRIS document should report the lower-bound on cancer potency and risk as well as the upper-bound. Otherwise, decision makers and others will not be fully informed of the range of possible cancer potency factors for — and potential cancer risks of — acrylamide exposures for humans.

To provide balance and perspective to its characterization of acrylamide's cancer risk, the document should be predicated in part on the findings of the several epidemiological studies, some of reasonably large populations. The majority of these studies found no statistically significant association between acrylamide exposures and cancer at assorted specific tumor sites. We find that the draft document has not drawn appropriate conclusions about these findings. An instance reporting a statistically significant association between estrogen-

positive receptor breast cancer in post-menopausal women and hemoglobin adducts of acrylamide has yet to be replicated, contains smokers as well as non-smokers (attempts were made to address this confounder), and is of unclear reliability. Consequently, the relevance of cancer findings from the high-dose acrylamide studies in rats have yet to be found definitively to be relevant to humans exposed to doses much lower than those used experimentally.

The Agency's draft is incomplete with regard to judging the relevance to humans of the tumors observed in acrylamide-exposed rodents. A Mode-of-Action/Human Relevance Framework exists to do so in considerable detail and with much reliability and transparency. USEPA has accepted this Framework as part of its assessment process. In this instance, USEPA has applied this Framework to an insufficient extent to acrylamide's toxicology review. Were it to do so, it would find that all rat tumors are not relevant to humans. Missing from USEPA's draft are (1) taking into proper account kinetic and dynamic factors, (2) plausibility of MoA for humans, and (3) concordance analysis of animal and human responses (notwithstanding USEPA's default view that no such concordance need exist). Such an analysis would prove to be essential for public health protection.

USEPA's PBTK model structure and parameters do not fully describe the various hemoglobin adducts used in model development, thereby rendering it very difficult to assess its accuracy for internal dosimetry. At a minimum, the recalibrated model for rats should include two binding terms for hemoglobin, one for the formation of terminal valine adducts and one for the formation of all other adducts, including those that reduce bioavailability. Furthermore, since USEPA's model appears to fail to predict kinetic behavior at high doses (as described on page E-9 of its draft), it will likely fail to predict repeat exposures at moderate doses due to changes associated with co-factor/protein depletion and enzyme induction. Efforts to validate the recalibrated rat model appear to be incomplete; and the documentation of the validation effort is certainly incomplete. USEPA should recalibrate its PBTK model to fit experimental data that do not appear to have been properly considered in development of the current (2007) model to fully assess the confidence in its predictions. Since completion of its model in 2005, several important publications have appeared (see our Specific Comments herein for references to these; however, these additional parameters and data have not been added to the PBTK model.

In addition, as part of its model, USEPA should explain in detail the large discrepancies in binding (hemoglobin, liver, blood, and other tissues) and metabolism terms between the Agency's assessment and the peer-reviewed publication of the PBTK model. The result appears to be an overstatement of hemoglobin adducts by 3- to 4-fold; also, USEPA's human model predicts that 50% of acrylamide is converted to glycidamide, a magnitude that makes no sense in light of the available data. Also, failure to perform a sensitivity analysis on the model is a glaring omission that casts doubt about the reliability on the model's output.

Finally, USEPA's integration of the results of the internal dosimetry (PBTK) modeling into the dose-response modeling appears incomplete, raising questions about the overall reliability of the internal human dose estimates.

The current draft acknowledges only a fraction of the detoxification mechanisms (both capacities and rates) present in the human body into its estimate of cancer potency and risk-specific doses. Particularly noteworthy is the realization that humans have a greater abundance of these defense mechanisms than do the rats used to test experimentally for the carcinogenicity of acrylamide (see Specific Comments herein). To address this one factor alone may reduce human cancer risk estimates from ingested acrylamide. Arguably, from the biological standpoint, such detoxification processes would be expected to play an important role for substances such as acrylamide that have been intrinsic to the human diet since the advent of cooking.

We find it particularly troublesome that the USEPA would put their draft document out for public review, let alone for consideration by an SAB Review Panel, without first providing complete disclosure of the development and validation of the PBTK model. Equally disturbing is that during interagency review, other agencies (USFDA, USDA, and OMB) would allow this assessment to go forward without complete transparency of the development of the tool used to conduct critical interspecies and route-to-route extrapolations. Full disclosure of the model code, assumptions used in altering parameters, and comparisons and critique of model predictions to all available data are required before an adequate evaluation, let alone acceptance, of the USEPA modeling efforts can be realized.

A shortcoming of the current proposal is that it fails to say "we could be wrong" or "we are not sure." Scientists live with this uncertainty all the time, and risk assessors try to do the best they can with the available data. But, it is extremely important to acknowledge to risk managers and to non-scientists that "we could be wrong." If a genotoxic mode-of-action were assumed and linear extrapolation were employed, the IRIS document should say "We could be wrong; if we are wrong, the cancer risk would be 10- to many orders of magnitude lower." Conversely, if a non-genotoxic mode-of-action is assumed and non-linear extrapolation is used, the draft document should say: "We could be wrong; if we are wrong, the cancer risk would be 10- to many orders of magnitude higher." <u>The weight-of-evidence for this group of studies suggests that, in the dose range experienced by humans, acrylamide is unlikely to cause cancer</u>.

Our detailed evaluation is presented below.

GENERIC COMMENTS —

Overall, USEPA is to be commended on a thoughtful and well organized effort in its review of the data relevant to interpreting their significance to human health.

In particular, we note the USEPA's treatment of acrylamide's neurotoxic properties demonstrated a sound understanding of the underlying science, an appropriate application of its methodology in generating a draft RfD, and a clear and understandable rationale of how it developed the draft RfD. We support the proposed RfD based on neurotoxicity, and suggest that USEPA consider the prospect that protecting against neurotoxicity may also protect against cancer (when the cancer potency is properly classified as non-linear, as noted in our comments). At this time, we offer no specific comments on this part of USEPA's draft.

With regard to draft's evaluation of acrylamide's carcinogenicity and relevance thereof for humans, we found that it was comprehensive in supporting documentation, described clearly the underlying data, and sought, though not always successfully, to apply state-of-the-art methodology to the estimation of carcinogenic potency and risk-specific doses. However, overall in this area, we find that the draft lacks considerations of key data and important evaluative approaches, resulting in our conclusion that the draft's resulting cancer potency and risk estimates per unit of dose are unjustifiably high, and lack the necessary validity to estimate cancer risk to humans ingesting acrylamide. Our comments and recommendations are aimed largely at complementing those in USEPA's draft Review and in assisting the Agency with its subsequent revisions to its draft.

1. The draft carcinogenicity assessment for acrylamide should be predicated in part on epidemiological findings and not solely on findings from laboratory animal data as presently structured. The human evidence is helpful in providing both balance and perspective to the laboratory animal findings, particularly for the hazard identification step and to a somewhat limited extent in the estimation of upper-bounds on carcinogenic potency. We note that precedence exists for relying on epidemiologic data for RfD. According to Persad and Cooper (2008) (authors are EPA employees);

> *"epidemiologic data, both observational and experimental, have also been used in the derivation of toxicological risk estimates (i.e., reference doses [RfD], reference concentrations [RfC], oral cancer slope factors [CSF] and inhalation unit risks [IUR]).....benzene, has utilized human data for derivation of all three quantitative risk estimates (i.e., RfC, RfD, and dose-response modeling for cancer assessment)."*

Numerous epidemiological studies, retrospective and prospective, have been performed and their findings published (Mucci *et al.*, 2003; 2004; 2005; 2006; Pelucchi *et al.*, 2003; 2006; 2007; Hogervorst *et al.*, 2007; Olesen *et al.*, 2008; Swaen *et al.*, 2007; Marsh *et al.*, 1999; 2007). For the most part, these are case-control studies, some of occupationally exposed workers and still others of populations exposed to acrylamide via the diet. Some have relied on cohorts numbering in the tens of thousands, while others have studied a few hundred individuals. Collectively, these studies have explored possible associations between acrylamide exposures and the following cancer sites: larynx, esophagus, breast (female), kidney, colon, rectum, pancreas, ovary, endometrium, thyroid[4], testis, and respiratory tract. The majority of these studies — some of highly exposed workers — reported <u>no statistically significant association</u> between acrylamide (at ascending dose levels) and specific tumor sites or tumor deaths. While one study has reported an association between dietary acrylamide and a specific type of mammary tumor in post-menopausal women, other studies, having looked closely for a such a link, have failed to do so.

