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

OFFICE OF THE ADMINISTRATOR
SCIENCE ADVISORY BOARD

June 28, 2007

EPA-SAB-07-008

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

Subject: Advisory on EPA's Assessments of Carcinogenic Effects of Organic and Inorganic Arsenic: A Report of the US EPA Science Advisory Board

Dear Administrator Johnson:

The U. S. Environmental Protection Agency's (EPA) Office of Pesticide Programs (OPP), Office of Water (OW), and Office of Research and Development (ORD) coordinated the development of two scientific documents that address the carcinogenicity of Dimethylarsinic Acid (DMA^V) and inorganic arsenic (iAs). In response to an Agency request, the Science Advisory Board (SAB) convened an expert panel to review and comment on key scientific issues presented in these two documents, including: (a) the metabolism and toxic responses of arsenic species; (b) mode(s) of carcinogenic action; (c) data selection for dose-response assessment; and (d) approaches and methods for low-dose extrapolation for DMA^V and iAs.

The SAB Panel supported the Agency's conclusion that on the basis of available data, human exposure to DMA^V appears to result in a narrower spectrum of active metabolites than those expected in the metabolic profile associated with exposure to iAs. Therefore, the Panel agreed with EPA that, in the absence of human data on DMA^V, the bladder tumor data from DMA^V rat bioassays is better suited for DMA^V cancer risk assessment than is epidemiology data from iAs exposure. The Panel, however, noted that there remain significant uncertainties associated with the use of animal data for DMA^V cancer risk assessment due to the observed metabolic differences between rats and

humans. The Panel agreed with the Agency's conclusion that DMA^V-induced bladder cancer in rats, at high dose, is mediated by a cytotoxic mode of action, and that this MOA should be considered relevant to humans. However, the Panel concluded there are not sufficient data to support a reactive oxygenated species-mediated mode of direct genetic action for DMA^V. The Panel supported the nonlinear approach for low dose extrapolation of DMA^V and the use of uncertainty factors to account for interspecies differences and human variability for sensitive human populations, and concluded that presently there is no arsenic-specific information that can inform the choice of specific values. This means that, at least for now, such choices must be based on more general considerations, including EPA's science policy judgment of the degree of precaution that it deems appropriate.

EPA concluded that the mechanisms by which inorganic arsenic induces bladder cancer in humans are not yet known, but they are likely to be mediated by multiple modes of action. The Agency used a linear default approach for low dose extrapolation because it lacked a full understanding of the iAs modes of carcinogenic action. The Panel agreed that available human and animal data do not fully describe the shape of the iAs carcinogenic dose-response curve at low doses. Given the considerable uncertainties regarding low dose extrapolation, the Panel supported the use of a linear cancer risk model for iAs as recommended by the National Research Council in its 2001 report. The Panel also supported the use of the epidemiologic data on the Taiwanese population for estimating human cancer risk for iAs especially to identify the potential range of responses of human populations. However, the Panel recognized limitations to these data, and that there is some evidence on iAs from animal toxicology, pharmacokinetics, and pharmacodynamics research, that suggests other than a linear bladder cancer dose-response. The Panel urged the Agency to consider other epidemiologic 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. The Panel made several suggestions for improvements in the currently applied risk model's programming and documentation conventions.

Finally, the Panel believes there is a critical need for a continued research effort to strengthen EPA's cancer risk assessment for DMA^V and iAs. The scientific bases for the Panel's conclusions and research recommendations are detailed throughout this report. We look forward to receiving your response to this review and we appreciate the

opportunity to provide EPA with advice on this important subject and stand ready to assist the Agency in any future efforts in updating the assessment.

Sincerely,

/Signed/	***/Signed/***
Dr. M. Granger Morgan, Chair EPA Science Advisory Board	Dr. Genevieve Matanoski, Chair EPA Science Advisory Board Arsenic Review Panel

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1. EXECUTIVE SUMMARY

New information has been developed on the metabolism, pharmacokinetics (PK) and mode of carcinogenic action of arsenic and its methylated species and new epidemiology studies have been conducted on inorganic arsenic since the publication of reviews by the National Research Council (NRC, 1999, 2001). EPA considered this new science in the development of the Office of Pesticide Programs' (OPP) *Draft Science Issue Paper: Mode of Action for Cacodylic Acid (Dimethylarsinic Acid) and Recommendations for Dose Response Extrapolation* (USEPA OPP, 2005) and the Office of Water's (OW) Draft *Toxicologic Review of Inorganic Arsenic* (USEPA OW, 2005). EPA's Office of Research and Development (ORD) further captured key scientific issues to be considered in its Issue Paper *Cancer Risk Assessment for Organic Arsenical Herbicides: Comments on Mode of Action, Human Relevance and Implications for Quantitative Dose-Response Assessment* (Appendix E of USEPA OPP, 2005, USEPA ORD, 2005). The Science Advisory Board (SAB) was asked to review these documents and offer advice on the metabolism, mode of action, dose-response, and approaches to low-dose extrapolation of cancer risk for Dimethylarsinic Acid (DMA^V) and inorganic arsenic (iAs). The full charge to the SAB is in Appendix A to this document.

In response to the Agency's request, the SAB convened an expert Panel to provide advice to the Agency on these scientific issues. In responding to the EPA Charge, the Panel reviewed the EPA assessments mentioned above, and considered comments and information that members of the interested public provided during each of the Panel's advisory meetings (during 2005 and 2006), and additional studies that are identified in the reference section of this report. The Panel considered expanding the Charge to include other health endpoints associated with arsenic and arsenic containing compounds. However, the Panel decided not to expand its activities beyond the EPA Charge that largely focused on bladder cancer and to some degree on lung cancer dose-response issues. It is important to recognize that the Panel did not conduct its own arsenic risk assessment. To do so would have required an updated literature search and exploration and resolution of many issues that are discussed throughout this report. The Panel leaves the larger activity of completing a full risk assessment of all relevant health endpoints associated with arsenic, and arsenic containing compounds, to the Agency itself when it conducts its final arsenic assessments.

