OAPTF

Organic Arsenical Products Task Force

Luxembourg-Pamol, Inc. • Drexel Chemical Company

March 29, 2010

Via E-mail and Hand Delivery

Dr. Sue Shallal
Designated Federal Officer
Science Advisory Board (1400F)
United States Environmental Protection Agency
1200 Pennsylvania Avenue, N.W.
Washington, D.C. 20460

Re: Toxicological Review of Inorganic Arsenic: In Support of the Summary Information on the Integrated Risk Information System -- Comments Submitted for Review by the SAB Workgroup

Dear Dr. Shallal

The Organic Arsenical Products Task Force (OAPTF) is pleased to submit the attached comments prepared by Gradient Inc. on the draft document entitled "Toxicological Review of Inorganic Arsenic: In Support of the Summary Information on the Integrated Risk Information System (IRIS) (US EPA, 2010)" (2010 Draft IRIS Report).

On April 6-7, 2010, a Science Advisory Board (SAB) workgroup will meet to review the current InAs assessment presented in the 2010 Draft IRIS Report. In a February 26, 2010, meeting, Dr. Paul Anastas, Assistant Administrator for Research and Development of the U.S. Environmental Protection Agency (EPA), stated that the workgroup is not bound to respond only to the Charge Questions posed to it by EPA, and that the workgroup is free to review and discuss any issue even if not included in those questions. It is in this spirit that we have reviewed the 2010 Draft IRIS Report in detail, and have identified several concerns with the analysis.

The revised CSF (25.7 mg/kg-d) is 17 times greater than the CSF that currently exists in IRIS (1.5 mg/kg-d). Such a steep CSF will likely have serious adverse implications for many sectors of the economy, municipal governments, and the U.S. population. In view of the serious ramifications that may result from the InAs assessment, it is crucial that the assessment is scientifically supportable and based on the state-of-the-art-information.

P.O. Box 33856, Washington, D.C. 20033-0856 • Tel. (800) 890-3301, Fax (202) 557-3836

Therefore, the workgroup should review the full breadth of comments received even if they are beyond the scope of the charge questions. It is crucial to ensure that the conclusions in the report are based on currently existing, valid scientific knowledge, and supported by a consensus of the scientific community.

Thank you for your considerations of our comments.

Sincerely,

Michal Eldan, Ph.D., Chair

Attachment

cc: The Honorable Paul Anastas (w/attachment) (via e-mail)
Mr. Nathan Gentry (w/attachment) (via e-mail)

P.O. Box 33856, Washington, D.C. 20033-0856 • Tel. (800) 890-3301, Fax (202) 557-3836

Comments on the Draft Document: "Toxicological Review of Inorganic Arsenic: In Support of the Summary Information on the Integrated Risk Information System (IRIS)"

Prepared by
Gradient
20 University Road
Cambridge, MA 02138

on behalf of
the Organic Arsenical Products Task Force

Submitted

March 29, 2010

Executive Summary

The Organic Arsenical Products Task Force (OAPTF) is pleased to submit the attached comments prepared by Gradient on the draft document entitled "Toxicological Review of Inorganic Arsenic: In Support of the Summary Information on the Integrated Risk Information System (IRIS)" (US EPA,) (2010 draft IRIS report).

On April 6-7, 2010, a Science Advisory Board (SAB) Work Group (Workgroup) will meet to review the current InAs assessment presented in the 2010 draft report. In a February 26, 2010 meeting, Dr. Paul Anastas, Assistant Administrator for Research and Development of the United States Environmental Protection Agency (US EPA), stated that the Workgroup is not bound to respond only to the Charge Questions posed to it by US EPA, and that the Workgroup is free to review and discuss any issue even if not included in those questions. It is in this spirit that we have reviewed the 2010 draft report in detail and have identified several concerns with the analysis.

The revised cancer slope factor (CSF) (25.7 mg/kg-d) is 17 times greater than the CSF that currently exists in IRIS (1.5 mg/kg-d). Such a steep CSF will likely have serious adverse implications for many sectors of the economy, municipal governments, and the U.S. population. It is, therefore, crucial to ensure that the conclusions in the report are based on currently existing, valid scientific knowledge, and supported by scientific community consensus. Also, it is, therefore, important for the Workgroup to consider the full breadth of comments received.

Because of the insufficient time allowed for public review, there are certain areas on which we could not expand. Therefore, this document will focus only on some of the key issues to which we would like to draw the attention of the Workgroup. The key issues, described in detail in this document, are the following:

1. **Failure to Adequately Address SAB Panel Comments:** In 2005, SAB reviewed the draft document and made numerous recommendations, which were published in a report in 2007 (SAB, 2007). While the 2010 draft report addresses some of the 2005 SAB comments, many issues have not been considered in the new report and remain unresolved. The recommendations of the 2005 SAB were inadequately incorporated into the 2010 IRIS draft report, particularly regarding the consideration of non-linear models and the evaluation of the applicability of the Taiwanese database for US populations. We understand that members of the 2005 SAB also made similar comments about the inadequacy of the 2010 draft IRIS report, in particular, with regard to the use of mode of action (MOA) and epidemiological data.
2. **Unresolved Scientific Issues:** The 2010 draft IRIS report contains several scientific issues that remain unresolved. Specific areas of concern include:

a. The continued reliance on the Taiwanese data set, especially given its recognized limitations, particularly for exposure characterization;

b. The remaining questions regarding the baseline cancer rate and other drinking water contaminants in the Taiwan study population;

c. The inadequacy of US EPA's alternative modeling approaches, especially concerning the selection of a comparison population and the use of a linear model;

d. The inadequate consideration of epidemiological studies other than the study from Taiwan, especially those of exposure to low InAs doses, and the failure to perform meta-analyses; and

e. The failure to consider the accumulating evidence from mechanistic studies that carcinogenicity MOA of InAs is likely non-linear.

3. **Overly Narrow Interpretation of US EPA Cancer Guidelines:** The 2010 draft report has applied a very narrow interpretation to the US EPA Guidelines for Carcinogen Risk Assessment (US EPA, 2005) by assuming that lack of a well established MOA necessitates the exclusive use of linear extrapolation. In fact, the Guidelines recommend the consideration of other biologically plausible alternatives, including non-linear approaches.

4. **Failure to Consider Available Literature:** During the nearly three years that have passed since the previous SAB review, new data have been published, providing significant new information relevant to the carcinogenicity of inorganic arsenic (InAs). US EPA's analyses do not consider data published after 2007, nor state-of-the-art information from ongoing active research programs some of which are sponsored by EPA and focused on InAs's MOA. As a result, the 2010 draft IRIS report is not based on the best available scientific information.

In view of the serious ramifications that may result from the InAs assessment, it is crucial that the assessment is scientifically supportable and based on the state-of-the-art information. Therefore, it is important for the Workgroup to review all these issues even if they are beyond the scope of the charge questions.

Table of Contents

Introduction

The Organic Arsenical Products Task Force (OAPTF) is pleased to submit the attached comments prepared by Gradient on the draft document entitled, "Toxicological Review of Inorganic Arsenic: In Support of the Summary Information on the Integrated Risk Information System (IRIS)" (US EPA, 2010) (2010 draft IRIS report). This document, which is intended to form the scientific basis for a revision to the inorganic arsenic (InAs) cancer slope factor (CSF) in IRIS, is a new version of a report released in 2005.

The revised CSF (25.7 mg/kg-d) is 17 times greater than the CSF that currently exists in IRIS (1.5 mg/kg-d) and seven times greater than the CSF calculated by the Office of Pesticide Programs (3.67 mg/kg-d) (US EPA, 2006)[1]. Such a steep CSF will likely have serious adverse implications for many sectors of the economy, municipal governments, and the US population. It is, therefore, crucial to ensure that the conclusions in the report are based on currently existing, valid scientific knowledge, and supported by scientific community consensus.

In 2005, a Science Advisory Board (SAB) Panel reviewed the draft document and made numerous recommendations, which were published in a report in 2007 (SAB, 2007). While the 2010 draft IRIS report addresses some of the 2005 SAB Panel comments, many issues have not been considered in the new report and remain unresolved. Additionally, during the nearly three years that have passed since the previous SAB review, new data have been published, providing significant new information relevant to the carcinogenicity of InAs.

On April 6-7, 2010, an SAB Work Group (Workgroup) will meet to review the current InAs assessment presented in the 2010 draft report. In a February 26, 2010 meeting, Dr. Paul Anastas, Assistant Administrator for Research and Development of the United States Environmental Protection Agency (US EPA), stated that the Workgroup is not bound to respond only to the Charge Questions posed to it by US EPA, and that the Workgroup is free to review and discuss any issue even if not included in those questions. It is in this spirit that we have reviewed the 2010 draft report in detail and have identified several concerns with the analysis. Because of the insufficient time allowed for public review, there are certain areas on which we could not expand. Therefore, this document will focus only on some of the key issues to which we would like to draw the attention of the Workgroup, and which we believe

[1] The CSF of 1.5 mg/kg-d is based on skin cancer in Taiwan, while the 3.67 mg/kg-d is based on bladder and lung cancer in Taiwan (the same study and data used in the present 2010 draft IRIS assessment).

the Workgroup should consider as it evaluates this updated analysis and makes recommendations to US EPA. We understand others will be providing detailed comments on other important issues such as recent epidemiological studies, or US EPA's analysis of drinking water consumption and food ingestion rates. It is important for the SAB to consider the full breadth of comments received.

The key issues, described in detail in this document, follow:

1. **Failure to Adequately Address SAB Panel Comments:** The recommendations of the 2005 SAB Panel were inadequately incorporated into the 2010 draft report, particularly regarding the consideration of non-linear models and the evaluation of the applicability of the Taiwanese database for US populations.
2. **Unresolved Scientific Issues:** The 2010 draft IRIS report contains several scientific issues that remain unresolved. Specific areas of concern include:
    a. The continued reliance on the Taiwanese data set, especially given its recognized limitations, particularly for exposure characterization;
    b. The remaining questions regarding the baseline cancer rate and other drinking water contaminants in the Taiwan study population;
    c. The inadequacy of US EPA's alternative modeling approaches, especially concerning the selection of a comparison population and the use of a linear model;
    d. The inadequate consideration of epidemiological studies other than the study from Taiwan, especially those of exposure to low InAs doses, and the failure to perform meta-analyses; and
    e. The failure to consider the accumulating evidence from mechanistic studies that carcinogenicity mode of action (MOA) of InAs is likely non-linear.
3. **Overly Narrow Interpretation of US EPA Cancer Guidelines:** The 2010 draft IRIS report has applied a very narrow interpretation to the US EPA Guidelines for Carcinogen Risk Assessment (US EPA, 2005) by assuming that lack of a well established MOA necessitates the exclusive use of linear extrapolation. In fact, the Guidelines recommend the consideration of other biologically plausible alternatives, including non-linear approaches.
4. **Failure to Consider Available Literature:** US EPA's analyses do not consider data published after 2007, nor state-of-the-art information from ongoing active research programs—some of which are sponsored by US EPA and focused on InAs's MOA. As a result, the 2010 draft IRIS report is not based on the best available scientific information.