One occupational mortality study of acrylamide (Marsh *et al.*, 1999) found slightly elevated pancreatic cancer deaths; however, these findings have not been replicated in other occupational or dietary studies. Further, a small dietary study of acrylamide reported an association between adducts of acrylamide-hemoglobin and glycidamide-hemoglobin and estrogen-receptor-positive breast cancer, with no indication of a dose-response (Olesen *et al.*, 2008). This study's measure of exposure is not only unusual but also suspect in as much as the adducts represent <u>inactive (non-bioavailable)</u> acrylamide and glycidamide with no indication of what fraction of the ingested dose, if any, they represent in the study group. Other larger studies looking for associations between acrylamide intake and breast cancer have found no statistically significant associations.

The weight-of evidence of this group of studies strongly suggests that in the dose range experienced by humans outside the workplace, acrylamide is unlikely to cause cancer. Furthermore, the limited suggestion of an association should be used not to infer that this compound might cause cancer in humans but rather to explore other forms of investigation to see if such an association was indeed substantive. Consequently, USEPA's draft should on balance conclude that the human evidence is at best very weak in suggesting any association with acrylamide and that, if there were any association at all, it would suggest that acrylamide is likely to be weakly carcinogenic for humans and that its carcinogenic potency is compatible with a non-linear dose-response.

2. Repeated and prolonged ("chronic") ingestion doses of acrylamide by humans have been misrepresented in the draft such that users aiming to estimate human cancer risks are likely

[4]The only site that corresponds with a site in the rat studies and that is considered relevant for humans.

to draw incorrect conclusions about the carcinogenic risks to humans from various sources of acrylamide.

Human exposures to acrylamide result from some drinking water, cigarette smoke, an assortment of foods (Shipp *et al.*, 2001), and perhaps others as well. We find that the draft as presented would not support accurate relative risk contributions that the Agency might generate from this information for application to dietary exposures and may, therefore, promote confusion and misinterpretation among stakeholders.

Presently, the draft offers only summary statistics for dietary exposure at the 50th and 95th percentiles for adults and young children, with little information on how these values were derived (particularly portion sizes, frequency of consumption, changes in consumption behaviors across life stages and special circumstances such as pregnancy and intercurrent disease). Such data are available in the nationwide food consumption survey.

The draft appears to assume (or hopes the reader the will assume) that daily exposure must be fixed over a lifetime with only body weight changing from childhood to adulthood. The draft should acknowledge the intermittent nature of dosing, and indicate how that will affect lifetime carcinogenic risk estimates. Likewise, the draft should provide analyses of how variances in daily doses will affect carcinogenic risk estimates under non-linear (including considerations of detoxification — see next generic comment) and linear (USEPA's default) dose-response relationships in the range of doses experienced by humans. Furthermore, these analyses should be described with great clarity to provide users of the information the opportunity to replicate the methodology with varying sources and conditions of acrylamide exposure.

Finally, the document has yet to fully describe, and needs to characterize, the means of estimating life-stage exposures via the diet. Such descriptions should encompass ranges or standard deviations — and even distributions from Monte Carlo simulations — so that users of the data can understand the level of reliability to assign to each portion of information.

3. Detoxification of acrylamide and of its primary metabolite glycidamide should be given increased importance in the draft's estimation of its carcinogenic potency at ingested doses encountered by humans. Certainly, the document lays out in detail the metabolism of acrylamide particularly to its reactive metabolite glycidamide which is strongly suspected of being the proximate carcinogen in rats exposed chronically to much high experimental doses of acrylamide than those obtained in everyday life.

As with most chemicals foreign to the body, detoxification is a means for the body protect itself from what might otherwise be potentially dangerous exposures. For acrylamide, several detoxification processes exist in humans; and while these processes are also present

in laboratory rodents, they are present to a lesser degree than in humans. This difference suggests that in general humans may be less susceptible to the toxic effects of foreign compounds than rodents; that is, a greater dose may be needed in humans to produce the same effects as in rodents. For acrylamide, a particularly important detoxification process is covalent binding to macromolecules[5] such as hemoglobin and albumin, and to glutathione (Kirman *et al.*, 2003; Young *et al.*, 2007). This binding essentially reduces, apparently quite readily, the availability of acrylamide and its reactive metabolite glycidamide in otherwise susceptible tissues (for instance, quite possibly the thyroid for acrylamide and glycidamide); the result at low doses of acrylamide and its primary metabolite encountered by humans is expected to preclude functional damage including tumor formation. Indeed, USEPA's PBTK model has taken some, but not necessarily all or even a large measure , of these factors into account.

For example, some of ingested acrylamide is expected to be oxidized in the GI tract, since the mucosa of the small intestine contains substantial amounts of xenobiotic metabolizing enzymes (P-450 family) that would convert acrylamide to glycidamide and subsequently break down that metabolite into less toxic moieties. Also, much protein is present in the small intestine and would be available to bind some of the acrylamide and glycidamide. While this capacity has not yet been measured or characterized, the opportunity do so first in animals then in humans exists. Incidentally, we find noteworthy that high experimental levels of acrylamide induced no tumors of the GI tract, despite the direct contact of this compounds and glycidamide with those tissues prior to absorption into the blood stream.

Binding capacities and rates of binding have been demonstrated to occur with some macromolecules in the bloodstream. And some of this information has been incorporated in USEPA's PBTK model. However, since completion of its model in 2005, no additional parameters and data have been added to the PBTK model, despite numerous publications describing data relevant to detoxification and kinetics (see Specific Comments herein). Clearly rates of reactions such as absorption into the blood stream and macromolecular binding are important in judging the concentration of acrylamide and glycidamide at potential target tissue sites. One can trace the path of acrylamide and its primary metabolite through membranes, tissues, and organs, and show that at each step the total ingested doses is gradually reduced. The amount of reduction requires the sophistication of PBTK modeling, which is why we find it so important for scientific community to have the opportunity to examine USEPA's acrylamide model to determine its robustness and validity. We highly recommend that the model code and documentation be made available as soon as possible to the public and that findings be shared with USEPA scientists prior to the Agency's next iteration of this IRIS draft document.

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[5]This process has been know to exist for many decades as can be seen in both the toxicological and pharmacological literature.

To demonstrate how quickly a single dose of ingested acrylamide and its metabolite glycidamide is reduced in the body from the time of consumption, we undertook an internal experiment in which we used the original Kirman model to compute how much of a dose of 0.1 micrograms per kg would be bound in or eliminated from the body of a rat in 24 hours. Our results indicated a reduction of the administered dose by 80%. We surmise that that fraction would change, perhaps increase, when all the relevant kinetic data of the past several years were included to update that model, maybe even more so in humans. While we do not suggest that we can presently estimate the amount of non-bioavailable acrylamide and glycidamide would remain from multiple daily doses, we do recognize that these detoxification processes are not only effective but also redundant, ample, readily replaceable (*e.g.*, constant replenishment of hemoglobin, to name but one), and widely distributed in the body. The expected impact of the detoxification mechanisms is to reduce the amount of reactive compounds, perhaps below the point where they are toxicologically effective. The importance of such a physiological process is, of course, the impact on the dose-response relationship: If the doses were insufficient to produce any initiation of a pathological event, then disease would be an unlikely result.

Subsequent revisions to the draft should include a quantitative evaluation of detoxification, particularly at dose levels actually encountered by humans, and estimate their overall impact on defining the dose-response characteristics of acrylamide and glycidamide for the carcinogenic potency in humans. The Agency, therefore, is encouraged to fully evaluate and incorporate all detoxification factors in its revision to the current draft.

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SPECIFIC COMMENTS —

A. Cancer Mode-of-Action Considerations

1. The modes of action (MoAs) for the cancer endpoints need to be re-evaluated in more detail and put into the Human Relevance Framework.

The USEPA is to be complimented on section *4.8.3 Mode of Action for Carcinogenicity* of the draft document for their use of the modified Hill criteria for organizing the evaluations of the MoAs for the cancer endpoints of concern and for following the 2005 Cancer Guidance (USEPA, 2005). To aid in clarifying the choice of tumor type and the relevance to humans, we recommend that the MoA evaluations be put more in line with the Human Relevance Framework (HRF), as described by the International Life Sciences Institute (ILSI) (Meek *et al.*, 2003) and by the International Programme on Chemical Safety (IPCS) (Boobis *et al.*, 2006). Specifically, it would be more effective and convincing to explicitly address the three questions that define the framework. These questions are:

1. *Is the weight of evidence sufficient to establish an MoA in animals?*

2. *Can human relevance of the MoA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans?*

3. *Can human relevance of the MoA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between experimental animals and humans?*

The USEPA has addressed question #1 above based on the evaluation performed using the modified Hill criteria, although as suggested below, a more thorough evaluation is in order. The other two questions need to be explicitly addressed in subsequent revisions of this document to better present the arguments, for and against, the relevance of the tumor types and MoAs to humans.