The Panel was organized into small groups of three to seven members to evaluate and respond to each specific charge question (see Appendix B to this report for a list of those members assigned to each charge question). The Panel's response to each question reflects consensus, though not necessarily unanimous agreement, among Panel Members that addressed each specific charge question. In addition, all Panel Members had the opportunity to participate in meeting discussions of each charge question and each was able to provide written comments on all questions during report drafting. Many Members

participated in this way and each response reflects adjustments that were considered to be appropriate by the specific charge group that led the Panel's efforts for each question. In that manner, this advisory report provides the Panel's judgments on each specific issue. This advice is intended to assist the Agency's continued efforts to complete its assessments on various arsenicals. There are many specific conclusions and recommendations on specific issues associated with each charge question, as well as recommendations for sensitivity analyses and additional research to answer many of the remaining questions on arsenic risk. The Panel's advice on each charge question is discussed in the remainder of this Executive Summary and discussed in detail in Section 3 of this report.

1.1 Metabolism and Toxic Responses of Arsenic Species

Charge Question A1

EPA concluded that available *in vivo* and *in vitro* metabolism and pharmaco-kinetic studies in humans and laboratory animals suggest that the efficiency of methylation reactions and cellular uptake varies with the arsenic compound administered exogenously. Most studies suggest a predominantly one-way process in mammals and that after DMA^V exposure, significant amounts of iAs^{III}, iAs^V, methylarsonous acid (MMA^{III}), or methylarsinic acid (MMA^V) are not expected at target tissues. EPA asked the SAB to comment on how best to consider the PK processes in cancer risk assessment based on data derived from direct dimethylarsinic acid (DMA^V) exposure versus direct inorganic arsenic (iAs) exposure.

Summary Response

The Panel agreed that:

i) Metabolism of iAs appears to be a one-way process in which iAs is converted to monomethylarsenic (MMA), dimethylarsenic (DMA), and in some species to trimethylarsenic (TMA) metabolites with arsenic in +3 or +5 oxidation states. Thus, significant amounts of MMA or iAs are not expected to be found in tissues or urine of rats or humans as a result of exposure to DMA^V, although iAs may be present in human tissues or urine from other sources.

ii) In contrast, exposure to iAs may result in production, tissue retention, and urinary excretion of a variety of tri- and pentavalent iAs and methylated arsenic species.

iii) The uptake and reduction of DMA^V to dimethylarsinous acid (DMA^{III}) are apparently critical steps in activation of DMA^V – though it is not clear if, where and to what extent these processes occur in humans exposed to DMA^V.

iv) The capacity to reduce DMA^V to DMA^{III} seems to exist in human tissues and the conversion of even a small amount of exogenous DMA^V to DMA^{III} is of toxicological concern.

v) Given the differences in the metabolic pattern for iAs and DMA^V, the Panel believes data derived from DMA^V exposure, not from iAs exposure, is better suited for cancer risk assessment of DMA^V.

vi) Significant uncertainties are associated with this approach. The toxicologic data on DMA^V are mainly from rat studies, and considering several key differences between rats and humans in the metabolism of arsenic, these uncertainties should be considered in the assessment of DMA^V cancer risk. Additional uncertainties include methylation and demethylation of arsenic compounds in humans by intestinal bacteria, co-exposures to other environmental contaminants, deficiencies in nutrients, and malnutrition.

vii) The physiologically based pharmacokinetic (PBPK) model under development by EPA may be a useful approach but it is not yet sufficiently robust to conduct interspecies extrapolations.

viii) EPA should continue developing the arsenic PBPK model and conducting research to obtain kinetic constants needed to describe rates of uptake, efflux, metabolism, and elimination of DMA^V in rats and humans.

ix) There is a need to validate such models for predicting tissue concentrations of active species regardless of the source of arsenic exposure.

Charge Question A2

EPA concluded that direct exposure to iAs^{III} or iAs^V is expected to result in a more complex mixture of toxic metabolites than with DMA^V exposure given that mixtures of metabolites vary based on which chemical is administered exogenously. EPA expects a less complex mixture of metabolites following DMA^V exposure than following iAs exposure. EPA further expects that the tumorigenic profiles vary with the arsenical compound administered. For its DMA^V assessment, EPA asked the SAB to comment on the use of data derived from rodent exposures to organic arsenicals versus data derived from direct human exposure to iAs.

Summary Response

The Panel agreed that:

i) Neither rodent laboratory data on organic arsenicals nor data from studies of human exposure to inorganic arsenic provide an optimal basis for the assessment of DMA^V exposure in humans because of differences between the metabolic profiles for inorganic arsenic and DMA and because of interspecies differences in their metabolism. Despite these uncertainties, for now, the data from rodent exposures to DMA^V appear to be the most reasonable approach for the DMA^V assessment, though this approach has a significant degree of uncertainty (see charge question A1).

ii) The metabolism of iAs yields a wide spectrum of metabolites which are apparently not produced during the metabolism of DMA^V.

iii) Production of iAs and MMA metabolites may be associated with specific toxic or cancer endpoints that are absent in DMA^V exposure to rats or humans.

iv) All published data on toxicological responses to DMA^V are from studies in rodents, mainly rats; no human data are available. As noted in the response to A1 above, these differences raise concerns for risk assessments based on these data.