In view of the serious ramifications that may result from the InAs assessment, it is crucial that the assessment is scientifically supportable and based on the state-of-the-art information. Therefore, we propose that US EPA:

- Incorporate the large body of currently published literature as well as ongoing research that provides further support for an MOA involving low-dose non-linearity;
- Re-evaluate the full range of epidemiological studies, including a more balanced assessment of the Taiwan data in the context of additional epidemiological studies, some of which are not cited by the 2010 draft IRIS report, and perform a meta-analysis or consider other meta-analyses that were performed recently (*e.g.*, Mink *et al*., 2008);
- Perform a weight of evidence analysis to assess the relative strength of the linear dose-response model *versus* other models that are based on available epidemiological and mechanistic information; and
- Conduct a more complete dose-response analysis, including the use of non-linear models, in quantification of cancer risks from InAs. In particular, we recommend that a Margin of Exposure (MOE) analysis be conducted, based on points of departure developed from epidemiological studies as well as from MOA studies.

Because of the insufficient time, we will discuss only part of these recommendations in more depth below.

1 2007 Scientific Advisory Board Panel's Suggestions that are not Adequately Addressed in the Current Draft

In response to the US EPA charge questions for the 2005 assessment, the SAB Panel made several recommendations for further analyses that would be necessary to address scientific deficiencies in the document. Many of these suggestions were ignored in the 2010 draft IRIS report. Key SAB recommendations that were not adequately addressed relate to the following:

- Consideration of physiologically-based pharmacokinetic (PBPK) and biologically-based dose response (BBDR) models in the overall evaluation of InAs carcinogenicity;
- Performance of a robust sensitivity analysis for the dose-response evaluation of the Taiwan data, including evaluation of the impact of alternative dose-evaluation and model-fitting assumptions (including consideration of non-linear models);
- Justification of dose-response model assumptions;
- Explicit and quantitative examination of *other* epidemiology data *in addition to* the Taiwan data set, including calculation of alternative potency estimates;
- Performance of an "integrative" analysis of the body of low-dose epidemiology evidence; and
- Consideration of the evidence that the InAs carcinogenic MOA is consistent with a non-linear action that "*implies a threshold.*"

Table A.1 (in Appendix A) compares the recommendations of the 2005 SAB Panel with the 2010 draft IRIS report and identifies SAB questions that were not addressed. Table A.1 includes the following items for issues relevant to this report:

a. US EPA's original charge questions posed to the 2005 SAB Panel (for relevant issues);

b. SAB's response and request for additional analyses as presented in the 2007 SAB Report;

c. The implementation of SAB's recommendations as presented in the 2010 draft IRIS report; and

d. Comments on the limitations in US EPA's response and/or implementation of SAB's suggestions in the 2010 draft IRIS report.

We understand that members of the 2005 SAB Panel also made similar comments about the inadequacy of the 2010 draft IRIS report, and in particular, with regard to the use of MOA and epidemiological data.

2 Key Scientific Issues that are not Adequately Addressed in the 2010 Draft IRIS Report

2.1 Inadequate Evaluation of Epidemiological Evidence

The SAB Panel specifically noted the epidemiological evaluation in the 2005 draft was inadequate. Key SAB (2007) recommendations included:

- "The Panel urged the Agency to consider other epidemiology studies from the U.S. and other countries, utilizing a uniform set of evaluative criteria. The Panel also recommended sensitivity analyses be conducted to account for human variability in drinking water consumption rates, dietary intake of iAs from food, and certain other assumptions currently used in EPA's assessment" (p. 2).
- "In view of the limitations of this database, the Panel recommends that the other relevant epidemiology databases from studies of arsenic-exposed populations be used to compare the unit risks at high exposure levels that emerge from the Taiwan data. Several of these studies had the advantage of data with excellent exposure assessment" (p. 38).

While the current IRIS support document contains an expanded discussion of epidemiological data sets outside of Taiwan, including the merits and limitations of each individual study, there is no meaningful synthesis of the data or any effort to reconcile disparate and similar data. For example, existing or *de novo* meta analyses, such as the one conducted by Mink *et al*. (2008), have not been explored or considered. Synthesis of the available data is critical, not only to provide perspective on what the literature collectively supports, but to quantify an integrated expression of cancer potency.

Instead of synthesizing information from multiple data sets to strengthen quantitative risk estimates, the 2010 draft IRIS report analysis appears to focus on defending the use of the data set from Taiwan as the basis for quantitative cancer potency estimates. This is in spite of rigorous analyses that have exposed the profound uncertainty in the data set, particularly in terms of background cancer rates and exposure characterization. As noted by the 2005 SAB Panel in the 2007 report, InAs epidemiology is a rich data set replete with studies with significantly more reliable exposure characterizations and study designs. These studies considered collectively can provide more reliable insights to exposures associated with InAs's cancer effects.

Overall, the literature that was available at the time of the 2010 draft IRIS report and the 2007 SAB review provided convincing evidence that the dose-response relationship for InAs is non-linear and that InAs is a human carcinogen only at high exposure levels (for review see Schoen *et al.,* 2004 ; Petito-Boyce *et al.,* 2008. Additionally, available US studies consistently found no relationship between InAs exposure and cancer, even in areas with relatively high InAs exposure (*e.g.*, Lewis *et al*., 1999; Steinmaus *et al.*, 2003). Recent studies, since the 2007 SAB Panel review, continue to provide further support that InAs has a non-linear dose response. While several studies confirm that InAs is associated with carcinogenic effects at doses greater than 400 µg As/L water [*e.g.*, Marshall *et al*., 2007 (lung and bladder cancer)], other recent studies provide further evidence that InAs is not associated with carcinogenicity at low dose levels, *i.e*., is likely to exhibit a threshold (*e.g.,* Baastrup *et al*. 2008; Mink *et al*., 2008; Meliker *et al*., 2010).

2.2 Outstanding Issues with Dose-Response Modeling

2.2.1 Use of the Taiwan Study

As discussed above, the quantitative assessment of the carcinogenic potency of InAs relies exclusively on the data set from Taiwan. Despite some advantages, the Taiwan study has features that complicate its use in dose-response analysis, particularly if risk interpretations are limited to any one analysis of the data. Specifically, there are questions about:

a) specific assumptions for assigning exposures to inhabitants of each village;
b) the effect of the model chosen for fitting to the data; and
c) the impact and appropriateness of using a population external to the study area as a reference population in model fitting.

The remedy for these questions is to thoroughly and forthrightly explore the issues using alternative analytical approaches and to compare the consistency of results from the Taiwan study with results obtained from other epidemiological studies. It is not a question of choosing another isolated study as an alternative, but rather of gauging whether conclusions from the Taiwan study are robust to choices of alternative reasonable assumptions and analytical methods, as well as to evaluate whether those results are consistent with observations in other populations.

The Taiwan study is large, with a substantial population that includes widely varying levels of chronic exposure to InAs among members of a geographically stable and ethnically uniform population. However, it is an ecological study, comparing village-by-village cancer rates with assumed typical InAs exposures assigned to all residents of each village, estimated from water well measurements of InAs, thus it has the limitations inherent to ecological study design. The specific implications with relying on ecological data in the Taiwan data set have been actively investigated, and the effect of the bias caused by the reliance on median well data was demonstrated to provide uncertain risk estimates (Brown, 2007).

Importantly, the variation in levels of InAs in well water among villages arises because of their geographic separation. Any other factor that varies geographically, and which may influence lung or bladder cancer rates, also has the potential of acting as a confounder to the apparent effect of InAs. With only 42 villages in the study, such a factor need not affect many villages to substantially confound and alter the apparent association of InAs exposure and cancer rates.

In fact, there are several observations indicating that such an alternative factor is indeed operating. First, the study area is characterized by varying levels of Blackfoot disease (BFD), a disease not found elsewhere in the world; some villages are in the BFD-endemic area while others are not (Guo, 2007; Lamm *et al*., 2006). Additionally, several analyses have suggested the presence of high levels of fluorescent humic acids in the artesian well water in this area. There is a further suggestion that these substances are the causative factors for the high levels of BFD and other disorders, including cancer, which are found in the BFD-endemic region of SW Taiwan (Chen *et al.*, 1962; Lu, 1990, 1988; Lamm *et al.*, 2003).

Second, even in villages with low InAs exposure, the levels of lung and bladder cancer seem elevated above the levels elsewhere in Taiwan; the effect of InAs on lung or bladder cancer seems to be on top of some other local factor that causes these diseases to appear at higher rates even in populations without any unusual exposure to InAs. Moreover, this higher-than-typical background cancer rate seems to be variable within the study area; examination of the villages with low InAs exposure (below 200 µg/L in well water) results in cancer rates that are statistically heterogeneous, varying significantly more than the variation which would be expected by chance alone. Finally, rates of cancers that have not been associated with InAs exposure appear somewhat higher in the study area than elsewhere in Taiwan. These observations suggest that there is a factor, other than InAs, which causes elevated cancer rates, operating in the study area, and its effect varies among villages.

The presence of these uncertainties in the Taiwan data set calls into question the relevance of this data set to the general population, and specifically to a population exposed to low levels of InAs, and whether it is scientifically justified to use this data set exclusively to characterize InAs cancer risk.

2.2.2 Issues in Modeling the Dose-Response of the Taiwan Data

Earlier modeling exercises on the Taiwan data by Morales *et al*. (2000 revealed profound differences in low-dose risk conclusions depending on whether linear or non-linear models were used, and on whether an external referent population outside of the Taiwan study area was used to set a "background." These results, plus the MOA data that suggest InAs has a threshold (see Section 2.3), led the 2005 SAB Panel to call for exploration of alternative modeling approaches and assumptions.

In view of this, the 2010 draft IRIS report should probe the potential for these complicating factors to distort analyses and be explicit how conclusions drawn from Taiwan comport with, or contradict, other evidence—specifically, the low-dose epidemiological studies in the US (which collectively show no effects, as revealed by meta-analysis), as well as the results of the MOA evaluation that strongly suggest an exposure threshold for carcinogenic effects (see Section 2.3). The issues that need to be explored, and will be discussed further below, are as follows:

1. The inclusion of an outside referent population in the 2010 draft IRIS report analysis to "anchor" the dose-response curve at the low end; if the chosen outside population is not consistent with the background levels in the study area, as we contend, then its inclusion distorts the low-dose shape and slope of the curve, as we illustrate below;
2. Linear *versus* non-linear models; in view of the non-linearity in dose-response expected from the MOA information, the ability of non-linear models to describe the Taiwan data is important to evaluate thoroughly;
3. The combined effect of the outside referent population effects and linear/non-linear models; these are not separable issues that have separate impact, as will be argued below; and
4. The consistency of the low-dose patterns modeled in Taiwan with the outcomes of human studies in the US; the US EPA draft 2010 report considers only individual US studies and says they have insufficient power to refute the preferred interpretation of the Taiwan data, yet meta-analysis of the US studies combined, which has sufficient power, still shows little effect for the US studies collectively, and should be considered in the 2010 assessment.

These issues will be discussed below. To aid the discussion, we provide Figure 1, a *diagrammatic* representation of patterns in the Taiwan study data. We stress that Figure 1 does not represent actual data

points or curve-fits—it is presented only to clarify *patterns* that we contend are present in the real data and to provide a framework for our discussion of the impact of modeling assumptions on estimates of low-dose risk. The aim of the following discussion is to show the reasons why certain analyses by themselves are misleading and to illustrate the need for full actual analyses of the Taiwan data that have not been included in the US EPA 2010 draft IRIS report.