Of greater concern is the discrepancy between the overall conclusions regarding MoA reached by the USEPA, including choice of tumor types used for dose-response assessment, and the results of a similar analysis of these same data by Shipp *et al.* (2006). The USEPA concluded that mutagenicity was the MoA for the thyroid follicular cell adenomas and carcinomas in male and female rats, tunica vaginalis testis (*i.e.*, scrotal sac) mesotheliomas in male rats, and mammary gland tumors (adenomas, fibroadenomas or fibromas) in female rats from oral bioassays. Shipp *et al.* (2006) went into meticulous detail in their MoA

analyses and convincingly, at least to these reviewers, eliminated all tumor types as being relevant to humans, except the thyroid tumors, and even the thyroid tumors might be considered not relevant. We advise that the USEPA also conduct a more in-depth evaluation for human relevancy of the tumor types found in the bioassays and to put these evaluations in the context of the Human Relevance Framework. Specifically, we foresee that the cancer potency would be sharply reduced for humans and that the dose-response relationship would eventually be defined as non-linear — in contrast to the Agency's present default view that acrylamide's dose-response curve for cancer is assumed to be only linear.

Regarding MoA, the USEPA presents a list of evidence to support a mutagenic MoA for acrylamide (see page 144-145), but should also explicitly list the data that do **not** support a mutagenic MoA as well (as per Agency Guidelines (USEPA, 2005 and USEPA, 2007)). We refer the USEPA to Shipp *et al.* (2006) where several key findings are discussed that weaken the support for mutagenicity (or at least indicate that other MoAs are as likely if not more likely to be operable) and indicate that a non-linear low-dose response is also likely to be pertinent. Several of these key findings are:

- The DNA binding found in various rat organs was higher in non-target organs than in target organs (thyroid, testes, mammary gland), which is contrary to what would be expected were genotoxicity the dominant MoA.

- Certain germ cell genotoxicity data (dominant lethal effects) do not support a linear genotoxic MoA and may involve binding to protamine.

- Acrylamide, in the absence of a classic tumor promoting agent,did not produce skin tumors in Sencar mice.

- The clastogenicity and aneuploidy of acrylamide may be non-linear or have a very shallow slope that at low doses may be indistinguishable from background.

- The majority of *in vivo* studies of chromosomal aberrations and micronuclei by a pertinent route of exposure (oral) were negative.

We direct the USEPA to either Shipp *et al.*(2006) and to Dourson *et al.* (2008) for a more complete summary of data indicating that mutagenicity is likely not the only operative MoA or to a full reexamination of the original data in its revisions to its present draft.

A recent review (Guyton *et al.*, 2008) indicated that the MoA frameworks are not meant to be "checklists" of criteria for choosing a MoA and that professional judgement does, necessarily, come into play. We agree with this review in that sense, and suggest careful use of checklists as the weight-of-evidence is evaluated in much more detail by USEPA in

revisions to its draft. However the MoA Framework is an important tool to provide transparency to the risk assessment process.

If indeed acrylamide's carcinogenicity is the result of mixed MoA as put forth by Dourson *et al.* (2008), we propose that USEPA develop a biologically-based low-dose extrapolation model that incorporates both MoAs with proper balance accommodated by the relative contribution of each MoA.

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2. A non-linear approach for extrapolating to low doses in the range experienced by humans is needed in addition to the linear default approach used by the USEPA for any pertinent cancer endpoints.

A growing literature indicates non-linear behavior for certain genotoxic and cancer dose-responses. Some of the most often cited processes leading to non-linear (or threshold) behaviors include saturable metabolism, overloading of oxidative defense mechanisms (*e.g.* GSH depletion), disruption of cell division and chromosome segregation, plasma binding capacities, decreased fidelity in DNA replication, loss of DNA repair capacity and cytotoxicity (Upton 1988; Kirsch-Volders *et al.,* 2003; Kirkland *et al.,* 2007; the entire special issue of *Mut. Res. 464,* 2000 reviewed this evidence, see specifically: Crebelli *et al.,* 2000; Henderson *et al.,* 2000; Moustacchi *et al.,* 2000; Muller and Kasper 2000; Parry *et al.,* 2000; Sofuni *et al.,* 2000; Speit *et al.,* 2000). Based on the toxicological review presented in the USEPA draft document and by Shipp *et al.* (2006), acrylamide is known to undergo saturable metabolism, is conjugated with GSH (including its metabolite, glycidamide), binds with plasma proteins and interacts with cellular proteins, all processes potentially leading to non-linear behavior. A very recent poster presented at the 2007 Society of Risk Analysis annual meeting (see Friedman *et al.* 2007) presented evidence for a non-linear dose response in the mouse micronucleus assay when using numerous very low doses of acrylamide. Clearly these and those data mentioned above should be given serious consideration in any re revisions to the Agency's Toxicological Review of Acrylamide.

As per the previous comment, Shipp *et al.* (2006) also describe a number of processes that can lead to non-linear cancer dose-response for acrylamide. The evidence was sufficient that they conducted both a linear and a RfD-type (non-linear) low-dose cancer extrapolation. Bolt *et al.* (2003) and an expert panel (German Federal Agency of Consumer Health Protection and Veterinary Medicine) has issued the opinion that, "despite acrylamide being a genotoxic carcinogen, a non-linear relation between dose and carcinogenic effect seems likely". This opinion was based on mechanistic arguments. Dybing and Sanner (2003) used a linear low-dose extrapolation in their cancer risk assessment of acrylamide, but they also gave several reasons why the low-dose cancer response for acrylamide might be non-linear as well, citing detoxification processes, cell cycle arrest, DNA repair, apoptosis, and the control of neoplastically transformed cells by the immune system, as reasons the linear default likely over-estimates, perhaps by a considerable amount, the risk of ingested acrylamide for humans.

Precedents exist for genotoxic carcinogens to have non-linear dose-response relationships (Kirman *et al.,* 2005; Albertini and Sweeney, 2007; and Kirman *et al.,* 2004).

Based on the above, it seems prudent that the USEPA conduct an RfD-type cancer assessment (*i.e.,* NOAEL or BMDL and Uncertainty Factors), in addition to the default linear

approach presently described, in the next version of their document. Another appropriate methodology would that called the Margin of Exposure (MoE), by which one compares a human equivalent NOAEL to known or anticipated human doses.

Another approach worthy of serious consideration is the formulation of a biologically-based model that incorporates mixed modes of action for low-dose extrapolation of cancer incidence in rats and subsequent application to humans.

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B. Internal Dosimetry (Toxicokinetic) Modeling Considerations

1. USEPA's model structure and parameters do not fully describe the various hemoglobin adducts used in model development.

The hemoglobin-binding portion of the recalibrated model addresses only terminal valine adducts, which serves as a useful biomarker of exposure, but does not include binding to other sites (cysteine) that are likely to be important to understand mass-balance and detoxification. The data of Ramsey *et al.* (1984) and Miller *et al.* (1982), that provide data for radiolabel binding in blood (*i.e.*, includes all adducts), were used in the original Kirman model, but are not used in the recalibrated model. At a minimum, the recalibrated model should include two binding terms for hemoglobin, one for the formation of terminal valine adducts and one for the formation of all other adducts, including those that reduce bioavailability.

Kirman *et al.* (2003) derived the hemoglobin binding rates for acrylamide and glycidamide by adjusting these rates to fit the data. In contrast, USEPA used hemoglobin binding rates determined *in vitro* to derive blood AUCs for acrylamide and glycidamide, and adjusted metabolism parameters to achieve fits to these AUCs. USEPA, therefore, assumes that the *in vitro* binding rates are applicable to *in vivo* exposure. This assumption is implicit, rather than being clearly stated, and has not been subjected to rigorous verification, as it should have been to assure the model's validity.

An example of the importance of this validation procedure can be seen for ethylene and ethylene oxide. Csanady *et al.* (2000) developed PBTK models for ethylene and ethylene oxide (EO) disposition in rats, mice, and humans that were validated with both direct EO measurements and EO-derived hemoglobin adducts. As the discrepancies between the EO AUC and adduct measurements were as much as 3-fold, hemoglobin binding rates measured *in vitro* should be used with caution for *in vivo* predictions, lest USEPA largely and unjustifiably overstate acrylamide's carcinogenic potency for humans by a factor of three or more.