1.2. Modes of Carcinogenic Action for DMA^V and Inorganic Arsenic

Charge question B1

EPA's approach to cancer risk assessment incorporates two key science policy assumptions when there are inadequate human data and it needs to rely on laboratory animal data: (a) animal tumor data are predictive of human cancer and (b) effects found at high experimental doses in animals predict human risk at lower exposure levels. Understanding a mode of action (MOA) for a chemical can help to inform the agency about these assumptions and the most appropriate approach to follow in low dose extrapolation. EPA asked the SAB to comment on the scientific soundness of the postulated MOA for DMA^V-induced bladder carcinogenesis in the rat.

Summary Response

The Panel concluded that:

i) There are adequate data to support an MOA for bladder carcinogenesis induced by high doses of DMA^V in the rat and that MOA involves cytotoxicity to the bladder epithelium and increased, sustained regenerative proliferation as key events.

ii) The rat metabolizes a significant fraction of exogenous DMA^V to trimethylarsine oxide (TMA^VO) and possibly trimethylarsine (TMA^{III}) and that these compounds cannot be excluded as additional mediators of the necrotic cytotoxicity in the bladder of exposed rats.

iii) There are not sufficient data to invoke reactive oxygen species (ROS)-induced DNA damage as a key event in the carcinogenic process associated with exposures to DMA^V and DMA^{III}.

iv) The Panel's postulated MOA for DMA^V is:
   a) Reductive metabolism of DMA^V to DMA^{III},
   b) High concentrations of DMA^{III} (and possibly DMA^V) in urine cause urothelial cytotoxicity, and
   c) Continuous exposure and persistent stress-associated regenerative cell proliferation leads to genomic instability, acquisition of genetic alterations, clonal expansion of altered cells and eventually tumors.

v) The Panel suggested several high priority research needs for this issue.

Charge question B2

EPA concluded that their postulated MOA for DMA^V induced bladder carcinogenesis in the rat would be relevant to humans as there are little or no data to suggest that key precursor events and ultimately tumor formation would not occur in exposed humans if sufficient DMA^{III} were present. EPA asked the SAB to comment on the relevance of the postulated key events to tumors in humans and how differences in humans and experimental animals should be accounted for in DMA^V risk assessments.

Summary Response

The Panel concluded that:

i) If high enough concentrations of DMA^V or DMA^{III} were present in human urine or the bladder after exposure to DMA^V it is plausible that a similar response would take place; however, no data are available to support or reject this assumption.
ii) The suggested greater conversion of DMA^V to TMA^VO or possibly TMA^{III} in rats vs. in humans, may contribute to induction of bladder cancer in rats, however, the extent of the contribution is unknown.
iii) No studies have been conducted to determine whether the DMA^V carcinogenic risk differs by life stage, e.g., among the young, or elderly.

Charge Question B3

EPA concluded that iAs causes human cancer most likely by many different modes of action. This is based on the observed findings that iAs 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. EPA asked the SAB to comment on the soundness of its conclusion.

Summary Response

The Panel concluded that:

i) Multiple modes of action may operate in carcinogenesis induced by iAs because there is simultaneous exposure to multiple metabolic products as well as multiple target organs and the composition of metabolites can differ in different organs.
ii) Each arsenic metabolite has its own cytotoxic and genotoxic capability.
iii) Inorganic arsenic (iAs^{III}) and its metabolites are not direct genotoxicants because these compounds do not directly react with DNA. However, iAS^{III} and some of its metabolites can exhibit indirect genotoxicity, induce aneuploidy, cause changes in DNA methylation, and alter signaling and hormone action. In addition, iAs can act as a transplacental carcinogen and a cocarcinogen.

iv) 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. In addition, a threshold has not been confirmed by epidemiological studies. This issue is an extremely important area for research attention, and it is an issue that should be evaluated in EPA's continuing risk assessment for iAs.

v) 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.

1.3. Selection of Data for Dose-Response Assessment

Charge Question C1

In the absence of human data**,** EPA proposed to use the bladder tumor data from the DMA^V rat bioassay for quantifying potential human cancer risk to DMA^V. EPA asked the SAB to comment on the appropriateness of this approach. The SAB was also asked to comment on whether the iAs epidemiology data can be used to inform the DMA^V dose-response assessment which is now based on data derived from studies in rats dosed with DMA^V.

Summary Response

The Panel agreed that:

i) Given the lack of human data, the bladder tumor data from DMA^V rat bioassays, are the most suitable data set for quantifying potential human cancer risk from DMA^V. The Panel stated that the available data suggest that the uncertainty associated with extrapolation across forms of arsenic in the DMA^V risk assessment would be greater than interspecies extrapolation.

ii) The Panel strongly suggested that EPA's DMA^V assessment discuss the key uncertainties in using data from studies in rats to conduct human health risk assessments. Panel responses to charge questions A1 and C1 discuss issues that members considered important to discuss in EPA's Science Issue Paper. These issues relate to the pharmacokinetic and pharmacodynamic similarities and differences between rats and humans in response to arsenic exposure, the use of rodent bladder tumor models in general, and issues in the use of rodent data for human risk assessment.

iii) The Panel considers research on these issues to be a high priority.

iv) The Panel concluded that without more detailed information on target tissue dosimetry for arsenic species, the iAs epidemiology data would be of limited use to inform the DMA^V dose-response assessment derived

from rat data with DMA^V. Additional details are contained in the Panel's response to charge question C1.

Charge Question C2

EPA reviewed the available epidemiologic studies including those published since the NRC 2001 review for U.S. populations exposed to inorganic arsenic via drinking water. EPA concluded that the Taiwanese dataset remains the most appropriate choice for estimating cancer risk in humans. The SAB was asked to comment on the soundness of this conclusion and also on whether these data provide adequate characterization of the impact of childhood exposure to iAs.