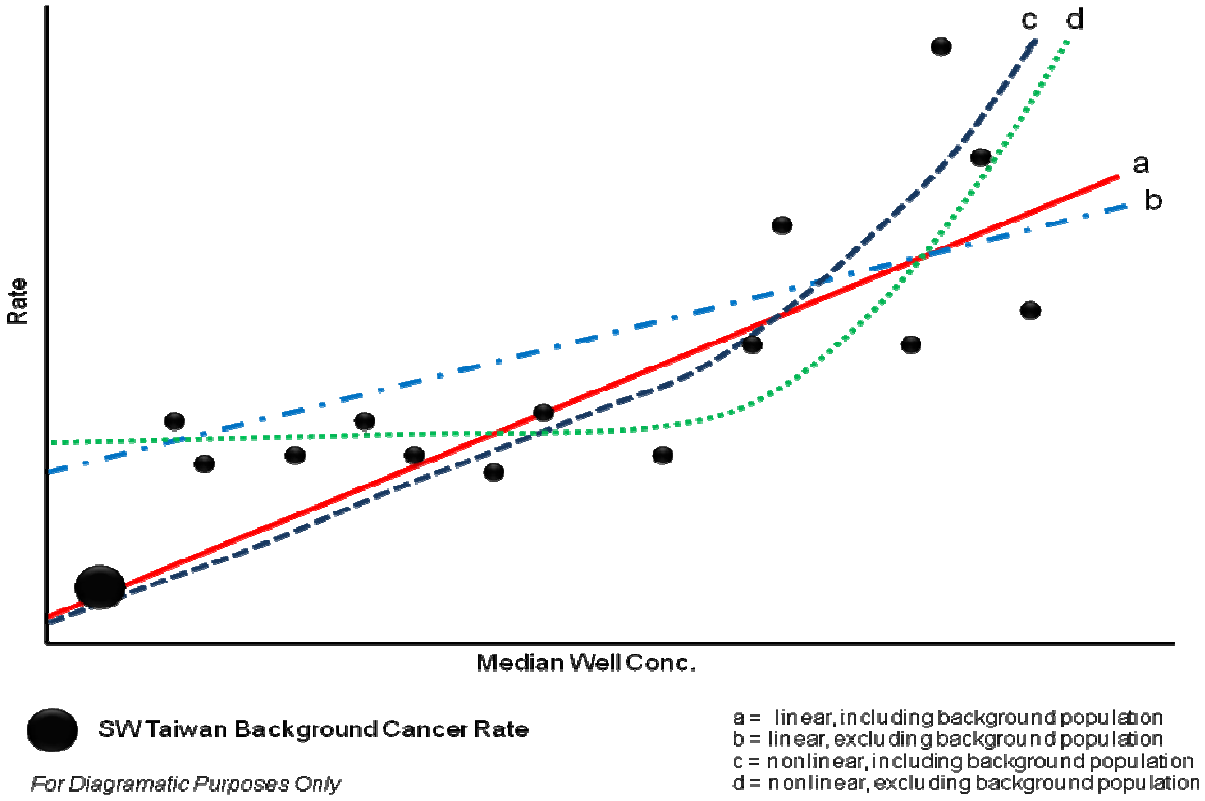

Figure 1. Diagrammatic representation of possible patterns of cancer rates among Taiwan Study villages—these points and curves represent patterns seen in the data for purposes of illustration but do not correspond to the actual data points or curve fits.

2.2.2.1 Inclusion of an Outside Referent Population

In the diagram above, the outside referent population (SW Taiwan) is represented by the single large dot, its size emphasizing that, owing to the large population it represents, it carries great weight in Poisson regression curve fits, such that any fitted curve is essentially compelled to go through this point. Figure 1 illustrates the concern that the cancer rates in this outside population are systematically lower than anywhere within the study area. This is also true if one uses all of Taiwan as an outside referent. As a consequence, any curve fitted to data when the referent population is included, is compelled to go down

sharply at low doses, to move from the higher cancer rates that exist even in villages with low InAs levels to the lower rates prevailing outside the study area. As a consequence, the low-dose shape of the curve is dominated by the difference between the outside referent population and the study area collectively, rather than by patterns among villages with different InAs levels. This phenomenon was seen in the Morales *et al*. (2000) modeling, and is what led the last SAB to suggest evaluating the role of the outside referent population.

If a linear curve is fit (red curve), the need to go through the referent population and through the elevated risk in high-dose villages (*i.e.*, the needs of the ends of the curve rather than its middle) results in a curve that has a steep slope and yet bears no relation to the actual patterns seen in the low-dose villages of the study area, which are presented by the smaller dots. It is this general form of the curve that forms the basis of the 2010 draft IRIS assessment's preferred measure of InAs potency.

If a linear curve is fitted to data that omit the outside reference population (blue-dashed curve), then the need to dip to accommodate the low outside rates is obviated. This results in a substantially lower slope than when the referent population is included; Tables 5-10 and 5-11 and Figure 5-2 of the draft reassessment show that setting the reference population to "none" lowers the slope of linear models for all endpoints, from a little for male lung, to three- to fourfold for female lung and male bladder, and a full 8.6-fold for female bladder.

The rationale for using an outside reference population is to stabilize the lower end of the curve when there are few data points. However, this method is valid only if the outside population represents the same background cancer rates (in the absence of elevated InAs exposure) as is seen within the study villages. This method cannot be used when the outside population has a different background cancer rate, as in the present case. When the outside population does not represent the background rate, then it biases the characterization of low-dose patterns by dragging the curve down to accommodate the outside point. If a model could have the following shape:

(*i.e*., curve upward sharply from the reference population, then flatten to fit the low-dose village results, and finally climb again to fit the high-dose village's increased risk at very high InAs levels) then such a model would describe the data well. But we are not aware that such a model exists, even among the non-linear models, or that there is a biological basis for such a dose-response relationship for InAs.

Substituting the whole-Taiwan population for the SW Taiwan population as the external reference does not alter this basic pattern, because the difference between the study area and the reference population continues to dominate and obscure the effect of the pattern of different InAs levels among villages actually within the study area.

2.2.2.2 Linear *vs*. Non-linear Models

The 2007 SAB Panel review asked for examination of non-linear models, prompted by the observations of the apparent patterns in the Taiwan data, by the results of the Morales *et al*. (2000) modeling, and by MOA considerations. While the current draft reassessment does examine some non-linear models, it only does so with the outside reference population included. There is no case of examining a non-linear model without inclusion of the outside population. The result is diagrammatically illustrated in Figure 1 by comparing the red line (linear model using outside reference population) and the short-dashed black line (non-linear model using outside reference population). Because the non-linearity of the non-linear model is constrained to be concave upward and because this curve is still constrained to go through the external reference population point, it cannot express the actual curvilinear patterns in the data. As a result, the non-linear model flattens and becomes only slightly different from the purely linear one. Such results are reported in the draft reassessment's Table F-2. Seen diagrammatically in Figure 1, it is evident that the essentially linear outcome even of non-linear models is not the result of patterns within the study area, but again the artifact of forcing the curve to drop sharply at low doses to accommodate the difference between even low InAs villages and the outside population in background cancer risks. That there is some curvature at high doses has little effect on the low-dose behavior of the curve. It is unfortunate that such an analysis has been used to dismiss the impact of considering non-linear dose-response models in the draft reassessment, despite the demonstration by Morales *et al*. (2000) that such models can give very different results if they are applied <u>without</u> the outside reference population.

The draft states (p. F-6), "when no reference population is included, and when inappropriate statistical models are employed, it is possible to find insignificant or negative dose-response relationships for InAs for some portions of the data. When appropriate models are used, however, the Taiwanese data show robust and significant positive associations…even in low-exposure groups." This statement evinces a tactic of dismissing the concerns of the earlier SAB review and its call for exploration of these issues through narrow technical considerations, attempting to address the letter of SAB's recommendation while making sure to avoid its substance and spirit. It is not evident that the analyses without an external

reference population are not "appropriate"; indeed, the questions about the comparability of the external populations used to the study area and the evident great impact that their inclusion has on the fitted curves, especially in view of the way that inclusion of an external point ruins the fit of the curve to the low-dose villages that are actually within the study area, argues that such analyses are very much a part of the proper characterization of the Taiwan study results.

2.2.2.3 Combined Effect of Non-linear Model and No External Reference Population

As discussed above, the 2010 draft IRIS report does not consider the case of fitting non-linear models to the Taiwan study data without using an external reference population. We propose that a non-linear model without an external reference population would have the pattern diagrammed in Figure 1 as the solid green curve (d), *i.e.*, a curve that is not forced down at the low end by the questionable external reference population. In this case, using a non-linear fit, the curve would show essentially no effect of InAs in villages below about 200 µg/L in InAs well water concentration, but would demonstrate that villages with much higher InAs levels do indeed seem to have elevated cancer risks. This would seem to be the most natural expression of the patterns seen in the actual data, without being thrown off by the artificial constraints of either (1) forcing a linear curve to fit the data; (2) forcing the curve to dive down to accommodate a qualitatively different external population; or (3) both of these effects together.

2.2.2.4 Consideration of Congruency of Taiwan Conclusions with Populations Elsewhere

A curve excluding an external comparison population not only describes the Taiwan study data without artificial constraints on the dose-response shape, it also would bring the Taiwan results into accord with the results of low-dose studies elsewhere in the world, including in the US. The draft reassessment dismisses other studies as having insufficient power, but this misses two points: (1) the studies collectively show no trends, as illustrated by a published meta-analysis (Mink *et al*., 2008) that was not included in the draft's discussion of literature, and the many studies collectively have more power than any one study alone; and (2) the call of the earlier SAB to consider other studies was not to find a single alternative study on which to do dose-response analysis, but rather to address the clear need for the conclusions drawn from the analysis to be in accord with the whole body of available data. SAB's earlier call was to examine the congruence among studies and to evaluate the degree to which conclusions drawn from Taiwan might reflect peculiarities of the Taiwan case rather than a generalizable pattern that applies

wherever InAs exposure occurs. Above, we have called attention to several reasons to be concerned that the Taiwan data are not fully representative of what would be seen elsewhere, and that peculiarities of the specific region of Taiwan, including variation in other influential factors among villages in the study region, could be the cause of results misleading about what would occur elsewhere.

Recognizing the limitations of exclusive reliance on the Taiwanese data set and questions about the applicability of this data set to InAs risks in the US, the 2005 SAB Panel called for several analyses addressing the issues described above. In its current state, however, the US EPA assessment fails to conduct this exploration; the 2010 draft IRIS report includes some additional analyses asked for by SAB, but these supplemental analyses aim at providing grounds for dismissal of alternatives rather than genuinely attempting to characterize the impact of the issues SAB was concerned about. That is, the revision fails to address the spirit and basis of SAB's concerns.