2. **Since USEPA's model fails to predict kinetic behavior at high doses (as described on page E-9 of its draft), it will likely fail to predict repeat exposures at moderate doses due to changes associated with co-factor/protein depletion and enzyme induction**.

We concur with the suggestion that the reaction rates for acrylamide and glycidamide conjugation with GSH be expressed as independent of GSH concentration in an updated version of the model. Based on the finding of Kurebayashi and Ohno (2006), nonenzymatic reaction does not appear to significantly contribute to the metabolic clearance of acrylamide or glycidamide, and we agree that the Km with respect to GSH concentration is likely to be low enough that the GST-mediated reaction can be considered zero-order with respect to GSH concentration at relevant doses.

3. USEPA's efforts to validate the recalibrated rat model appear to be incomplete; our limited validation effort indicates that USEPA needs to further revise the model.

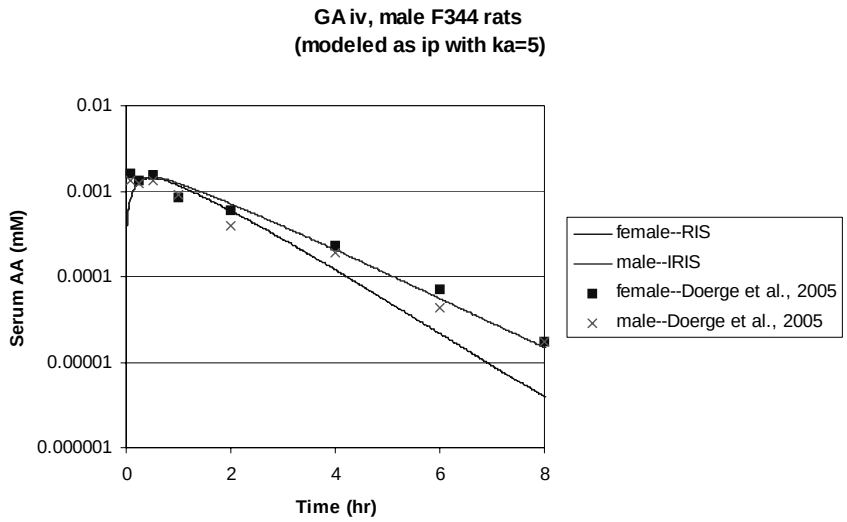
USEPA's recalibrated model was achieved by fitting to a small number of data sets for hemoglobin binding, primarily those of Fennell *et al.* (2005) and Sumner *et al.* (2003). In the recalibration process, the model was also said to be constrained to be consistent with urinary elimination of certain acrylamide metabolites, though no model outputs were provided to illustrate this assertion in either Appendix E or in Walker *et al.* (2007) (which appears to be the published version of the application of USEPA recalibrated model) . A limited comparison to other hemoglobin binding data sets was provided by USEPA. No indication was found that comparisons had been made to the numerous other data sets available, which include many tissue sites other than blood. Specifically, some of the data used in calibrating the original Kirman *et al.* (2003) model were not used (Ramsey *et al.*, 1984 and Miller *et al.*, 1982). Strangely, USEPA's recalibration used the apparent volume of distribution of Doerge *et al.* (2005 a, b, c) to help calibrate the partition coefficients, yet did not use the blood and tissue concentration data therein to calibrate the model. Also, the USEPA model did not use the urinary data of Doerge *et al.* (2007), as it should have. Preferably, the fit of the model to all of the data noted would have been considered in developing the model. At a minimum, the recalibrated rat model should be compared to all of these data and the comparisons documented in the USEPA assessment. These comparisons would allow the peer reviewers and the public to more fully assess the confidence in the model predictions.

USEPA also failed to note the scaled kinetic parameters derived by Kurebayashi and Ohno (2006) from incubated isolated hepatocytes. The data of Kurebayashi and Ohno (2006) support the higher KM derived by USEPA, but a Vmax for acrylamide epoxidation that is closer to the value of Kirman *et al.* (2003). The impact of these changes are not clear at this time as changes to model parameters like the acrylamide epoxidation Vmax may require changes to other model parameters to maintain the fit to other data sets.

Because we have the original Kirman *et al.* (2003) model code, we are able to conduct simulations that should replicate simulations that USEPA's model would produce simply by changing model parameters to match those reported in the draft assessment. (We note, however, that since USEPA has not yet made their model code available to the public, there is no guarantee that our current simulations will be found to replicate USEPA's.) We have limited our comparisons to a subset of the data presented in Doerge *et al.* (2005a). We have chosen to simulate the serum time courses for acrylamide and glycidamide after *iv* or gavage administration of acrylamide to rats (**Figures 2A-D** of Doerge *et al.*, 2005a) and *iv* administration of glycidamide to rats (**Figure 3A** of Doerge *et al.*, 2005a) because these dosing scenarios were either already embedded in the model code (acrylamide iv and gavage)

or could be reasonably approximated (glycidamide *iv* was approximated as *ip* dosing with rapid uptake). USEPA should have made these comparisons before using the recalibrated model in their assessment, and should have provided the comparisons in their model documentation. Based on our simulations (**Figure 1**), use of the USEPA IRIS model appears to adequately simulate the glycidamide *iv* data, with a better fit to male rate data than female rat data. Since USEPA used the volume of distribution derived from this study to set their glycidamide tissue:blood paritition coefficients, it was expected that the early time points should be accurately simulated, as these early points (<1 hr) are dictated primarily by partitioning and not by metabolism. The fit to the later time points indicates that, in general, total glycidamide clearance is adequately represented in the model, though no validation of the rates for the different means of glycidamide clearance (GST metabolism, hydrolysis, and renal elimination) can be inferred from these data.

Figure 1. Simulation of serum glycidamide concentration following iv administration of 0.12 mg/kg glycidamide to F344 rats. Symbols: data of Doerge et al, 2005. Lines: simulations using a reconstruction of USEPA's (2007) recalibration of Kirman *et al.* (2003)



The USEPA (2007) model is not nearly as successful at simulating serum acrylamide and glycidamide concentrations following acrylamide administration (**Figure 2**). Since the "glycidamide alone" simulations were fairly accurate, it stands to reason that the discrepancies are due to inadequate description of the uptake, distribution and/or metabolism of acrylamide. In the *iv* simulations (**Figures 2a** and **2b**), serum acrylamide concentrations were consistently underpredicted by the model, suggesting that acrylamide tissue:blood partition coefficients were overestimated. The initial overestimation of glycidamide concentrations indicates that acrylamide metabolism may be overestimated in the USEPA (2007) model. The overpredictions of serum acrylamide levels at early timepoints in the

gavage studies (**Figures 2c** and **2d**) indicate that a slower absorption rate (KA) would be more appropriate.

Figure 2. Simulation of serum acrylamide (AA) and glycidamide (GA) concentrations in F344 rats after administration of 0.1 mg/kg acrylamide. (A) iv administration to males. (B) iv administration to females. (C) gavage administration to males. (D) gavage administration to females. Symbols: experimental data of Doerge et al. (2005). Lines: simulations using a reconstruction of USEPA's (2007) recalibration of Kirman *et al.* (2003).