Summary Response

The Panel concluded that:

i) Because of various factors (e.g., size and statistical stability of the Taiwanese database relative to other studies, the reliability of the population and mortality counts, the stability of residential patterns, and the inclusion of long-term exposures), this database remains, at this time, the most appropriate choice for estimating bladder cancer risk among humans, though the data have considerable limitations that should be described qualitatively or quantitatively to help inform risk managers about the strength of the conclusions.

ii) There are other epidemiologic databases from studies of populations also exposed at high levels of arsenic, and the Panel recommends that these be used to compare the unit risks at the higher exposure levels that have emerged from the Taiwan data.

iii) The Panel also suggests that published epidemiology studies of US and other populations chronically exposed from 0.5 to 160 μg/L inorganic arsenic in drinking water be critically evaluated, using a uniform set of criteria and that the results from these evaluations be transparently documented in EPA's assessment documents. If, after this evaluation, one or more of these studies are shown to be of potential utility, the low-level studies and Taiwan data may be compared for concordance. Comparative analyses could lead to further insights into the possible influence of these differences on population responses to arsenic in drinking water.

iv) Regarding childhood exposure to iAs, it was the Panel's view that, based on available data, it is not clear whether children differ from adults with regard to their sensitivity to the carcinogenic effects of arsenic in drinking water. However, the possibility of a different response in degree or kind should not be ignored and needs to be investigated.

1.4 Approaches to Low-Dose Extrapolation for iAs and DMA^V

Charge Question D1

EPA's Guidelines for Carcinogen Risk Assessment underscore the importance of understanding the MOA as the basis for making judgments on how to best extrapolate cancer risk at lower exposures. EPA concluded that available data on DMA^V are not sufficient to support development of biologically-based models and therefore opted to use a default nonlinear low-dose extrapolation method. The SAB was asked to comment on the Agency's scientific rationale in support of this approach and how uncertainty should be incorporated into low-dose extrapolation.

Summary Response

The Panel concluded that:

i) Though there are adequate data to support the proposed EPA MOA, neither the MOA postulated by the Panel, nor those postulated by EPA's ORD or OPP contain key events expected to be a linear function of dose of DMA^V.

ii) Several processes important to some postulated key events would have non-linear components or are non-linear (e.g., saturable metabolic processes, cytotoxicity, formation of heritable alterations in DNA by ROS, cell proliferation, repair of ROS-induced DNA damage).

iii) The linear approach would be consistent with evidence for direct genotoxicity of $DMA^{III/V}$; however, it is generally accepted that DMA^V is not directly genotoxic and neither DMA^{III} nor DMA^V react directly with DNA.

iv) There are insufficient data to invoke ROS-induced DNA damage as a key event in the carcinogenic process associated with exposures to DMA^V or DMA^{III}.

v) The nonlinear approach is more consistent with available DMA^V data and current concepts of chemical carcinogenesis.

vi) Uncertainty is best incorporated through the use of uncertainty factors that capture pharmacokinetic and pharmacodynamic differences across species and differences associated with sensitive populations.

vii) There are not sufficient data on comparative dosimetry in rats and humans to make any conclusive statements about species differences in pharmacokinetics, though available data on uroepithelial cell cytotoxicity might allow EPA to assemble a case for pharmacodynamic equivalency. There is presently no arsenic-specific information that can inform the choice of uncertainty factors for sensitive human populations. Thus, at least for now, such choices must be based on more general considerations including EPA's science policy judgment of the degree of precaution that it deems appropriate.

Charge Question D2

EPA determined that the most prudent approach for modeling cancer risk from iAs is to use a linear model because of the remaining uncertainties regarding the ultimate carcinogenic metabolites and whether mixtures of toxic metabolites interact at the site(s) of action. EPA asked the SAB if it 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.

Summary Response

The Panel concluded that:

i) Inorganic arsenic has the potential for a highly complex mode of action.
ii) Until more is learned about the complex PK and PD properties of iAs and its metabolites there is not sufficient justification for the choice of a specific nonlinear form of the dose-response relationship.
iii) The NRC (2001) recommendation to base risk assessments on a linear dose response model that includes the Southwestern Taiwan population as a comparison group seems the most appropriate approach.
iv) The Panel also recommends that 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.

Charge Question D3

EPA employed the Microsoft Excel software that was previously used by the NRC (2001) to project estimated cancer risks from iAs exposure. The SAB was asked to comment on the precision and accuracy of this program.

Summary Response:

The Panel concluded:

i) That the EPA program conformed to the NRC (2001) recommendation for modeling cancer hazard as a function of age and the average daily dose of exposure to arsenic through drinking water sources.
ii) The panel did, however, identify and report to the EPA on two potential discrepancies in the data inputs and one computational error in the portion of the program that employs the BEIR-IV formula to evaluate excess lifetime cancer risk from arsenic exposure.
iii) The panel made several suggestions for improvements in the model's programming and documentation conventions as well as recommendations for specific sensitivity analyses designed to test the robustness of the

model to alternative formulations of the hazard function and aggregate population data inputs.

Charge Question D4

In calculating estimated cancer risk to the US general population from drinking water exposure to iAs, the EPA utilized epidemiologic data from Taiwan. EPA followed the NRC (2001) recommendations to account for the differences in the drinking water consumption rates for the Taiwanese population and U.S. populations. On the basis of more recent data (noted in USEPA, 2005b), EPA utilized water intake adjustments for 2 to 3.5 liters/day. EPA asked the SAB to recommend a drinking water value.