2.2.2.5 Weight of Evidence

The potential low-dose cancer risks need to be evaluated based on a weight-of-evidence evaluation of all the pertinent data, from epidemiological studies conducted in different geographic locales, as well as MOA data. This need to evaluate corroboration and consistency is especially important in view of the many questions regarding whether the Taiwanese study area has special local factors other than InAs exposure that skew its cancer results, and questions about whether the preferred linear dose-response analysis using an external reference population addresses the question of association between cancer and low InAs doses in the Taiwanese study area, or rather about differences between the area as a whole and other places in Taiwan. If the modeling of the Taiwanese data is conducted in a way that makes a forthright investigation of possible issues, it becomes clear that the study area has uncharacteristically high background levels of several cancers, including the lung and bladder cancers that were analyzed, that the causes of this high background probably vary within the study area, and that the possibility of confounding of InAs results by variation among villages in these other factors is a real concern. Moreover, if the demonstrably different external reference population is omitted and curvilinear dose-response models entertained as serious possibilities, it is clear that the Taiwanese data can be compatible with the US low-dose studies and the MOA data in finding little or no increase in cancer risk at chronic exposures below those associated with about 200 ug/L in well water.

These questions were of concern to SAB in its previous review of the agency's InAs assessment, and they were the motivation behind the SAB's call for broader investigation of modeling alternatives,

greater use of the MOA understanding (even if not definitive regarding any single mode), and comparison of the results from Taiwan with a broad and collective view of other epidemiological studies, including the US low-dose studies. The spirit and motivating questions of SAB's earlier concerns have yet to be met in the revised analysis, and these questions remain unaddressed in the draft document as it now stands.

2.3 MOA Considerations

A key issue in characterizing the dose-response relationship for InAs carcinogenicity relates to the MOA. Accumulating animal and *in vitro* evidence supports that InAs carcinogenicity would be expected to exhibit a non-linear dose-response at low doses, with a possible threshold. There is vast experimental evidence that InAs does not cause direct DNA damage (*i.e.*, is not mutagenic) and all proposed key events (*e.g.*, cytotoxicity, modulation of cell signaling pathways, changes in DNA methylation patterns, *etc.*) are non-linear biological responses. Additionally, current scientific literature has characterized a vast array of cellular adaptations and hormetic behaviors in response to InAs insults.

As discussed in more depth below, a probable non-linear dose-response relationship has been recognized for InAs by regulatory and scientific organizations evaluating InAs carcinogenicity for over a decade (NRC, 1999, 2001; US EPA, 2001). The most recent efforts to evaluate InAs's MOA—starting with the 2005 InAs IRIS support document, continuing with the 2007 SAB InAs report and this most recent effort in 2010—continue to present evidence that overwhelmingly supports a non-linear MOA for InAs carcinogenicity. Moreover, important research specifically directed at understanding InAs's MOA in the context of risk assessment decisions has been published since the SAB and latest US EPA review. This key new research not only confirms evidence for key event non-linearities, but provides greater clarity on the specific key events involved in InAs's carcinogenic MOA.

2.3.1 SAB 2007 Conclusion Regarding a Non-linear Dose-response Relationship for InAs with a Possible Threshold

In 2007, SAB responded to charge questions on the 2005 version on the IRIS draft report (SAB, 2007). Specifically, regarding the MOA, US EPA asked if SAB "concurred with the selection of a linear model following the recommendations of the NRC (2001) to estimate cancer risk in light of the multiple modes of carcinogenic action for iAs." Several key statements from the SAB report regarding this issue are listed below:

- "At present the experimental evidence on MOA of inorganic arsenic supports a possible nonlinear dose-response at low exposure levels yet there is no clear indication of what shape a nonlinear dose-response would take for application to human cancer risks at low exposures (< 50 or < 100 ppb)…it is clear that effects are only seen at doses that induce cytotoxicity. **This implies a threshold**" (p. 44).
- "Studies of indirect genotoxicity strongly suggest the possibility of a threshold for arsenic carcinogenicity. However, the studies discussed herein do not show where such a threshold might be, nor do they show the shape of the dose-response curve at these low levels" (p. 6).
- "Arsenic essentiality and the possibility of hormetic effects are in need of additional research to determine how they would influence the determination of a threshold for specific arsenic-associated health endpoints" (p. 6).
- "[T]he Panel discusses studies of indirect genotoxic effects associated with iAs and/or its metabolites, as well as the notion that iAs might have some beneficial effects at very low doses. Taken together, these studies suggest the possibility of a threshold" (p. 27).

Finally, SAB concluded:

> "The Panel recognizes the potential for a highly complex mode of action of iAs and its metabolites, and until more is learned about the complex PK and PD properties of iAs and its metabolites there is not a sufficient justification for the choice of a specific nonlinear form of the dose-response relationship" (p. 43).

From the comments, it is clear that, based on its review of the mechanistic literature (which included published literature through 2006), SAB formed a consensus that InAs MOAs were likely non-linear, with the potential for a threshold. However, because no definitive MOA could be established, and, at the time, pharmacokinetic (PK) and pharmacodynamic (PD) information was deficient, SAB believed there was insufficient justification for using a non-linear model exclusively to characterize InAs risks. Since SAB published its opinions in 2007, advancements in the understanding of InAs's MOA have progressed and provide further support for non-linearities in dose response. A significant portion of this information is summarized by US EPA in the 2010 draft IRIS report [including PBPK modeling research by a US EPA scientist (El-Masri and Kenyon, 2008; Kenyon *et al*., 2008a)]; however, studies published in 2009 and 2010 are not included in the report, and these could address many data gaps noted by SAB. This new research and implications for the dose-response, which is discussed below, should be considered in a current assessment of InAs carcinogenicity.

2.3.2 Support for Non-linear Dose Response in the 2010 Draft IRIS Report

The 2010 draft IRIS report contains a comprehensive review of mechanistic InAs data (in humans, animals, and *in vitro*) from 2004 through August 2007, plus a review of key literature from earlier years (p. C-1). Based on the evaluation of the literature, the US EPA 2010 draft IRIS report specifically notes that there is no evidence of InAs-induced mutagenicity (p. 100), and proposes several other "key events" that are likely to be involved in the InAs MOA. These proposed key events are summarized in Table 4-1 (p. 71) in the 2010 draft IRIS report, which is reproduced below.

Table 4-1. Summary of Number of Rows Derived From Peer-Reviewed Publications for Different Hypothesized Key Events[a]

Hypothesized Key Events	Number of Rows in Tables		
	In Vivo Human Studies (Table C-1)	*In Vivo* Experiments Using Laboratory Animals (Table C-2)	*In Vitro* Experiments (Table C-3)
Aberrant Gene or Protein Expression[b]	6	32	124
Apoptosis	1	6	78
Cancer Promotion	0	3	3
Cell Cycle Arrest or Reduced Proliferation	0	1	29
Cell Proliferation Stimulation	0	18	21
Chromosomal Aberrations and/or Genetic Instability	13	3	83
Co-carcinogenesis	0	2	3
Co-mutagenesis	0	1	21
Cytotoxicity	0	2	118
DNA Damage	5	6	35
DNA Repair Inhibition or Stimulation	2	0	11
Effects Related to Oxidative Stress (ROS)	2	30	69
Enzyme Activity Inhibition	0	0	5
Gene Amplification	0	0	5
Gene Mutations	1	2	7
Hypermethylation of DNA	2	1	2
Hypomethylation of DNA	1	2	7
Immune System Response	1	0	46
Inhibition of Differentiation	0	0	13
Interference With Hormone Function	0	1	7
Malignant Rransformation or Morphological Transformation	0	0	13
Signal Transduction	1	2	51

[a] Details of the studies are presented in Appendix C.
[b] Some hypothesized key events are shown in boldface to emphasize that in at least one of the tables they contain much more data than the other categories.

Except for certain types of DNA damage, all of the key events listed in this table are considered to have a non-linear dose-response. Direct DNA damage (*i.e.*, mutagenicity) is the only mechanism considered to have linear dose response. With regard to this endpoint, US EPA hypothesizes that, while there is no evidence of mutagenicity from InAs exposure (which would have potential to cause a linear dose-response), "chromosomal aberrations can be induced, and if a chromosome happened to break, for example in a tumor suppressor gene, that mutation might provide an important step in a MOA." Although

this form of DNA damage can be documented in mechanistic studies, there is no evidence that, among the possible MOAs, chromosomal damage in the tumor suppressor gene is a critical key event *in vivo*. Moreover, even if chromosomal damage to a tumor suppressor gene were a key event, there is no conclusive evidence that this would result in a linear dose-response, given that multiple hits would typically be required for chromosomal damage.

According to US EPA's Table 4-1, one of the key events with the greatest support is cytotoxicity (118 *in vitro* experiments).

As noted by US EPA, cytotoxicity is a clear non-linear process:

> Probably the most important observation related to cytotoxicity…is that exposure of a large number of different cell lines to trivalent arsenicals results in significant cytotoxicity at molarities smaller than what would be found in urine, or even in the blood streams, of individuals exposed to high levels of inorganic arsenic in drinking water in places like Bangladesh….Also, from the numerous dose-response curves published in those papers, it is apparent that cytotoxicity generally has a threshold below which there is no apparent effect. (p. 88)

Recognition of cytotoxicity as a key event has important implications for characterizing low-dose InAs risk, and suggests a non-linear model might best describe low-dose InAs toxicity. Literature published after 2007, which is not included in the 2010 draft IRIS report, further supports cytotoxicity as the key event in the overall carcinogenic process, especially in bladder carcinogenesis. These studies, discussed in more depth in Section 2.3.3, suggest that trivalent InAs metabolites cause cytotoxicity of the bladder, which leads to a regenerative response and eventual tumor formation.

The 2010 draft IRIS report provides overwhelming support for non-linear key events, and specifically cytotoxicity, as a key event in the MOA for InAs carcinogenicity. However, this support, which includes US EPA's own research, is not reflected in the weight-of-evidence evaluation, and in the interpretation of the data for use in risk assessments. Instead, US EPA focuses on the fact that no one MOA has been definitively determined for InAs, and, ultimately, decides to model InAs risks at low-dose exposure, using a linear model. US EPA justifies this decision through reliance on the US EPA cancer guidelines (US EPA, 2005). Assuming low dose linearity as a default in the absence of a specific MOA, however, is a narrow interpretation of the US EPA cancer guidelines, especially when there is a consensus that the one possible MOA that is linear (*i.e.*, mutagenicity) has been excluded as a key event (for a more detailed discussion, see Section 3 of this document). Consistent with the recommendations of the 2005

SAB in the 2007 report, non-linear models should also be explored and used when there is sufficient evidence of potential non-linearity at low-dose exposures. This approach would be consistent with literature and additional research published after 2007 supporting low-dose InAs non-linearity.

2.3.3 Recent Published Studies not Included in the 2010 Draft IRIS Report

Numerous publications have been issued since 2007, which are not included in the 2010 draft IRIS report. These include publications on InAs's MOA, as well as information on InAs's possible hormetic effects. These publications are described in the following sections.