Figure 2a

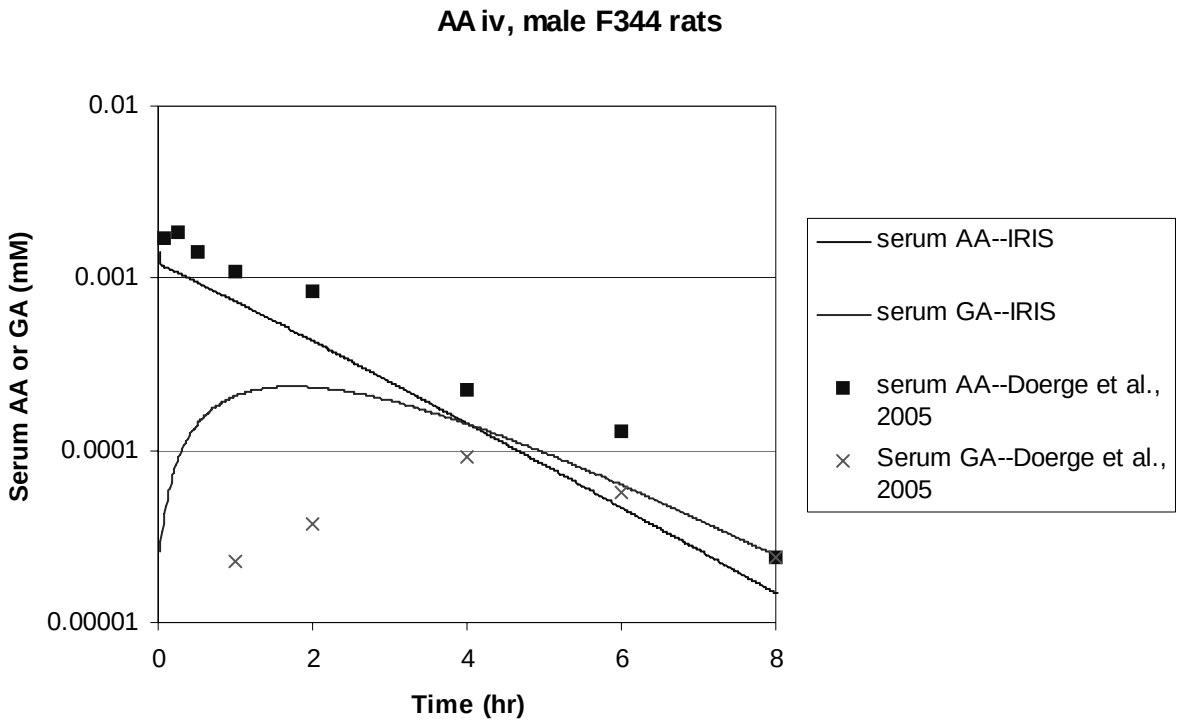


Figure 2b

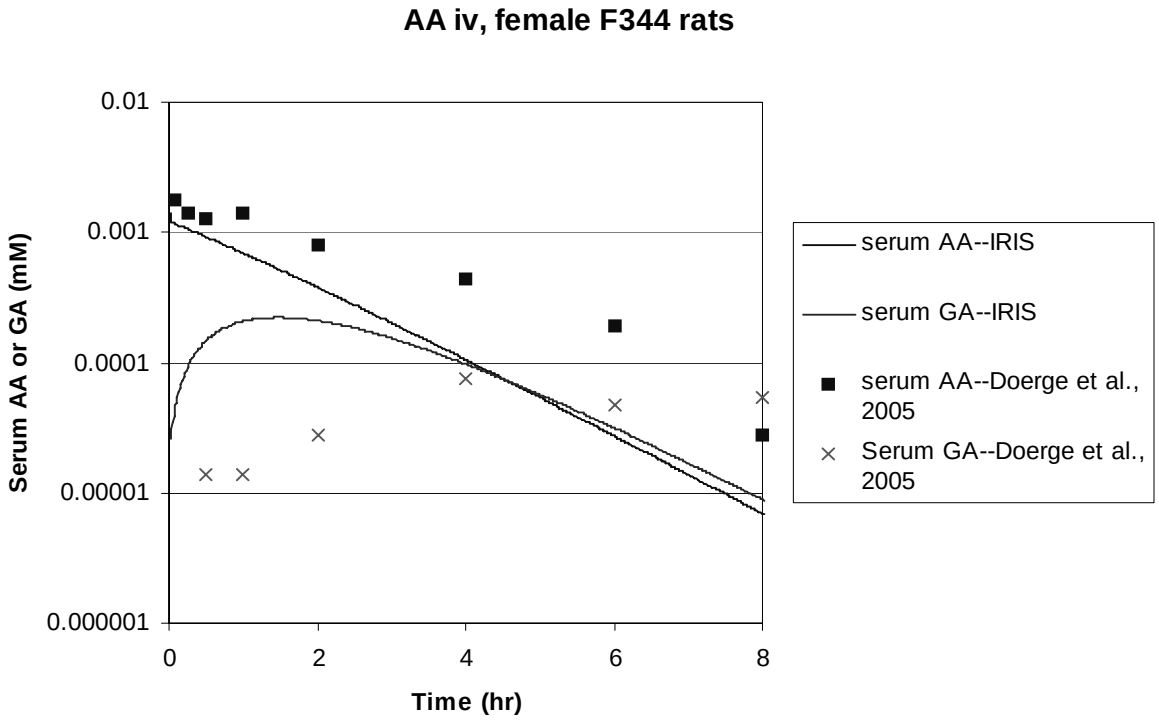


Figure 2c

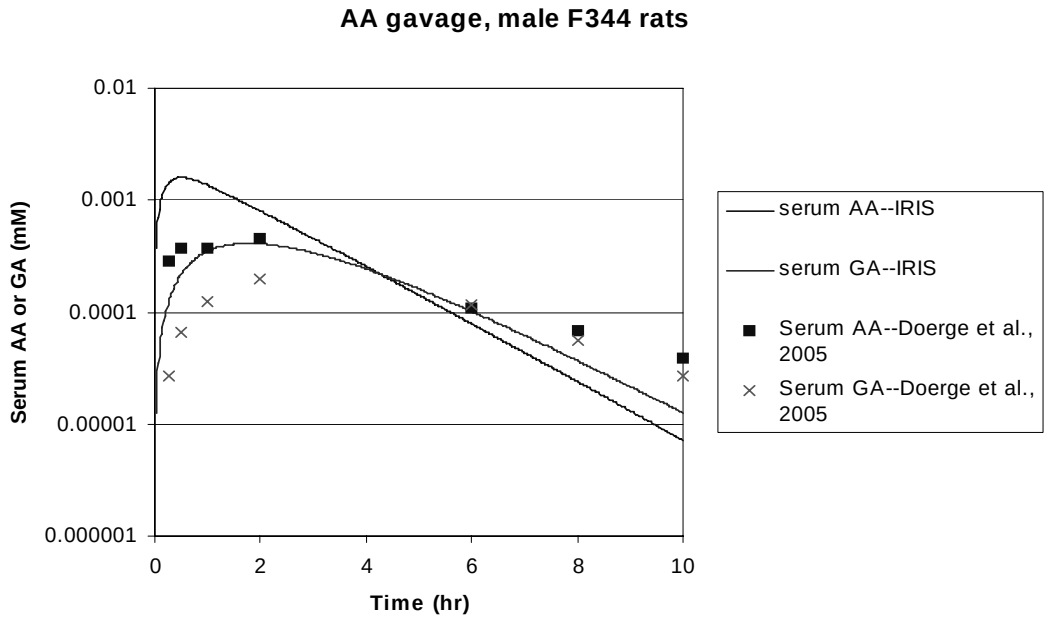
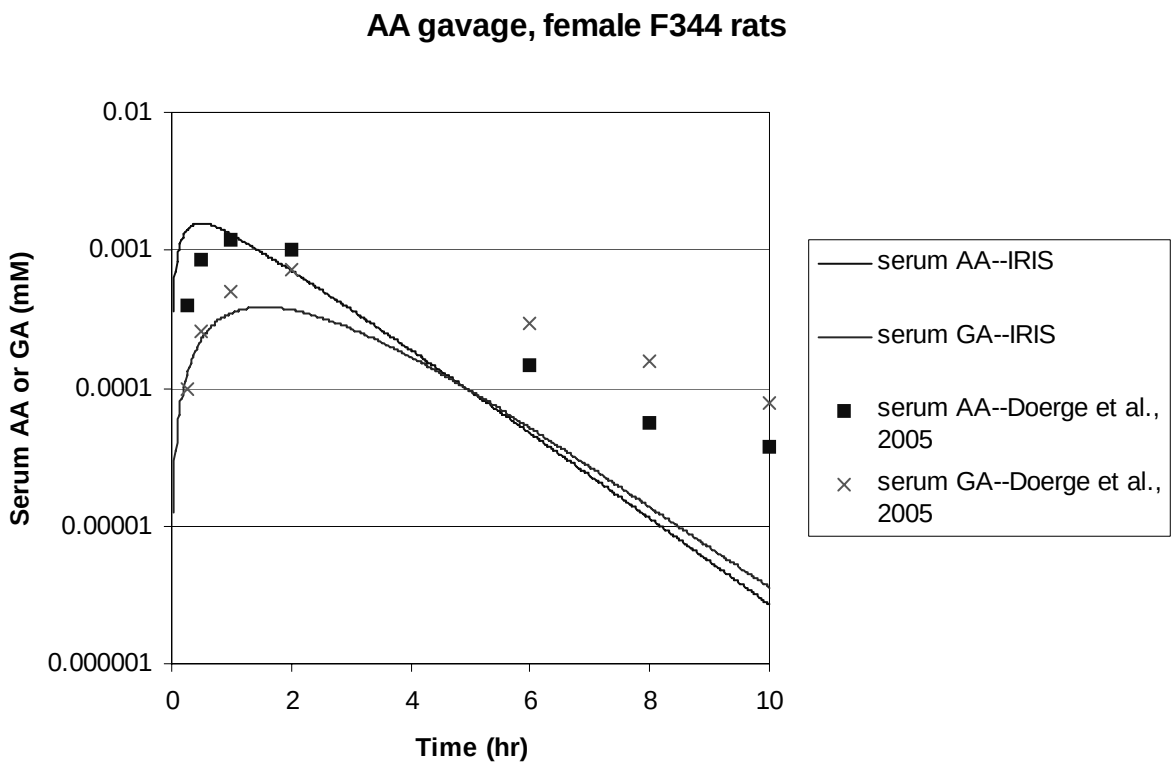