Summary Response

The Panel agreed that water consumption (via drinking as water, in beverages, or in cooking water) assumptions have a substantial impact on the assessment of arsenic's risk. However, the Panel did not recommend specific values for EPA to use in evaluating dose-response in the Taiwanese study nor for levels of exposure in the U.S. population risk estimates. It did recommend that uncertainty in this parameter be evaluated for both the Taiwanese study population and the U.S. populations at risk. The Panel recommended that EPA should:

i) Evaluate the impact of drinking water consumption rates associated with more highly exposed population groups with differing exposures and susceptibilities (e.g., children, pregnant women).
ii) Incorporate variability parameters for individual water consumption into their analysis for dose-response in the Taiwanese population as they have done for the U.S. population.
iii) Conduct sensitivity analyses of the impact of using a range of consumption values for the Taiwanese population.
iv) Provide a better justification for assuming different consumption levels by gender or in the absence of such a justification, conduct additional sensitivity analyses to examine the impact of equalizing the gender-specific consumption level.
v) More fully articulate and document how different sources of water intake, as well as variability, are incorporated into the risk model (e.g. data for intake from beverages and cooking water).

Charge Question D5

As recommended by the NRC (2001) EPA considered the background dietary intake of iAs and incorporated adjustment values of 0, 10, 30, and 50 μg per day into the cancer modeling based on available new data. The SAB was asked to recommend a value for the background dietary intake of iAs for both the control population and study population of Southwestern Taiwan.

Summary Response

The Panel agreed that arsenic levels in food are important considerations for EPA's assessment of lung and bladder cancer risk associated with exposures to arsenic in drinking water. However, the Panel did not recommend a specific value for EPA to use in its base risk assessment. It did recommend a range of values for consideration by EPA in its sensitivity analysis and the Panel offered suggestions to EPA for additional analytical steps to clarify the impact of food levels of arsenic on dose-response and exposure as it revises its risk estimates. These Panel recommendations include that EPA should:

i) Conduct sensitivity analyses using a range of total arsenic food intake values from at least 50 to 100 µg per day to perhaps as high as 200 µg per day to assess the impact of this range of dietary intakes on risk of lung and bladder cancer from exposure *via* drinking water in the Taiwan cohort.
ii) Not assume that the control population has an intake value of zero arsenic from food.
iii) Apply greater rigor in their discussions of data used in these assessments (e.g., sources, methodological and analytical issues, bioavailability).
iv) Give immediate research attention to the issue of arsenic bioavailability.

2. INTRODUCTION

2.1. Process for Developing this Report and Structure of the Report

In response to the Agency's request (USEPA, 2005a), the SAB convened an expert Panel to review the Agency's hazard and dose-response assessments for dimethylarsinic acid (DMA^V) and inorganic arsenic (iAs). The full EPA charge to the SAB is in Appendix A to this advisory report. The Panel was established in accordance with the SAB *Panel Formation Process: Immediate Steps to Improve Policies and Procedures* (EPA-SAB-EC-COM-02-003). The Panel held public telephone conference meetings and a face-to-face meeting to plan for and conduct its advisory activities. The Panel met on September 12-13, 2005 to discuss the issues and deliberate on its response to the charge questions. The Panel held three subsequent public telephone conference meetings on January 24, 2006, February 23, 2006 and February 28, 2006 (see GPO, 2005a; 2005b; 2005c; 2005d; 2005e; and 2005f). The Panel considered written comments and oral statements from the public during each of these advisory meetings. This was also the case for the chartered SAB's public telephone conference meeting held on November 27, 2006 to conduct a review of the draft Panel report (see GPO 2006). Written public comments submitted for consideration at these meetings are available on the SAB web page.

Several individuals from the public requested that the Panel consider broadening its charge to include other human health endpoints associated with exposure to arsenic and to undertake a quantitative dose-response assessment for arsenic induced bladder cancer. While the Panel recognized that there is a need for the Agency to conduct a complete and thorough review of all health effects endpoints associated with arsenic exposure, the Panel limited its advisory to issues relevant to carcinogenicity of various arsenicals as requested by EPA. The Panel did not conduct a full risk assessment of the arsenicals of interest itself, because this was beyond the scope of the project and the resources available to the SAB to conduct such an analysis.

Throughout the Panel's report writing and editing process, and during the Chartered SAB's quality review, some Panel Members, and some of the interested public, suggested that additions to literature subsequent to the Panel's deliberations be evaluated and referenced in the panel's report. The Panel did not add such studies to its discussions because its active deliberations were complete. The Panel's advice to EPA considers the review of other relevant studies to be within its suggestion to EPA to evaluate other endpoints, and other studies, as the Agency completes its arsenic assessments.

This advisory report is structured according to the charge questions submitted by EPA. Subgroups of Panel members were assigned to focus on specific charge questions (see Appendix B), and each such group was responsible for leading the discussions for specific charge questions during the public meetings and for drafting the written responses to the charge questions based upon the Panel's discussions and deliberations at the meetings. All Panel members were encouraged to participate in all discussions as well as to provide comments on the draft responses for each question during the entire

advisory process. The Panel's draft report (dated September 15, 2006) was reviewed by the chartered SAB and approved conditional to the Panel Chair making the series of clarifying editorial revisions; that are now reflected in this final report which has been approved by the Panel Chair and the SAB Chair.

2.2 Background

EPA's Office of Research and Development (ORD), the Office of Water (OW) and the EPA Office of Pesticide Programs (OPP), requested that the EPA SAB evaluate certain components of the EPA draft assessment of potential human carcinogenicity associated with arsenic, and arsenic containing compounds. Information from the EPA request is summarized in the remainder of this section of the Panel's report.