2.3.3.1 Publications on InAs's MOA

There is a significant amount of literature published after 2007 regarding InAs's MOA that needs to be considered in a thorough and contemporaneous assessment of InAs carcinogenicity (for further details, see Section 4 of this document). Overall, these studies provide evidence that InAs (or its metabolites) does not cause oxidative damage to DNA by a direct interaction, has a low-dose (< 1 µm) adaptive phase, and has an MOA involving cytotoxicity followed by regeneration (in the bladder). It should be noted that this specific research aimed at developing a BBDR model is consistent with SAB recommendations and has been sponsored by US EPA. The work is expected to be completed later in 2010 or early 2011 (*via* communication at EPRI Arsenic SRP Meeting, 2010). In addition to the specific research aimed at defining mechanistic key events that will underlie BBDR modeling, other research, including human studies, has provided support of similar transcriptional changes that occur in response to InAs (for example, Fry *et al*., 2007).

Taken together, all of these studies further support a non-linear dose-response relationship with a possible threshold. It should also be mentioned that in the literature published after 2007, we did not find any publication supporting an MOA of direct DNA damage or linear dose-response. Examples of key papers that provide important information about the shape of the dose-response curve at low doses, and which are not included in the 2010 draft IRIS report, are summarized below.

Kitchin and Wallace (2008), of the US EPA's National Health and Environmental Effects Research Laboratory, conducted experiments examining whether oxidative damage caused by InAs and/or its metabolites could directly damage nuclear DNA. Using radiolabeled InAs in *in vitro* experiments, the researchers showed that InAs did not bind DNA or any proteins usually found closely

associated with DNA (*i.e.*, histones) or ferritin in the nucleus. The authors concluded that the lack of binding made it "highly unlikely that *in situ* binding of trivalent arsenicals, followed by *in situ* oxidative damage can account for arsenic's carcinogenicity." The experiments did not rule out a role for oxidative stress, but indicated that any oxidative stress would be secondary to other biological reactions.

Gentry *et al*. (2010) recently published an analysis that synthesized information from 160 studies on the mechanism of InAs carcinogenicity. The goal of integrating the available information was to try to understand dose-dependent changes in gene and protein expression related to key events in InAs's MOA. Overall, the study demonstrated that the lowest concentrations of InAs (*i.e.*, < 0.1 μM) are associated with adaptive responses that do not compromise DNA integrity (*i.e*., genes that control DNA repair and cell cycle control become down-regulated). At concentrations ranging from about 0.1 to 10 μM, evidence of unrepaired DNA damage in conjunction with cytotoxicity became evident, but further adaptive responses are also induced. At about 1 μM, DNA repair goes from being down- to up-regulated (although DNA ligase in particular begins to become inhibited at the higher end of the range). In this range, genes associated with proliferation also get up-regulated. At the highest concentrations, characteristic responses included cell cycle stasis and apoptosis.

The work published by Gentry *et al.* (2010) is part of a larger effort to develop a BBDR model for InAs, which is an active area of research being sponsored by the Electrical Power Research Institute (EPRI), with input from US EPA. Developing a BBDR model was recommended by the 2005 SAB Panel. Work in this area is advancing rapidly and is scheduled to be completed by the end of 2010. The results of this work will offer important insights into low-dose InAs effects that can have important implications for InAs risk assessment and identification of a threshold. For example, at a recent conference (Alliance for Risk Assessment Workshop), Clewell (2010) presented a synthesis of work-to-date on this effort, which included *in vivo* data to support *in vitro* findings. Clewell presented several observations including that critical changes in gene expression generally occur after exposure to about 10 mg/L InAs in drinking water in mice (*i.e.*, benchmark doses [BMDs] for gene ontology categories generally ranged from 9-15 mg/L As for week one and 6-11 mg/L As for week 12). He hypothesized that critical key events in InAs's MOA based on changes in gene expression are as follows:

> **Binding of InAs to proteins → oxidative stress → inflammation→ cell proliferation → increased DNA mutations → tumor formation** (Also involved is inhibition of DNA repair which facilitates increased DNA damage)

In a preliminary analysis of an InAs-exposed population in Bangladesh, Fry *et al*. (2007) examined differences in gene expression in the fetal cord blood in infants born to mothers with and without InAs exposure. Distinct genetic profiles were predictive of InAs exposure (which was defined as mother having a toenail InAs content > 0.5 μg/g, ranging up to 68.63 μg/g, which approximately equates to exposure to approximately 10 to over 1,700 μg/L). More specifically, genes associated with NF-κB, inflammation, cell proliferation, stress, and apoptosis were up-regulated in fetal cord blood from mothers with InAs exposure (Fry *et al*., 2007).

Studies specifically designed to evaluate cytotoxicity followed by regeneration as an MOA have also been published since the 2007 SAB report and are not included in the US EPA 2010 draft IRIS report. This MOA is consistent both with US EPA's (2010) review, as well as EPRI's research, which underscores cytotoxicity as a key event in InAs carcinogenicity. Suzuki *et al*. (2008) characterized the urothelial effects of InAs in both mouse and rat models. In several short-term experiments of two to 10 weeks, these researchers administered InAs(V) and InAs(III) in the diet and/or drinking water of mice and rats. Cytotoxicity and necrosis of the urothelial superficial layer as well as hyperplasia were observed in both animal species. Greater bladder cell toxicity (as well as overall toxicity) was observed when InAs (3) was administered *via* drinking water compared to in the diet. Female rats were more sensitive to InAs than male rats, but gender differences were not observed in mice. More recently, these investigators found that cytotoxicity, cell proliferation, and hyperplasia increased in a dose-dependent manner in the urothelial cell of rats exposed to up to 50 mg/kg in feed (labeling index was decreased at 100 mg/kg). No evidence of urothelial changes in response to InAs exposure was seen at 1 mg/kg InAs in feed, indicating a threshold. The authors suggested that InAs operates by an MOA similar to that of DMA(V), with the urothelial cytotoxicity being mediated by DMA(III) for both compounds. In the 2007 report, the 2005 SAB Panel concluded that this was the MOA for DMA(V), that this MOA had a threshold, and that cancer risks should be evaluated using an MOE approach.

2.3.3.2 Publications on Hormesis

The 2005 SAB Panel specifically recommended that US EPA consider the potential hormetic effect of InAs in its evaluation of InAs carcinogenicity. In the 2007 report it stated:

- "Arsenic essentiality and the possibility of hormetic effects are in need of additional research to determine how they would influence the determination of a threshold for specific arsenic-associated health endpoints" (p. 6).

- "Hormetic effects of iAs (beneficial effects at very low doses) have also been suggested and need further investigation even if arsenic should not be essential" (p. 33).

To support these statements, the SAB (2007) report included several citations to studies that demonstrate InAs's hormetic potential (*e.g.*, Snow *et al*., 2005). Despite SAB's suggestion to further evaluate the potential hormetic properties of InAs, the US EPA 2010 draft IRIS report mentions hormesis only briefly and does not make any attempt to evaluate InAs-related hormesis or even acknowledge that evidence of hormesis might provide important information about the shape of the dose-response curve at low doses. In fact, only one study on hormesis is mentioned in the text of US EPA 2010 draft IRIS report and this was a study highlighted by the SAB 2007 report. Studies continue to be published providing support for a hormetic component to low-dose InAs exposure. Overall, these studies suggest that hormesis may be observed in various homeostatic processes including apoptosis, DNA repair, cell proliferation, altered DNA methylation, and other non-DNA genotoxic responses (Klein *et al*., 2007). Examples of specific key recent studies are summarized below.

Several researchers have found that lung fibroblasts display a hormetic dose-response following exposure to InAs (He *et al*., 2007; Sykora and Snow, 2008; Yang *et al*., 2007). For example, He *et al.* (2007) demonstrated that cell viability in human fibroblast cells increased at lower concentrations (0.1 and 0.5 μM InAs) and was inhibited at higher concentrations (5 and 10 μM) when exposed to InAs. Similar results are reported with apoptosis as the endpoint. Similarly, Sykora and Snow (2008) have observed hormetic dose-responses in lung fibroblasts and keratinocytes at physiologically-relevant doses of InAs. At low doses, DNA repair activity [and specifically DNA polymerase-associated base excision repair (BER) activity] was increased, whereas at higher doses (above 1 μM) there was significant down-regulation of BER activity. The authors suggested that changes in BER activity at high *vs.* low doses of InAs support the idea of an InAs threshold.

Yang *et al.* (2007) described how cell viability resulting from InAs-induced oxidative stress may display a hormetic dose-response. Using lung fibroblast cells exposed to InAs, the researchers examined the levels of reactive oxygen species (ROS), lipid peroxidation, and heat-shock proteins, and the activities of glutathione peroxidase and superoxide dismutase. The Yang *et al*. data showed a hormetic dose-response with significant stimulation in cell viability at low concentrations (0.5 μM for 12, 24, and 48 h) and inhibition at high concentrations (5 and 10 μM for 24 and 48 h). The authors offered several alternative mechanistic explanations for the bi-phasic dose-response. For example, they proposed that, at

low doses, InAs induces ROS (without cytotoxicity) that results in cellular protection, while at higher concentrations, InAs induces increased ROS formation, marked oxidative stress, and cellular damage.

It is clear that an analysis of potential hormetic effects, which is consistent with evidence of an adaptive stage of cellular responses, provides critical information about the dose response for InAs at low doses. Ignoring this information represents a failure to fully evaluate a key question in InAs cancer risk assessment—namely, is there sufficient evidence to support a non-linear, threshold approach to characterize InAs risks or must we resort to default linear approaches with little scientific support?

In summary, the US EPA 2010 draft IRIS report provides a review of individual studies on the potential MOA of InAs's carcinogenicity, which were published until 2007, with just a few publications from 2008. While the description of each of the cited studies is quite extensive, the synthesis of the data is deficient. The weight-of-evidence discussion on the MOA information offered by the 2010 draft IRIS report largely consists of general (non InAs-specific) information on different types of MOAs and study summaries selected with no clear rationale. Overall, only a small fraction of the studies reviewed in Appendix C of the 2010 draft IRIS report are mentioned and studies included in the discussion are not criticality evaluated for strengths and weaknesses. A proper weight-of-evidence requires consideration of all the available data in an attempt to reconcile disparate information to reach an overall conclusion. MOA information is critical to understanding the shape of the dose-response curve and the potential for non-linearities in the dose response. The current draft simply reports that multiple plausible MOAs exist and uses this to support low-dose linear extrapolation as a default approach. Given the importance of MOA in low-dose extrapolation decisions, it is critical that US EPA evaluate the totality of the evidence of plausible MOAs and what the MOAs suggest about appropriate approaches for low-dose extrapolation, using state-of-the-art information, including publications from 2008-2010.

2.3.4 Recommendations

In the sections above we provided comments on the extent to which the current draft adequately considers epidemiological evidence, approaches to dose-response modeling, and MOA studies. Based on these comments, we recommend that US EPA:

- Evaluate the full range of epidemiological evidence, and provide a more balanced assessment of the Taiwan data in the context of other epidemiological studies and a recent meta-analysis (Mink *et al*., 2008);

- Conduct a complete dose-response analysis, including assessment of the combined effect of a non-linear model with no external reference population and the congruency of the Taiwan conclusions with populations elsewhere. A weight-of-evidence analysis should be conducted to assess the relative strength of the US EPA-proposed dose-response model *versus* other approaches; Perform an integrated analysis of MOA studies, including an evaluation of dose-dependent transitions; and
- Conduct an MOE analysis using alternative points of departure (PODs) from current MOA research. This represents a scientifically supportable approach for analyzing chemical risks with possible thresholds of toxicity and will provide risk manag*ers wi*th a fuller understanding of the uncertainty of InAs dose-response modeling, particularly with respect to US EPA's present quantification of dose-response.