Figure 2d



Since some parameters derived from the rat model fits were used in the human modeling effort (either unchanged, or as a starting point for further adjustment), it is unclear what the rat model failures are on the risk value derivations. However, based on this limited evaluation (there are additional data that could have been modeled), it is clear that <u>the rat model used in the assessment does not adequately reproduce the available data, and this model ought to be updated before it should be used in the assessment</u>.

If USEPA does not update the 2007 model, differences between the rat model described in the USEPA document and the publication of Walker *et al.* (2007) (discussed below) should be clarified in a revised assessment as the Walker *et al.* (2007) model appears to be the published version of the USEPA model.

4. USEPA should explain the discrepancies in binding and metabolism terms between the assessment and the peer-reviewed publication of the PBTK model.

Inspection of Table E-4 reveals that parameter values for physiology, compartment volumes, and absorption are identical for both the Kirman *et al.* (2003) and recalibrated rat models. In the recalibrated model, small changes were made to the partition coefficients (~3-fold decrease in glycidamide partitioning to tissues), metabolism (~2-fold increase in oxidation, ~2-fold decrease in hydrolysis), and glycidamide binding (~2-fold decrease). However, based on the values in Table E-4, large changes were made to binding terms for acrylamide to hemoglobin, liver, other tissues, and blood (~370-fold decrease) during the recalibration process. By contrast, in the Walker *et al.* (2007) publication derived from this effort, the binding terms are frequently two orders of magnitude higher than those in Table E-4, and are thus generally similar to the Kirman *et al.* (2003) values. Smaller discrepancies were also noted for values of metabolism parameters in the assessment vs. Walker *et al.* (2007) (*e.g.*, Vmax for acrylamide oxidation rate of 4.0 vs. 3.52 mg/hr-kg$^{0.7}$).

The recalibrated rat model tended to overestimate the amount of hemoglobin adducts (up to 3- to 4-fold), suggesting that the validity of the recalibrated model is questionable. Furthermore, the human model prediction that approximately 50% of an acrylamide dose is metabolized to glycidamide appears unrealistically high. The urinary metabolite data of Sumner *et al.* (1992) indicate that, in rats, approximately twice as much acrylamide is conjugated with GSH as is metabolized to glycidamide. Since the hemoglobin adduct data of Fennell *et al.* (2005) indicate that humans produce less glycidamide than rats, it does not make sense that in humans the flux through glycidamide should be so high. In future revisions of its draft, USEPA should resolve this matter, and make appropriate changes to the model before generating estimates of cancer potency and of dose-specific risks.

5. As presented, the validation and documentation of the human PBTK model lacked completeness and lacked important details, making it unclear how the USEPA arrived at their current set of parameters.

On page E-19, the additional scaling factor (ratio of 0.04/0.195) for liver oxidation rate in humans is unusual and quite possibly in error; and it appears contrary to data supporting a lower rate of metabolism in humans compared to rats (Fennell and Friedman, 2005), an important consideration in estimating risk-specific ingestion doses for humans. A lack of transparency exists as to how this ratio was derived, and more importantly, why it is justified. One cannot adjust "upward by a factor of (0.04/0.195)" (page E-19) since 0.04/0.195 is less than one. Perhaps the correct ratio is 0.04/0.0195, the fractional liver volumes specified in Walker *et al.* (2007); however, the correct values need to be described and used by USEPA.

The human liver volume listed in Table E-8 is 0.183, which happens to be the same as the fractional liver blood flow in this table. Likewise, the fractional tissue blood flow and fractional tissue volumes in Table E-8 are also identical (0.8842). These values are problematic as the sum of tissue volumes and blood flow to tissues in humans are greater than 100%, a physical impossibility. Since the model code was not provided, we cannot be certain that these clearly erroneous values listed in the table were not also used in the simulations. In general, the scaling adjustments were difficult to follow; examples should be provided.

It is also unclear how much impact the initial scaling adjustments had on the final model parameters because "various" unspecified model parameters were iteratively adjusted to fit the data. USEPA should specify which model parameters were adjusted and how. As with the recalibrated rat model, there are small, unexplained differences between the values presented in Appendix E and the peer-reviewed publication of Walker *et al.* (2007) — raising questions about the reliability of the outputs. USEPA has used a very limited data set to parameterize the human model, and the data of Fuhr *et al.* (2006) was not used. The impact cannot be determined until we know whether or not USEPA's model output is consistent with the data of Fuhr *et al.*

In light of the limitations of the human model, the failure to perform (or, at least, document) a sensitivity analysis is a glaring omission and perhaps fatal flaw. When faced with highly uncertain parameter values with limited validation, USEPA should conduct sensitivity analyses of the dose metrics used in the assessment. Without this information, model confidence cannot be assigned, and thus the confidence in the quantitative aspect of the assessment cannot be assessed.

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C. Carcinogenic Potency (Dose-Response) Considerations

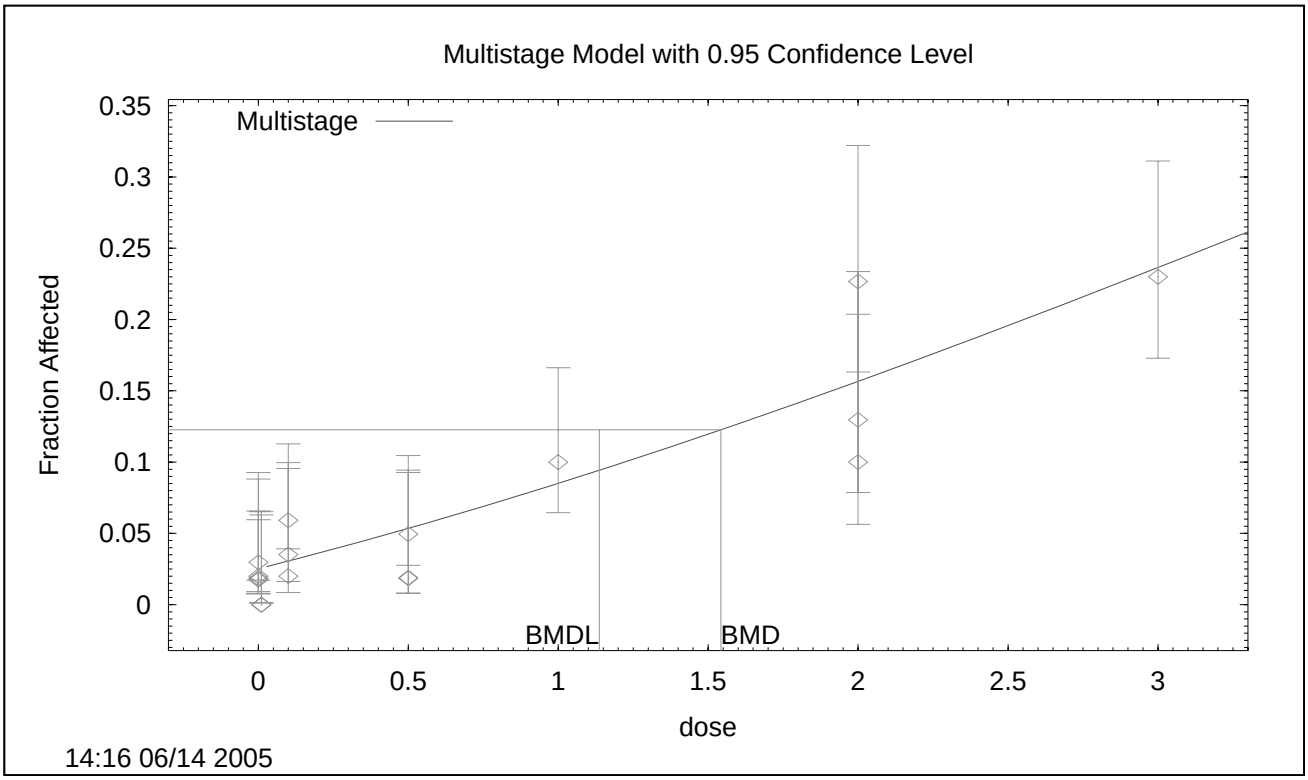
1. Some dose-response data from the Johnson *et al.* (1986) study may be useful for estimating the cancer potency of acrylamide, and should be considered for inclusion in the quantitative assessment.