Inorganic arsenic (iAs) is found naturally in the environment and it is typically present in soil and water at some determinate level. Human exposure to inorganic arsenic can come from drinking water, food, air and anthropogenic sources such as wood preservatives, industrial wastes, and certain pesticides containing organic arsenic.

Specific statutory mandates require that EPA consider human health risks associated with arsenic and arsenic containing compounds. The Safe Drinking Water Act (SDWA) directs EPA to establish national standards for arsenic containing compounds, among other contaminants, in public drinking water supplies. EPA's Superfund and Resource Conservation and Recovery Act (RCRA) programs require the evaluation of exposure to arsenic containing compounds at locations undergoing clean up or remediation, and the Clean Air Act, requires EPA to set air emissions standards for certain sources of arsenic. EPA's OPP evaluates the exposure and health risks associated with arsenicals used as pesticides in the U.S. and under the mandate of the Food Quality Protection Agency (FQPA) is reevaluating tolerances for arsenicals, and other pesticides. Tolerances are legal limits of pesticides on or in food or animal feed. Several organic arsenic containing herbicides are undergoing reregistration and/or tolerance reassessment (e.g., cacodylic acid which is often referred to as dimethylarsinic acid or DMA^V, and the monosodium, disodium, and calcium salts of methanearsonate acid --MSMA, DSMA, and CAMA, collectively as referred as MMA^V).

Arsenic, and arsenic containing compounds, have been the focus of many EPA assessments as the above statutory authorities suggest. In addition, the National Research Council (NRC) of the National Academy of Sciences (NAS) has conducted comprehensive health sciences reviews of arsenic on at least two occasions (NRC, 1999; NRC, 2001). EPA SAB Panels have also considered inorganic arsenic issues (US EPA SAB, 2000; USEPA SAB, 2001).

Since the 2001 NAS review, new information has been developed on the mode of carcinogenic action, metabolism and pharmacokinetics (PK) of arsenic and its methylated species, and new epidemiology studies have been conducted on inorganic arsenic. EPA considered this new information in its hazard characterization for tolerance assessment of dimethylarsinic acid (DMA^V) and methylarsonic acid (MMA^V)(USEPA OPP, 2005 and

USEPA ORD, 2005). EPA also developed a revised hazard and dose response assessment for inorganic Arsenic (USEPA OW, 2005) which relies on the two NRC reviews and provides an updated human health effects and dose-response assessment for inorganic arsenic. In its charge to the SAB, EPA asked for advice on the soundness of the major science conclusions in these two documents. These documents focus on the assessment of DMA^V and inorganic arsenic carcinogenicity (more specifically, metabolism, mode of action, dose-response, and approaches to low-dose extrapolation of cancer risk (see the specific Charge questions in subsections 2.2.1 through 2.2.4 and in Appendix A to the report).

2.2.1 Metabolism and Toxic Responses of Arsenic Species

Charge Question A1. Metabolism and pharmacokinetics: Please comment on how pharmacokinetic processes are best considered regarding the use of data derived from direct DMA^V exposure versus direct iAs exposure for cancer risk assessment.

Charge Question A2. Response to mixtures of metabolites: Given the toxicological response profiles observed following direct exposures to iAs versus MMA^V and DMA^V, and the differences in human and rodent toxicologic responses to arsenicals, please comment on the use of data derived from rodent exposures to the organic arsenicals versus use of data derived from direct iAs human exposure, in the DMA^V assessment.

2.2.2 Modes of Carcinogenic Action for DMA^V and Inorganic Arsenic

Charge Question B1. Mode of action of DMA^V: Please comment on the sufficiency of evidence to establish the animal mode of carcinogenic action for DMA^V. Are the scientific conclusions sound and consistent with the available evidence on DMA^V and the current state of knowledge for chemical carcinogenesis. Please comment on whether the key events in DMA's mode of action are supported by the available data. Specifically comment on the role of: a) reactive oxygen species in producing chromosomal damage and the strength of the evidence supporting oxidative damage as a causal key event in DMA^V/DMA^{III}'s mode of carcinogenic action versus an associative event or a secondary consequence of cytotoxicity; b) cell proliferation and cytotoxicity and the strength of the evidence as causal key events in DMA^V/DMA^{III}'s mode of carcinogenic action versus associative or secondary events, and c) other potential modes of action that have substantial scientific support that may be contributing to the carcinogenicity of DMA.

Charge Question B2. Human relevance of animal DMA^V MOA: Please comment on the relevance of the postulated key events (see B1) to tumors in humans. Please comment on how, if at all, differences in the human population vs. experimental animals should be accounted for in the risk assessment for DMA^V. Please comment on the Agency's conclusion that the young are likely to respond like the adult to the formation of bladder tumors following exposure to DMA.

Charge Question B3. Modes of carcinogenic action from exposure to inorganic arsenic: Please comment on the conclusion that the available data support the hypothesis that multiple modes of action may be operational following exposure to inorganic arsenic.

2.2.3 Selection of Data for Dose-Response Assessment

Charge Question C1. Use of animal data for DMA^V: Please comment on the use of the bladder tumor data from the DMA^V rat bioassay as the most suitable dataset for quantifying potential human cancer risk to DMA^V, including the weight of evidence to support this conclusion. Please comment on whether the iAs epidemiology data can be used to inform the DMA^V dose-response assessment derived from rat data with DMA^V. If so, please discuss how such information might be used.

Charge Question C2. Use of human epidemiological data from direct iAs exposure: Does the SAB agree that the Taiwanese dataset remains the most appropriate choice for estimating cancer risk in humans? Please discuss the rationale for your response. Do these data provide adequate characterization of the impact of childhood exposure to iAs? Please discuss the rationale for your response.