3 US EPA's interpretation of the US EPA Cancer Guidelines is Overly Narrow

The US EPA 2010 draft IRIS report used linear extrapolation to characterize InAs cancer potency at low doses. The basis for this decision was presented in a single paragraph, referring to criteria from the US EPA Guidelines for Carcinogen Risk (cancer guidelines) assessment. US EPA concluded that, in the absence of a well-characterized MOA, linear extrapolation should be used as a default assumption. This choice reflects an overly narrow interpretation of the guidelines. As described in this section, a more complete reading of the cancer guidelines demonstrates their flexibility and that consideration of other biologically plausible alternatives, including non-linear approaches, are to be explored. We recommend that US EPA conduct a complete dose-response analysis, including the use of non-linear models, in its quantification of cancer risks from InAs.

3.1 Need to Present Biologically Plausible Alternatives

The cancer guidelines recommend that risk assessments consider all of the available scientific information. Specifically, in defining appropriate dose-response approaches, US EPA stresses that risk evaluations should take into account information on the MOA and that all biologically plausible alternatives to characterize more fully the range of possible risks should be considered. The guidelines state:

> Where alternative approaches have significant biological support, and no scientific consensus favors a single approach, an assessment may present results using alternative approaches. A nonlinear approach can be used to develop a reference dose or a reference concentration. (US EPA, 2005; Section 1.3.4, p. 1-15)

This statement clearly and unambiguously indicates that a non-linear approach can be used when several alternative approaches are biologically feasible, even when the MOA is not clearly defined. Additional statements in the cancer guidelines further support the presentation of alternative plausible dose-response models when scientifically justified. The following are some examples:

> When risk assessments are performed using only one set of procedures, it may be difficult for risk managers to determine how much health protectiveness is built into a particular hazard determination or risk characterization. When there are alternative procedures having significant biological support, the Agency encourages assessments to

be performed using these alternative procedures, if feasible, in order to shed light on the uncertainties in the assessment. (Section 1.3.1, p. 1-8)

If critical analysis of agent-specific information is consistent with one or more biologically based models as well as with the default option, the alternative models and the default option are both carried through the assessment and characterized for the risk manager. (Section 1.3.1, p. 1-9)

Extrapolation is based on extension of a biologically based model if supported by substantial data…Otherwise, default approaches can be applied that are consistent with current understanding of mode(s) of action of the agent, including approaches that assume linearity or nonlinearity of the dose-response relationship…. (Section 1.3.4, p. 1-14)

Both linear and nonlinear approaches are available…when multiple estimates can be developed, the strengths and weaknesses of each are presented. (Section 3, p. 3-1)

An assessment that omits or underestimates uncertainty can leave decision makers with a false sense of confidence in estimates of risk.… Model uncertainty is expressed through comparison of separate analyses from each model, coupled with a subjective probability statement, where feasible and appropriate, of the likelihood that each model might be correct…. Some aspects of model uncertainty that should be addressed in an assessment include…the use of effects observed at high doses as an indicator of the potential for effects at lower doses, [and] the effect of using linear or nonlinear extrapolation to estimate risks. (Section 3.6, p. 3-29)

[I]n situations where there are alternative models [for analysis of dose-response data] with significant biological support, the decision maker can be informed by the presentation of these alternatives along with their strengths and uncertainties. (Section 3.2.3, p. 3-15; repeated at Section 5.1, p. 5-3)

3.2 Selection of a Form of Dose-Response Model

The cancer guidelines clearly recommend selection of a non-linear dose-response model when supported by the MOA:

A nonlinear approach should be selected when there are sufficient data to ascertain the mode of action and conclude that it is not linear at low doses and the agent does not demonstrate mutagenic or other activity consistent with linearity at low doses. (US EPA, 2005; Section 3.3.1, p. 3-22)

A nonlinear extrapolation method can be used for cases with sufficient data to ascertain the mode of action and to conclude that it is not linear at low doses but with not enough data to support a toxicodynamic model. (US EPA, 2005; Section 3.3.4, p. 3-23)

Nonlinear extrapolation having a significant biological support may be presented in addition to a linear approach when the available data and a weight of evidence evaluation

> support a nonlinear approach, but the data are not strong enough to ascertain the mode of action applying the Agency's mode of action framework. (US EPA, 2005; Section 3.3.4, p. 3-23)

3.3 Need to Use Non-Linear Dose-Response Models

Given the epidemiological and mechanistic support for potential non-linearities in the dose response (as described in Section 2), and the recommendations of the *Guidelines for Carcinogen Risk Assessment* (US EPA, 2005) (described in Sections 3.1 and 3.2), the critical decision to use linear extrapolation reflects a superficial interpretation of the available data as well as of the *Cancer Guidelines.*

To be consistent with the *Cancer Guidelines,* we recommend that alternative modeling analyses be conducted. In addition to a range of statistical models (discussed in Section 2.2), we propose that an MOE analysis also be conducted, using alternative PODs. This represents a scientifically supportable approach for analyzing chemical risks with possible thresholds of toxicity and will provide risk managers with a fuller understanding of the uncertainty of InAs dose-response modeling, particularly with respect to US EPA's present quantification of dose-response. Possible PODs should be developed from key epidemiological studies, as well as recent MOA studies.

4 Studies Published Since US EPA's Review and Ongoing Active Research Programs Continue to Provide Important Information that will Shape InAs Risk Assessment

US EPA's formal review of available MOA literature has been performed through August 2007. Thus, US EPA's assessment is lacking over two years of the most relevant, updated literature, which reflects the state-of-the-art information. During this time several new studies have been published that add an important understanding to InAs's MOA and important research is still ongoing, some of it sponsored by US EPA. We recommend that US EPA incorporate the new information into its analysis (even if it has not yet been published) to ensure its evaluation contains state-of-the-art information on InAs's MOA. Some examples of active research programs and key literature that should be included in the current assessment are described below.

Mechanistic research aimed at understanding InAs responses at the genetic and cellular levels is advancing rapidly, providing important insights to low-dose InAs risk. The most active and relevant research program is being sponsored by EPRI and involves scientists from a variety of institutions, including US EPA. The collaborative program involves investigations into PBPK models and mechanistic MOA studies with the ultimate goal to develop a BBDR model for human health risk assessment application. This type of research has been encouraged by US EPA, and the 2007 SAB report has highlighted the importance of such research in a robust evaluation of the dose-response of InAs carcinogenicity.

It is noteworthy that some of the most important work in the area is being performed by scientists at US EPA's National Health and Environmental Effects Research Laboratory. This research is state-of the-art and should not be ignored. As noted by the US EPA 2010 draft IRIS report, there have been significant advancements in PBPK modeling, embodied mainly in the research of Elaine Kenyon and co-workers, who published two important papers on InAs PK in 2008 (El-Masri and Kenyon, 2008; Kenyon *et al*., 2008a). Work in this area continues to be further developed and refined. This work is being complemented by the work of scientists outside of US EPA who have been conducting *in vitro* studies and animal experiments and synthesizing a vast amount of mechanistic data in an effort to define the dose-dependence molecular pathways involved in InAs carcinogenesis. This work (*i.e.*, Clewell *et al.*, 2007, 2010 and Gentry *et al*., 2010) has been successful in identifying exposure ranges associated with

adaptive response pathways, as well as those signaling networks that may be involved in carcinogenic response.

While integration of this work with PBPK models is not complete, these studies do provide important insights that can be used in risk assessments. Studies targeted at completion of a BBDR model, which will mark an important advancement in InAs risk assessment, are ongoing. The work is expected to be completed later in 2010 or early 2011 (*via* communication at EPRI Arsenic SRP Meeting, 2010). Other key research efforts at US EPA's labs and in the Lab of Dr. Sam Cohen (*e.g.*, Kitchin and Wallace, 2008; Suzuki *et al.*, 2008, 2010) are active and continue to clarify InAs's MOA.

While the specific MOA has not yet been determined from this research, the POD can be clearly defined. It is possible that several modes of actions are involved in InAs carcinogenicity and, therefore, a single specific MOA would never be defined. However, since all these MOAs have a threshold, the dose-response relationship would still be expected to exhibit a threshold.

In summary, the current analysis in US EPA's 2010 draft IRIS report contains very little information from 2008 through 2010, even though some of the studies published during this time provide important information about low-dose InAs dose-response and the existence of a threshold. It is important to make sure that the most relevant information on InAs carcinogenicity is included in the IRIS assessment. Key studies, focused on MOA information that should be considered in US EPA's 2010 draft IRIS report are listed below.

1. Clewell, H. 2010. "Modeling of Early Key Events Based on Genomics and potential applications for nuclear-receptor-mediated toxicity." The Hamner Institutes for Health Sciences, Research Triangle Park, NC. http://www.tceq.state.tx.us/implementation/tox/workshop-presented-by-the-alliance-for-risk-assessment.
2. Gentry, PR; McDonald, TB; Sullivan, DE; Shipp, AM; Yager, JW; Clewell, HJ III. 2010. "Analysis of genomic dose-response information on arsenic to inform key events in a mode of action for carcinogenicity." *Environ. Mol. Mutagen.* 51(1):1-14.
3. Suzuki, S; Arnold, LL; Pennington, KL; Chen, B; Naranmandura, H; Le, XC; Cohen, SM. 2010. "Dietary administration of sodium arsenite to rats: Relations between dose and urinary concentrations of methylated and thio-metabolites and effects on the rat urinary bladder epithelium." *Toxicol. Appl. Pharmacol.* doi:10.1016/j.taap.2009.12.026.
4. Beyersmann, D; Hartwig, A. 2008. "Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms." *Arch. Toxicol.* 82(8):493-512.
5. Kitchin, KT; Wallace, K. 2008. "Evidence against the nuclear *in situ* binding of arsenicals – oxidative stress theory of arsenic carcinogenesis." *Toxicol. Appl. Pharmacol.* 232(2):252-7.

6. Kenyon, EM; Klimecki, WT; El-Masri, H; Conolly, RB; Clewell, HJ; Beck, BD. 2008. "How can biologically-based modeling of arsenic kinetics and dynamics inform the risk assessment process? - A workshop review." *Toxicol. Appl. Pharmacol.* 232 :359-368.

7. Salnikow, K; Zhitkovich, A. 2008. "Genetic and epigenetic mechanisms in metal carcinogenesis and carcinogenesis: nickel, arsenic, and chromium." *Chem. Res. Toxicol.* 21(1):28-44.

8. Suzuki, S; Arnold, LL; Ohnishi, T; Cohen, SM. 2008. "Effects of inorganic arsenic on the rat and mouse urinary bladder." *Toxicol. Sci.* 106(2):350-63.

9. Sykora, P; Snow, ET. 2008. "Modulation of DNA polymerase beta-dependent base excision repair in cultured human cells after low dose exposure to arsenite." *Toxicol. Appl. Pharmacol.* 228(3):385-94.