Although we generally agree with the decision to exclude Johnson *et al.* (1986), there still appear to be some useful data in this study. In particular, the thyroid data for male and female rats from both Johnson *et al.* (1986) and Friedman *et al.* (1995) describe a reasonably consistent dose-response curve (**Table 1**). This consistency suggests that the thyroid tumor data were not significantly affected by deficiencies in the Johnson *et al.* study (*e.g.*, viral infection). More importantly, the Johnson *et al.* study provides dose-response information for thyroid tumors at doses lower than those tested by Friedman *et al.* (1995). Data for thyroid tumors from male and female animals from both studies could be combined to estimate potency (as shown in **Figure 3**). The combined data set provides a more complete depiction of the dose-response relationship at low doses, and can be used to support a point of departure below the default value (10%). This approach was adopted by Dourson *et al.* (2008), who derived a linear dose-response for cancer for acrylamide in terms of administered dose of 0.03 $(mg/kg\text{-}day)^{-1}$ for the pooled rat thyroid tumor data. This alternative value is <u>73-87% smaller</u> than the values derived in terms of administered dose for this tumor type in Appendix D of the draft (*i.e.*, 0.11-0.23 per mg/kg-day).

Table 1. Incidence of Thyroid Tumors in Rats Exposed to Acrylamide (Johnson *et al.*, 1986; Friedman *et al.*, 1995)

Reference	**Sex**	**Dose**	**Thyroid**
Friedman *et al.* (1995)	Male	0	3/202
		0.1	9/203
		0.5	5/101
		2	12/75
	Female	0	2/100
		1	10/100
		3	23/100
Johnson *et al.* (1986)	Male	0	1/60
		0.01	0/58
		0.1	2/59
		0.5	1/59
		2	7/59
	Female	0	1/58
		0.01	0/59
		0.1	1/59
		0.5	1/58
		2	5/60

Figure 3. Multistage Model Fit to Combined Dose-Response Data for Thyroid Tumors in Male and Female Rats (Johnson *et al.*, 1986; Friedman *et al.*, 1995).



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2. Low-Dose extrapolation models other than the multistage model used exclusively by USEPA should be employed in the dose-response assessment, with selection based on goodness-of-fit/AIC comparison.

USEPA relied upon the multistage model from USEPA's Benchmark Dose Software (BMDS) in estimating the cancer potency of acrylamide. However, other models (gamma, logistic, probit, quantal linear, Weibull) are also available in the software program, and their use should be considered for inclusion in the dose-response assessment, an approach that would be consistent with USEPA guidelines. For example, a comparison of AIC values and goodness of fit (**Table 2**) could be used to support USEPA's decision to use the multistage model for combined female rat tumor data. These results demonstrate that the multistage model provides an adequate fit to the data by comparing to the other available models.

Table 2. Comparison of BMDS Models Fit to Combined Tumor Data in Female Rats

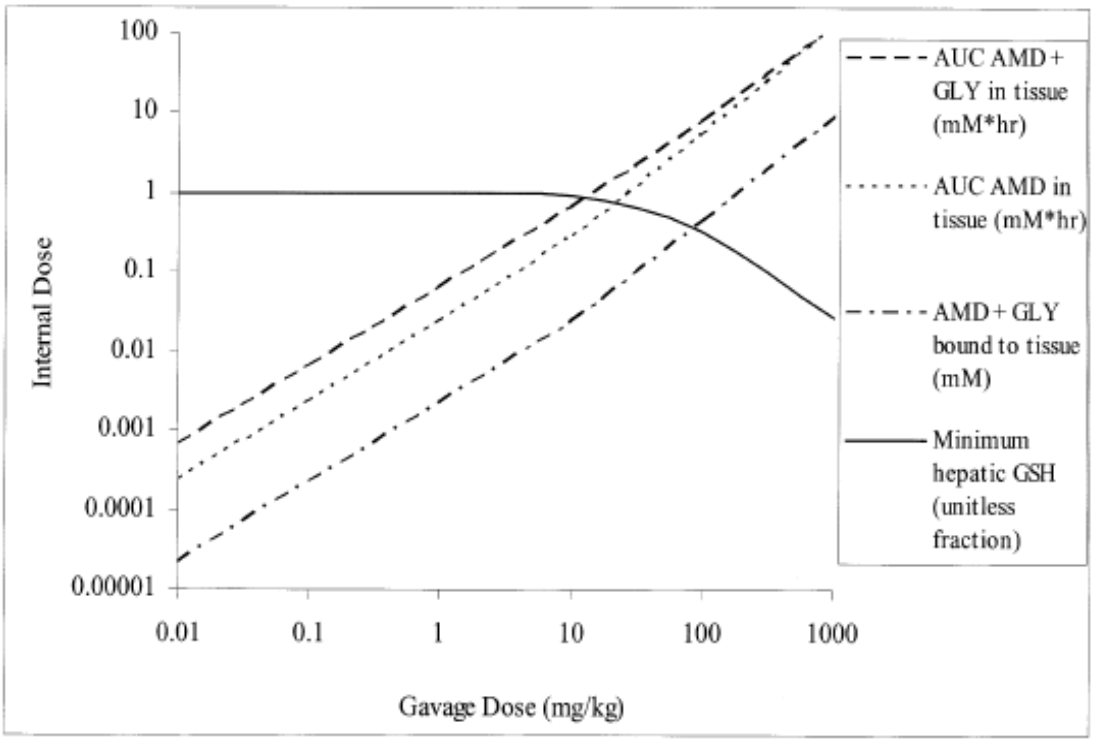
Model	**AIC**	**p-value**	**BMD10**	**BMDL10**
Gamma	320.8	0.379	0.55	0.42
Logistic	322.2	0.210	0.92	0.77
Multistage	320.8	0.379	0.55	0.42
Probit	322.0	0.232	0.87	0.73
Quantal Linear	320.8	0.379	0.55	0.42
Weibull	320.8	0.379	0.55	0.42

A similar comparison table should be prepared for the male rat tumor data (note - combined tumor incidence data are not provided in Table D-1 of USEPA's Appendix D and should be included).

3. USEPA's integration of the results of the internal dosimetry (PBTK) modeling into the dose-response modeling is incomplete.

Human equivalent doses for each dose group based upon PBTK model are not presented in USEPA's draft (specifically, Appendix D). Instead, all dose-response modeling appears to have been performed using unscaled, administered dose. Application of the PBTK model results appears in the main body of the report (Section 5.4.4). USEPA appears to have applied the PBTK model to the points of departure (POD) only, rather than to each dose group. This approach might be sufficient if acrylamide kinetics were linear across the range of observation defined by the key study. However, if nonlinear kinetics occur in the range of observation (as is likely to be the case), then use of an internal dose measure would alter the spacing of the dose groups, which in turn can alter the fit of the dose-response model(s) used. Kirman *et al.* (2003) reported that the metabolism of acrylamide become saturated as doses approach 10 mg/kg (see curve deflections in **Figure 4**). It is not clear at what doses acrylamide kinetics become non-linear using the modified PBTK model. USEPA should incorporate the PBTK internal dose measures into Appendix D, and furthermore needs to either (1) demonstrate that the toxicokinetics of acrylamide, as predicted by their PBTK model, are linear across the range of observation; or (2) apply the dose-response model to each dose group prior to fitting a dose-response model to the data. If USEPA did so, the resulting outcome would change, but in a direction and to a degree that cannot be predicted with the information in the current draft.

Figure 4. Behavior of Internal Doses as a Function of Administered Dose of Acrylamide (from Kirman *et al.* 2003)



In addition, we recommend that USEPA consolidate the age-dependent-adjustment factor (ADAF) adjustments (along with PBTK adjustments) within Appendix D, to avoid potential confusion by the reader between the values derived in this Appendix and those described in the main body of the report. A summary table is provided in **Table 3**.

Table 3. Summary of Cancer Potency Estimates For Acrylamide

				Point of Departure		**Linear Potency (Cancer Slope Factor, per mg/kg-day)**		**Nonlinear Potency (Cancer Reference Dose, mg/kg-day)**	
Tumor Data Set	**Dose Measure**	**ADAF (Duration)**	**BMR**	**ED**	**LED**	**BMR/ ED10**	**BMR/ LED10**	**ED/ UF***	**LED/ UF***
Thyroid or TVM in male rats	Administered	No	0.1	0.70	0.30	0.14	0.33	0.002	0.001
		Yes (30-year)	0.1	0.66	0.28	0.15	0.36	0.002	0.0009
		Yes (70-year)	0.1	0.43	0.18	0.23	0.55	0.001	0.0006
	PBTK	No	0.1	0.52	0.22	0.19	0.45	0.002	0.0007
		Yes (30-year)	0.1	0.42	0.20	0.24	0.49	0.001	0.0007
		Yes (70-year)	0.1	0.27	0.13	0.36	0.75	0.0009	0.0004
Thyroid or Mammary Gland in female rats	Administered	No	0.2	1.2	0.88	0.17	0.23	0.004	0.003
		Yes (30-year)	0.2	1.1	0.8	0.18	0.25	0.004	0.003
		Yes (70-year)	0.2	0.71	0.52	0.28	0.38	0.002	0.002
	PBTK	No	0.2	0.75	0.64	0.27	0.31	0.003	0.002
		Yes (30-year)	0.2	0.69	0.59	0.29	0.34	0.002	0.002
		Yes (70-year)	0.2	0.45	0.38	0.44	0.52	0.002	0.001

*UF = 300

As can be observed, the non-linear estimates of cancer potency are <u>2 to 3 orders of magnitude less</u> than those estimated based on a linear assumption. The non-linear approach is consistent with evidence indicating that the cancer mode-of-action for acrylamide / glycidamide is at least as likely, if not more so, to be based on non-genotoxic phenomena than genotoxicity. At the very least, USEPA should present both cancer potency estimates with a statement of the degree of likelihood that either one may be dominant to the other.

4. USEPA's use of a time-to-response model for acrylamide carcinogenicity is not warranted.

USEPA used a multistage-Weibull time-to-response model from K.S. Crump and Co. in its dose-response assessment for the carcinogenic effects of acrylamide. Unfortunately, this software program (Multi-Weib, 1985) is no longer available or supported, and, therefore, the transparency and reproducibility of USEPA's assessment is adversely affected. More importantly, use of the time-to-response model for male rats does not appear to be supported for tunica vaginalis mesothelioma data since the time function coefficients fit by the model (T0, J) remained at default values (0 and 1, respectively).

Although use of a time-to-response model for thyroid tumors was explained by USEPA as being due to increased mortality, the consistency of the dose-response data for male and female rats (**Table 1, Figure 3**) suggests that the increased mortality in male rats did significantly alter the dose-response relationship for this tumor type. The simpler models (multistage and others in BMDS) should be presented, at least for comparison. Furthermore, consideration should also be given to using the poly-3 adjusted incidence rates (Portier and Bailer, 1989) to address potential concerns with early mortality. If the effects on survival are not large at the estimated points of departure (POD), then the simpler models would serve as an appropriate basis for estimating cancer potency, and the time-to-response model can be dropped.

5. USEPA's assumption that the different tumor types are statistically independent is likely <u>not valid</u> for acrylamide, and may <u>not have been calculated correctly</u>.

Because the rat tumors are generally attributed to the formation of glycidamide, which is dependent upon systemic factors (*e.g.*, hepatic formation and subsequent distribution to target tissues), the individual tumor types are not expected to be statistically independent. Specifically, a rat with a higher rate of glycidamide formation and/or lower rate of glycidamide detoxification is expected to be at a greater risk of either tumor type than a rat with a lower rate of glycidamide formation and/or higher rate of glycidamide detoxification. However, because they summed risk two different ways (one which does not require the independence assumption) and yielded essentially the same result, this assumption does not appear to have a large impact on the combined potency estimate in this case. However, under an assumption of statistical independence, the combined risk needs to be summed as follows to avoid double counting animals with multiple tumors:

$$Combined\ Risk(Tumor\ A\ or\ B) = Risk(A) + Risk(B) - Risk(A)*Risk(B)$$

It is unclear if USEPA included the last term in this equation (which is likely to be small) in their calculations for combining risk across tissues. If excluded, then USEPA has not assumed independence, but instead has assumed that the tumors are mutually exclusive. USEPA should either: (1) confirm that tumor risks were summed appropriately using the equation above; or (2) state that mutual exclusivity was assumed, if this is the case. The value in doing so would be to improve the clarity of USEPA's dose-response assessment.

6. Lower-bound estimates of cancer potency need to be presented along with the central tendency and upper bound limits as specified by Agency Guidelines.

Although USEPA presented central tendency and upper-bound estimates of cancer potency for acrylamide, no lower bound estimates were presented. For consistency with USEPA (2005) cancer guidelines which states, "*...risk assessors should calculate, to the extent practicable, and present the central estimate and the corresponding upper and lower statistical bounds (such as confidence limits) to inform decisionmakers.*", these values should be included.

References

Environmental Protection Agency. (2005). Guidelines for carcinogen risk assessment. EPA/630/P-03/001B.

Kirman, C. R., Gargas, M. L., Deskin, R., Tonner-Navarro, L., and Andersen, M. E. (2003). A physiologically based pharmacokinetic model for acrylamide and its metabolite, glycidamide, in the rat. J Toxicol Environ Health A **66**, 253-274.

Portier, C. J. and Bailer, A. J. (1989). Testing for increased carcinogenicity using a survival-adjusted quantal response test. Fundam.Appl Toxicol **12**, 731-737

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7. Heritable Germ Cell Toxicity is not applicable to acrylamide

USEPA has included in its IRIS draft for acrylamide a section on "heritable germ cell studies." The section summarizes reviews and studies that relate acrylamide to this phenomenon. USEPA hypothesizes that exposures to genotoxic agents (the Agency includes acrylamide in this group) by parents may lead to mutations in male and/or female germ cells and that such mutations might lead to any number of diseases (including cancer) in offspring of multiple generations.

NTP-CERHR concluded that data such as that cited by EPA should not be used to quantify risks to the general population exposed to low doses of acrylamide because of a lack of dose levels below the reproductive and developmental toxic effects observed (NTP, 2005).

The studies that bear on the question for acrylamide do not meet important criteria to suggest, much less demonstrate, that ingested acrylamide (including glycidamide) can elicit heritable mutations in human germ cells. The *in vivo* studies have been performed at very high doses, doses capable of causing frank neurotoxicity, were administered mostly by intraperitoneal injection and occasionally via dermal application (neither of which is relevant to ingestion), and, if dosing had been continued for a lifetime, would have greatly exceed the carcinogenic doses rates in rats. Such studies are incapable of being used to estimate risks at low doses in test animals much less at low doses experienced by humans, based on professional standards in the field of risk assessment.

Were USEPA to apply its own "Guidelines for Mutagenicity Risk Assessment" to these data (USEPA, 1986), it might conclude that acrylamide met the qualitative requirements of the hazard assessment step of risk assessment, yet fail to come even close to meeting the criteria of the more important dose-response step. Indeed, those guidelines admit that effects from the tests addressing heritable mutations depart "from linearity at low exposure and exposure rates," implying the presence and acceptance of toxicological thresholds for heritable mutations that would be applicable to acrylamide.

We strongly recommend that USEPA, in its next draft of the IRIS document, clearly state that such heritable effects have not been documented with existing acrylamide data. While USEPA may wish to identify and promote opportunities for future laboratory investigation (a position already established by USEPA in the first draft document) and development of risk assessment methodologies, this assessment is not an appropriate forum. Neither the data nor the risk assessment methodologies are yet suitable for characterizing risks of heritable germ cell effects on human health.

References

Environmental Protection Agency. (1986). Guidelines for mutagenicity risk assessment. EPA/630/R-98/003.

National Toxicology Program Center for the Evaluation of Risks to Human Reproduction. (2005). NTP-CERHR Monograph on the potential human reproductive and developmental effects of acrylamide. NIH Publication No. 05-4472.

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