10. Fry, RC; Navasumrit, P; Valiathan, C; Svensson, JP; Hogan, BJ; Luo, M; Bhattacharya, S; Kandjanapa, K; Soontararuks, S; Nookabkaew, S; Mahidol, C; Ruchirawat, M; Samson, LD. 2007. "Activation of Inflammation/NF-κB Signaling in Infants Born to Arsenic-Exposed Mothers." *PLoS Genet* 3(11):e207.

11. He, XQ; Chen, R; Yang, P; Li, AP; Zhou, JW; Liu, QZ. 2007. "Biphasic effect of arsenite on cell proliferation and apoptosis is associated with the activation of JNK and ERK1/2 in human embryo lung fibroblast cells." *Toxicol. Appl. Pharmacol.* 220(1):18-24.

12. Kumagai, Y; Sumi, D. 2007. "Arsenic: Signal transduction, transcription factor, and biotransformation involved in cellular response and toxicity." *Annu. Rev. Pharmacol. Toxicol.* 47:243-262.

13. Lu, M; Wang, H; Li, XF; Arnold, LL; Cohen, SM; Le, XC. 2007. "Binding of dimethylarsinous acid to cys-13alpha of rat hemoglobin is responsible for the retention of arsenic in rat blood." *Chem. Res. Toxicol.* 20(1):27-37.

14. Yang, P; He, XQ; Peng, L; Li, AP; Wang, XR; Zhou, JW; Liu, QZ. 2007. "The role of oxidative stress in hormesis induced by sodium arsenite in human embryo lung fibroblast (HELF) cellular proliferation model." *J. Toxicol. Environ. Health A.* 70(11):976-83.

References

Baastrup, R; Sørensen, M; Balstrøm, T; Frederiksen, K; Larsen, CL; Tjønneland, A; Overvad, K; Raaschou-Nielsen, O. 2008. "Arsenic in drinking-water and risk for cancer in Denmark." *Environ. Health Perspect.* 116(2):231-237.

Brown, KG. 2007. "How creditable are cancer risk estimates from the S.W. Taiwan database for arsenic in drinking water?" *Hum. Ecol. Risk Assess.* 13:180-190.

Chen, KP; Wu, HY; Wu, TC. 1962. "Epidemiologic studies on black-foot disease in Taiwan. 3. Physicochemical characteristics of drinking water in endemic Blackfoot disease areas." *Mem. Coll. Med. Nat. Taiwan Univ.* 8(1-2):115-129.

Clewell, H. 2010. "Modeling of Early Key Events Based on Genomics and potential applications for nuclear-receptor-mediated toxicity." The Hamner Institutes for Health Sciences, Research Triangle Park, NC. See: http://www.tceq.state.tx.us/implementation/tox/workshop-presented-by-the-alliance-for-risk-assessment

Clewell, HJ; Thomas, RS; Gentry, PR; Crump, KS; Kenyon, EM; El-Masri, HA; Yager, JW. 2007. "Research toward the development of a biologically based dose response assessment for inorganic arsenic carcinogenicity: A progress report." *Toxicol. Appl. Pharmacol.* 222:388-398.

El-Masri, HA; Kenyon, EM. 2008. "Development of a human physiologically based pharmacokinetic (PBPK) model for inorganic arsenic and its mono- and di-methylated metabolites." *J. Pharmacokinet. Pharmacodyn.* 35:31-68.

EPRI Arsenic SRP Meeting. 2010. Hilton-Raleigh Durham Airport at Research Triangle Park. Durham, NC. January 28-29.

Fry, RC; Navasumrit, P; Valiathan, C; Svensson, JP; Hogan, BJ; Luo, M; Bhattacharya, S; Kandjanapa, K; Soontararuks, S; Nookabkaew, S; Mahidol, C; Ruchirawat, M; Samson, LD. 2007. "Activation of Inflammation/NF-κB Signaling in Infants Born to Arsenic-Exposed Mothers." *PLoS Genet* 3(11):e207.

Gentry, PR; McDonald, TB; Sullivan, DE; Shipp, AM; Yager, JW; Clewell, HJ III. 2010. "Analysis of genomic dose-response information on arsenic to inform key events in a mode of action for carcinogenicity." *Environ. Mol. Mutagen.* 51(1):1-14.

He, XQ; Chen, R; Yang, P; Li, AP; Zhou, JW; Liu, QZ. 2007. "Biphasic effect of arsenite on cell proliferation and apoptosis is associated with the activation of JNK and ERK1/2 in human embryo lung fibroblast cells." *Toxicol. Appl. Pharmacol.* 220(1):18-24.

Kenyon, EM; Hughes, MF; Adair, BM; Highfill, JH; Crecelius, EA; Clewell, HJ; Yager, JW. 2008a. "Tissue distribution and urinary excretion of inorganic arsenic and its methylated metabolites in C57BL6 mice following subchronic exposure to arsenate in drinking water." *Toxicol. Appl. Pharmacol.* 232:448-455.

Kenyon, EM; Klimecki, WT; El-Masri, H; Conolly, RB; Clewell, HJ; Beck, BD. 2008b. "How can biologically-based modeling of arsenic kinetics and dynamics inform the risk assessment process? - A workshop review." *Toxicol. Appl. Pharmacol.* 232:359-368.

Kitchin, KT; Wallace, K. 2008. "Evidence against the nuclear *in situ* binding of arsenicals – oxidative stress theory of arsenic carcinogenesis." *Toxicol. Appl. Pharmacol.* 232(2):252-7.

Klein, CB; Leszczynska, J; Hickey, C; Rossman, TG. 2007. "Further evidence against a direct genotoxic mode of action for arsenic-induced cancer." *Toxicol. Appl. Pharmacol.* 222:289-297.

Lamm, SH; Byrd, DM; Kruse, MB; Feinleib, M; Lai, S. 2003. "Bladder cancer and arsenic exposure: Differences in the two populations enrolled in a study in Southwest Taiwan." *Biomed. Environ. Sci.* 16:355-368.

Lamm, SH; Engel, A; Penn, CA; Chen, R; Feinleib, M. 2006. "Arsenic cancer risk confounder in southwest Taiwan data set." *Environ. Health Perspect.* 114:1077-1082.

Lewis, DR; Southwick, JW; Ouellet-Hellstrom, R; Rench, J; Calderon, RL. 1999. "Drinking water arsenic in Utah: A cohort mortality study." *Environ. Health Perspect.* 107(5):359-365.

Guo, HR. 2007. "Cancer risks associated with arsenic in drinking water (Letter)." *Environ. Health Perspect.* 115 (7) :A339-A340.

Lu, FJ. 1990. "Blackfoot disease: Arsenic or humic acid?" *Lancet* 336:115-116.

Lu, F-J; Yamamura, Y; Yamauchi, H. 1988. "Studies on fluorescent compounds in water of a well in blackfoot disease endemic areas in Taiwan: Humic substances." *J. Formosan Med. Assoc.* 87:66-75.

Marshall, G; Ferreccio, C; Yuan, Y; Bates, MN; Steinmaus, C; Selvin, S; Liaw, J; Smith, AH. 2007. "Fifty-year study of lung and bladder cancer mortality in Chile related to arsenic in drinking water." *J. Natl. Cancer Inst.* doi:10.1093/jnci/djm004.

Meliker, JR; Slotnick, MJ; Avruskin, GA; Schottenfeld, D; Jacquez, GM; Wilson, ML; Goovaerts, P; Franzblau, A; Nriagu, JO. 2010. "Lifetime exposure to arsenic in drinking water and bladder cancer: A population-based case-control study in Michigan, USA. " *Cancer Causes Control* doi:10.1007/s10552-010-9503-z.

Mink, PJ; Alexander, DD; Barraj, LM; Kelsh, MA; Tsuji, JS. 2008. "Low-level arsenic exposure in drinking water and bladder cancer: A review and meta-analysis." *Regul. Toxicol. Pharmacol.* 52:299-310.

Morales, KH; Ryan, L; Kuo, TL; Wu, MM; Chen, CJ. 2000. "Risk of internal cancers from arsenic in drinking water." *Environ. Health Perspect.* 108(7):655-661.

National Research Council (NRC). 1999. "Arsenic in drinking water." National Academy Press, Subcommittee on Arsenic in Drinking Water, Washington, DC. 310p.

National Research Council (NRC). 2001. "Arsenic in drinking water: 2001 update." National Academy Press, Subcommittee on Arsenic in Drinking Water, Washington, DC. 189p., September.

Petito Boyce, C; Lewis, AS; Sax, SN; Eldan, M; Cohen, SM; Beck, BD. 2008. "Probabilistic analysis of human health risks associated with background concentrations of inorganic arsenic: Use of a margin of exposure approach." *Hum. Ecol. Risk Assess.* 14 :1159-1201. Accessed on July 02, 2009 at http://www.informaworld.com/smpp/content~db=all?content=10.1080/10807030802493966

Schoen, A; Beck, B; Sharma, R; Dube, E. 2004. "Arsenic toxicity at low doses: Epidemiological and mode of action considerations." *Toxicol. Appl. Pharmacol.* 198:253-267.

Snow, ET; Sykora, P; Durham, TR; Klein, CB. 2005. "Arsenic, mode of action at biologically plausible low doses: What are the implications for low dose cancer risk?" *Toxicol. Appl. Pharmacol.* 207(2 Suppl. 1):S557-S564. In *Living in a Safe Chemical World: Proceedings of the 10th International Congress of Toxicology,* July 11-15, 2004, Tampere, Finland, (Eds: Husgafvel-Pursiainen, K; Rautio, A; Savolainen, K, Sorsa, M, Vähäkangas, K; Ylitalo, P, Tähti, H).

Steinmaus, C; Yuan, Y; Bates, MN; Smith, AH. 2003. "Case-control study of bladder cancer and drinking water arsenic in the western United States." *Am. J. Epidemiol.* 158:1193-2001.

Suzuki, S; Arnold, LL; Ohnishi, T; Cohen, SM. 2008. "Effects of inorganic arsenic on the rat and mouse urinary bladder." *Toxicol. Sci.* 106(2):350-63.

Suzuki, S; Arnold, LL; Pennington, KL; Chen, B; Naranmandura, H; Le, XC; Cohen, SM. 2010. "Dietary administration of sodium arsenite to rats: Relations between dose and urinary concentrations of methylated and thio-metabolites and effects on the rat urinary bladder epithelium." *Toxicol. Appl. Pharmacol.* doi:10.1016/j.taap.2009.12.026.

Sykora, P; Snow, ET. 2008. "Modulation of DNA polymerase beta-dependent base excision repair in cultured human cells after low dose exposure to arsenite." *Toxicol. Appl. Pharmacol.* 228:385-394.

US EPA Science Advisory Board (SAB). 2007. "Advisory on EPA's Assessments of Carcinogenic Effects of Organic and Inorganic Arsenic: A Report of the US EPA Science Advisory Board." Report to US EPA. EPA-SAB-07-008. 88p., June 28.

US EPA. 2001. "National primary drinking water regulations; Arsenic and clarifications to compliance and new source contaminants monitoring (Final rule)." *Fed. Reg.* 66:6975-7066. January 22.

US EPA. 2005. "Guidelines for Carcinogen Risk Assessment (Final)." EPA/630/P-03/001B, Risk Assessment Forum. Accessed on March 30, 2005 at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=116283 166p., March.

US EPA. 2006. "Reregistration Eligibility Decision for MSMA, DSMA, CAMA, and Cacodylic Acid." EPA-HQ-OPP-2006-0201-0079; EPA 738-R-06-021. Office of Prevention, Pesticides and Toxic Substances. 69p., July (Revised August 10, 2006).

US EPA. 2010. "Toxicological Review of Inorganic Arsenic in Support of Summary Information on the Integrated Risk Information System (IRIS) (Draft)." EPA/635/R-10/001. Accessed on February 22, 2010 at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=219111 575p., February

Yang, P; He, XQ; Peng, L; Li, AP; Wang, XR; Zhou, JW; Liu, QZ. 2007. "The role of oxidative stress in hormesis induced by sodium arsenite in human embryo lung fibroblast (HELF) cellular proliferation model." *J. Toxicol. Environ. Health A.* 70(11):976-83.

Appendix A

Table A.1 presents the following information:

a) US EPA's original charge questions posed to the 2005 SAB Panel (for relevant issues);

b) SAB's response and request for additional analyses as presented in the 2007 SAB Report;

c) The implementation of SAB's recommendation as presented in the 2010 draft IRIS report; and

d) Comments on the limitations of US EPA's response and/or implementation of SAB's suggestions in the 2010 IRIS draft report.

This table presents just a few examples of instances where US EPA did not adequately consider points raised by SAB. We recognize that additional examples, outside of the scope of this report, may also exist.

Table A.1
Examples of the Limitations in IRIS's Implementation of SAB Recommendations

Charge Question A1: "US EPA asked the SAB to comment on how best to consider the PK processes in cancer risk assessment based on data derived from direct DMA(V) exposure versus direct inorganic arsenic (InAs) exposure."
SAB Recommendations: • With regard to InAs, SAB commented that "exposure to iAs may result in production, tissue retention, and urinary excretion of a variety of tri- and pentavalent iAs and methylated arsenic species" (p. 2) and that "the physiologically based pharmacokinetic (PBPK) model under development by EPA may be a useful approach when it is sufficiently robust to conduct inter species extrapolations" (p. 3).
US EPA 2010 Draft IRIS Report: • The 2010 draft IRIS report presents some information on more recent refined PBPK models for InAs, but US EPA ultimately concludes that, "Although there are several PBPK models available (see Section 2.3), none have sufficiently addressed the complex nature of the kinetics associated with Asi carcinogenesis; therefore, this is an ongoing effort along with BBDR modeling" (p. 99).
Comments on Limitations of US EPA's Response: • As recognized in the 2010 draft IRIS report, since the SAB review, new information that supports PBPK and BBDR modeling for use in risk assessment has been published (*e.g.*, Clewell *et al.*, 2007; Kenyon *et al*., 2008a; Kenyon *et al*., 2008b). Although an operational BBDR model has not been fully developed, the development of a BBDR model for InAs is an active research area both within US EPA and through a research program sponsored by the Electric Power Research

Institute (EPRI) (Gentry *et al*., 2010) (see Section 2.3 and 4 of this report for more information).

- Information from these research efforts is constantly evolving and has made significant advances from 2005 to the present. In light of the substantial developing work in this area, US EPA should consider using some of the available underlying information more explicitly in the present assessment, or delaying the InAs carcinogenicity assessment until the BBDR is operational and can be used to quantitatively characterize dose-response relationships (see Section 4 of this report for more information).

Charge Question B3:

US EPA concluded that InAs causes human cancer most likely by many different modes of action (MOAs). This is based on the observed findings that InAs undergoes successive methylation steps in humans and results in the production of a number of intermediate metabolic products and that each has its own toxicity. US EPA asked SAB to comment on the soundness of its conclusion.

SAB Recommendations:

a) Multiple MOAs may operate in carcinogenesis induced by InAs;

b) Each InAs metabolite has its own cytotoxic and genotoxic capability;

c) InAs and its metabolites are not direct genotoxicants because these compounds do not directly react with DNA. However, InAs and some of its metabolites can exhibit indirect genotoxicity, induce aneuploidy, cause changes in DNA methylation, and alter signaling and hormone action;

d) Studies of indirect genotoxicity strongly suggest the possibility of a threshold for InAs carcinogenicity. However, the studies discussed herein do not show where such a threshold might be, nor do they show the shape of the dose-response curve at these low levels. This issue is an extremely important area for research attention, and it is an issue that should be evaluated in US EPA's continuing risk assessment for InAs; and

e) InAs essentiality and the possibility of hormetic effects are in need of additional research to determine how they would influence the determination of a threshold for specific arsenic-associated health endpoints.

US EPA 2010 Draft IRIS Report:

- The 2010 draft IRIS report provides a comprehensive evaluation of mechanistic data that informs key events in the MOA. This information is summarized in Chapter 4 and tabulated in Appendix C.
- In Appendix A of the draft IRIS report, US EPA states that InAs's MOA is not understood well enough to support its use in quantitative risk assessment.

Comments on Limitations of US EPA's Response:

- The 2010 draft IRIS report presents a significant amount of current information on the possible key events involved in InAs's MOA. None of the key events in InAs's MOA include direct mutagenicity (the only MOA with linear dose-response). Consistent with the SAB assessment, all possible key events supported by available science (*e.g.*, cytotoxicity, changes in signal transduction, DNA repair, *etc.*) have a clear non-linear dose-response. More recent research since US EPA's evaluation continues to provide support for an MOA that does not involve direct genotoxicity (see Section 2.3 of this report for more details). Although all of the literature that US EPA reviews is consistent with InAs as an indirect genotoxin, US EPA makes no assertion as to the probable shape of the dose-response curve based on mechanistic data in the body of the report.

- SAB made a specific recommendation to explore the potential hormetic effects of InAs and implication for the dose-response curve. However, the 2010 draft IRIS report failed to consider the potential influence of hormesis and essentiality on the shape dose-response curve.

Charge Question C2:

US EPA reviewed the available epidemiological studies including those published since the NRC 2001 review for US populations exposed to InAs *via* drinking water. US EPA concluded that the Taiwanese data set remains the most appropriate choice for estimating cancer risk in humans. SAB was asked to comment on the soundness of this conclusion.

Key SAB Recommendations:

a) SAB commented that Taiwanese data had considerable limitations (both qualitatively and quantitatively), yet, it ultimately decided that database remains the most appropriate for estimating bladder cancer risk among humans, at that time.

b) SAB was explicit, however, that other studies of populations exposed at high levels of InAs be used to compare the unit risks at the higher exposure levels from the Taiwan data. SAB added that this was because "[s]everal of these studies had the advantage of data with excellent exposure assessment" (p. 38).

c) SAB also suggested that:

   i. "published epidemiology studies of US and other populations chronically exposed to 0.5-160 µg/L of iAs should be critically evaluated using a uniform set of criteria, and these results should be documented by EPA. If one or more of these studies are of potential utility, the low-level studies and Taiwan data maybe compared for concordance. This may lead to further insights into the possible influence of these differences on population responses to arsenic in drinking water" (pp. 7, 39).

d) The Panel further suggested:

   i. "that if findings from a critical review of "low-level" studies indicate that some or all studies are potentially of value in further analyses, that results from these studies should be explored in secondary analyses, particularly on bladder cancer risk, and compared with the main analysis for concordance. Analyses integrating health outcome information from a number of epidemiology studies can result in improved statistical power and precision of the estimates; these factors represent an additional advantage of utilizing a larger dataset" (p. 39).

US EPA 2010 Draft IRIS Report:

To comply with this recommendation, US EPA completed a comprehensive review of available epidemiological studies, which is tabulated in Appendix B of the 2010 draft IRIS report. Each study was critically evaluated individually, by study type, population size, and study "strengths and weaknesses."

Comments on Limitations of US EPA's Response:

- The 2010 draft IRIS report included review of a large number of human studies that were not included in earlier drafts. A critical evaluation of these individual studies, however, and how they should be use in risk assessment is not explained. The evaluation and selection of epidemiological studies was inconsistent and was not conducted using clearly defined *a prior* and transparent criteria. What constituted a significant weakness or strength was not presented, and some of the weaknesses listed also appear to be incorrect. Justification for the exclusion of each study within

the context of the criteria-based review was not provided. Also, potential confounding and unmeasured confounders are not discussed in a systematic manner. Finally, the data set from Taiwan has not been examined using the same criteria as the other available studies. Unfortunately, the comment period was too short to allow more detailed comments on this issue. We understand that other interested parties will comment on specific deficiencies in the epidemiological analysis.

- US EPA fails to consider existing meta-analyses (*e.g.*, Mink *et al.*, 2008) or conduct "Analyses integrating health outcome information from a number of epidemiology studies," as suggested by SAB.

Charge Question D2:

US EPA asked SAB if it concurred with the selection of a linear model following the recommendations of the National Research Council (NRC, 2001) to estimate cancer risk in light of the multiple modes of carcinogenic action for InAs.

Key SAB Recommendations

a) InAs has the potential for a highly complex MOA.

b) Until more is learned about the complex pharmacokinetic (PK) and pharmacodynamic (PD) properties of InAs and its metabolites there is not sufficient justification for the choice of a specific non-linear form of the dose-response relationship. The NRC (2001) recommendation to base risk assessments on a linear dose response model that includes the SW Taiwan population as a comparison group seems the most appropriate approach.

c) SAB also recommended that US EPA perform a sensitivity analysis of the Taiwanese data with different exposure metrics, with the subgroup of villages with more than one well measurement, and using a multiplicative model that includes a quadratic term for dose.

US EPA 2010 Draft IRIS Report:

- The 2010 draft IRIS report includes an extensive review of literature related to the MOA of InAs. Although all of the possible MOAs of InAs have a non-linear dose response, US EPA used a linear extrapolation to low doses (exclusively) arguing that no single definitive MOA is established. US EPA argues that this approach is consistent with the 2005 *Cancer Guidelines*.
- The risk assessment in the 2010 draft IRIS report was modeled using numerous models, including models that allowed for non-linearity. The exploration of non-linear models uses an external comparison population.
- Similarly, US EPA (2010) examined the dose-response relationship at the low-end of the dose response data, but again, this assessment appears to include an external reference population.

Comments on Limitations of US EPA's Response:

- Although a definitive MOA has not been established for InAs, all plausible MOAs are non-linear, with the one possible linear MOA (*i.e.*, direct DNA damage) being excluded. The *Cancer Guidelines* are explicit that non-linear models should be explored where there is sufficient scientific evidence to demonstrate an MOA may be non-linear or have a threshold. Based on the *Cancer Guidelines,* US EPA should explore all viable potential non-linear forms on the model (based on either epidemiological or mechanistic data) (see Section 2.3 and Section 3 of this report for more details).
- US EPA's assessment of potential non-linear models in the Taiwan data set is inadequate to

evaluate the utility of non-linear models, as its model is constrained to pass through an external reference population, which has notably lower risk than the background of even the low-dose villages. The use of an external comparison population also confounds an assessment restricted to the low-dose villages; it is only when the curve is not forced to drop below the risk levels found in low-dose villages to accommodate the outside reference population that the non-linear dose-response within the range of low-dose villages can be seen. Please refer to Section 2.2 of this report for a detailed explanation of our comments.