2.2.4 Approaches to Low-Dose Extrapolation for Inorganic Arsenic and DMA^V

Charge Question D1. Mode of carcinogenic action understanding for $DMA^{V/III}$ and implications for dose response extrapolation to estimate human cancer risk: Please comment on the scientific evidence and biological rationale in support of nonlinear versus linear low dose extrapolation approaches, which approach is more consistent with the available data on DMA^V and current concepts of chemical carcinogenesis, and how scientific uncertainty should most appropriately be incorporated into low-dose extrapolation.

Charge Question D2. Implementation of the recommendations of the NRC (2001): Does the panel concur with the selection of a linear model following the recommendations of the NRC (2001) to estimate cancer risk at this time? Please discuss your response in light of the highly complex mode of action for iAs with its metabolites.

Charge Question D3. EPA re-implemented the model presented in the NRC (2001) in the language R as well as in an Excel spreadsheet format. In addition, extensive testing of the resulting code was conducted: Please comment upon precision and accuracy of the re-implementation of the model.

Charge Question D4. Evaluation of Available literature describing drinking water consumption rates for the southwestern Taiwanese study population: What drinking water value does the panel recommend for use in deriving the cancer slope factor for inorganic arsenic?

Charge Question D5. Selection of an estimate of dietary intake of arsenic from food: What background dietary intake (of arsenic) value does the panel recommend for both the control population and study population of Southwestern Taiwan used in deriving the cancer slope factor for inorganic arsenic?

3. RESPONSE TO THE CHARGE

3.1 Overview

The SAB Arsenic Review Panel was asked to comment on the i) toxicity/ metabolic profile/bioavailability for different arsenic species, ii) the Agency's understanding of the mode of action of arsenic carcinogenesis and implications of that on dose response extrapolation for DMA^V and inorganic arsenic, and iii) the implications of newer epidemiology studies as well as the 2001 National Research Council recommendations on modeling of the human cancer slope factor for inorganic arsenic. The SAB Panel's advice is contained in sections 3.2 through 3.5 that follow.

3.2. Metabolism and Toxic Responses of Arsenic Species

3.2.1 Metabolism and pharmacokinetics (Charge Question A1)

EPA's charge states that, **"**Evidence from *in vivo* and *in vitro* metabolism and pharmacokinetic studies with humans and laboratory animals suggests that the efficiency of the methylation reaction(s) and cellular uptake varies based on which arsenical compound is administered exogenously. Most available studies suggest that the metabolic process in most mammals is primarily a one-way process and that following direct exposure to DMA^V significant amounts of [arsenite] (iAs^{III}), [arsenate] (iAs^V), [methylarsonous acid] (MMA^{III}), or [methylarsonic acid] (MMA^V) at the target tissue are not expected" (USEPA, 2005a). *Charge Question A1 asks the SAB to "…comment on how pharmacokinetic processes are best considered regarding the use of data derived from direct DMA^V exposure versus direct iAs exposure for cancer risk assessment."*

Charge questions A1 and A2 address exposure to and the metabolic fate of DMA^V associated organoarsenic-containing herbicides. DMA^V from these herbicides can be degraded by microorganisms, both in the environment and in the intestinal tract, to yield a variety of methylated and inorganic arsenic species, which have specific metabolic fates and toxicities. The Panel's responses to questions A1 and A2 do not take into consideration potential byproducts of the microbial degradation of DMA^V in the environment. This is because EPA representatives stated during the September, 2005 Arsenic Review Panel meeting that the environmental conversion of DMA^V from organoarsenic pesticides, and the risk associated with exposures to these conversion products, will be addressed later by EPA in a separate document.

The panel agrees with the Agency's reasoning behind this question which is summarized at the beginning of this subsection (3.2.1). In mammalian tissues/cells (including human), the metabolism of inorganic arsenic (iAs) appears to be a one-way process in which iAs is converted to MMA, DMA and in some species to TMA metabolites containing arsenic in +3 or +5 oxidation states (Vahter, 1999; Thomas, et al., 2001). There is no evidence for demethylation of methylated arsenic species in either animal or human tissues, though as noted in the preceding paragraph and in subparagraph

"d" below microbial transformation is possible in the intestine. However, this issue needs further investigation. While the step-wise addition of methyl groups is likely a one-way process, a cycling between +3 and +5 arsenic species may occur at each of the methylation steps due to a spontaneous oxidation of +3 species (Gong, et al., 2001; Aposhian, et al., 2003) and non-enzymatic (Delnomdedieu, et al., 1994; Scott et al., 1993) or enzymatic (Zakharayn and Aposhian, 1999; Radabaugh and Aposhian, 2000; Waters et al., 2004) reduction of +5 species. Given the likely one-way character of arsenic methylation, significant amounts of MMA or iAs are not anticipated as products of DMA^V metabolism in either rat or human tissues or urine on the basis of available data.

In contrast, exposure to iAs may result in the production, tissue retention, and urinary excretion of all the above iAs and methylated arsenic species. Based on data from rodent studies, both the uptake and reduction of DMA^V to DMA^{III} are apparently critical steps in the activation of exogenous DMA^V. It is not clear, where and to what extent (if at all) these processes occur in humans exposed to DMA^V, although it appears that uptake may be rate limiting for further metabolism of DMA^V. DMA^{III} is a urinary metabolite in individuals chronically exposed to iAs (Le et al., 2000; Aposhian, et al., 2000; Mandal, Ogra and Suzuki, 2001; Del Razo et al., 2001; Valenzuela, *et al.*, 2005), indicating that the capacity to reduce DMA^V to DMA^{III} exists in human tissues. The Panel pointed out that even the conversion of a small amount/fraction of exogenous DMA^V to DMA^{III} is of toxicological significance due to the significant toxicity of DMA^{III}. Thus, strictly from the point of view of the metabolic pattern, data derived from DMA^V exposure (in the rat), not from iAs exposure in humans, is better suited for cancer risk assessment of DMA^V. However, this approach is uncertain because of specific metabolic differences between rats and humans, and other factors, including:

a) The uptake pathway or pathways for DMA^V in humans is/are unidentified. The expression or properties of DMA^V transporters may differ in rats and humans, leading to differences in uptake of DMA^V in tissues and organs.
b) Results of laboratory and epidemiological studies suggest that the pattern for DMA^V metabolism in rats is different from that in humans (Figure 1). Rats metabolize DMA^V to DMA^{III}, trimethylarsine oxide (TMA^VO) (Yoshida et al., 1997; Yoshida et al., 1998; Cohen et al., 2002), and possibly, trimethylarsine (TMA^{III}) (Waters et al., 2004). DMA^V, DMA^{III}, and TMA^VO are urinary metabolites of DMA^V in the rat. In addition, TMA^VO was also detected in urine of rats chronically exposed to iAs (Yoshida et al., 1998). In contrast, little or no TMA^VO was found in human urine after a single dose of DMA^V (Marafante et al, 1987; Buchet et al., 1981) or after acute (Mahieu, et al., 1981; Apostoli et al., 1997; Benramdane et al., 1999) or chronic exposures to iAs (Vahter, 1999; Thomas et al., 2001). These data suggest that the capacity to produce TMA^VO from iAs or DMA^V or to excrete TMA^VO in urine is lower in humans compared to rats. Thus, while it is possible that the urinary $TMA^{V/III}$ metabolites significantly affect the

overall toxic or cancerous outcomes in the bladder of rats exposed to DMA^V, the relative lack of these metabolites in human urine suggests that the outcome in humans would not be as severe as in rats. However, because the suggested toxicity differences above reflect a very limited human data set, more research is needed to characterize the role of $TMA^{V/III}$ metabolites in bladder carcinogenesis induced in rats by chronic exposures to DMA^V. Research is also needed to determine whether the apparent absence of these metabolites in humans is associated with a decreased susceptibility to the carcinogenic effects of DMA^V.

c) Accumulation of DMA^{III} in rat erythrocytes, due to a high-affinity for binding to hemoglobin (Lu et al., 2004) contributes to a specific kinetic pattern for DMA^V in rats. It is not clear how and to what extent this factor affects the yield and concentration of the active arsenic species (e.g., DMA^{III}, TMA^VO, or $TMAs^{III}$) in urine or in target tissues of rats and how lower accumulation in human erythrocytes would alter the kinetic pattern for DMA^V and toxic/cancerous outcomes of DMA^V exposure in humans.

d) Microorganisms, including intestinal bacteria, have a capacity to either methylate or demethylate arsenicals (Hall et al., 1997; Cullen et al., 1984; Cullen et al, 1989; Lehr et al., 2003; Bently and Chasten, 2002; Tamaki and Frankenberger, 1992; Mukhopadhyay et al, 2002; Ridley et al., 1977; Qin, et al., 2006). Although the patterns and extent of DMA^V metabolism by human intestinal microflora are not known, it is possible that oral exposure to DMA^V results in the absorption of a wide spectrum of arsenic metabolites produced by bacteria in the gastrointestinal tract of exposed individuals. In contrast, bacterial metabolism would not affect the absorption of DMA^V after inhalation or dermal exposures. Thus, arsenic species found in tissues may differ with different routes of exposure. Interspecies differences in endogenous intestinal bacteria may further complicate extrapolation from rats to humans.

e) Additional factors may affect the metabolic profiles for DMA^V in humans, including co-exposures to other environmental contaminants, deficiencies of specific nutrients (e.g., selenium) or malnutrition. For example, poor nutrition has been shown to induce expression of aquaglyceroporin-9 (AQP9), an iAs^{III}/MMA^{III} transporter (Liu et al., 2002; Liu et al., 2004; Liu et al., 2006), 20-fold (Carbrey et al., 2003).

All the above concerns should be considered in the risk assessment of DMA^V exposure.

EPA's briefing documents presented information on a physiologically based pharmacokinetic (PBPK) model for arsenic disposition and metabolism that is under development. PBPK modeling might be a useful approach for integrating tissue and excreta concentrations of arsenic metabolites resulting from exposure to the various

forms of arsenic, including DMA^V, in laboratory animals and humans. For now, the modeling work described by EPA is in the developmental stage and is not considered sufficiently robust to conduct interspecies extrapolations. However, the Panel strongly encourages the Agency to proceed with PBPK model development, including laboratory studies to obtain the kinetic constants needed to describe rates of uptake, efflux, metabolism, and elimination of DMA^V in both rats and humans. When sufficiently validated, this model could simulate concentrations of active (toxic or carcinogenic) metabolites in urine and bladder tissue following exposure to DMA^V. This approach could be used for dose response analysis in cancer risk assessment. Such models must be validated for predicting tissue concentrations of active species regardless of the source of arsenic exposure.

3.2.2 Response to mixtures of metabolites (Charge Question A2)

EPA's Charge stated that, **"**Tumorigenic profiles vary based on which arsenical compound is administered exogenously. *In vivo* and *in vitro* studies indicate that each of the arsenical compounds exhibit similarities and differences in their profiles of biological activities. Direct exposure to iAs^{III} or iAs^{V} is expected to result in more of a mixture of toxic metabolites than for direct exposure to DMA^V; the mixture of metabolites is expected to vary based on which chemical is administered exogenously. The potential mixture of metabolites following direct exposure to DMA^V appears less complex as compared to iAs" (USEPA, 2005a). *Charge Question A2 asks, "Given the toxicological response profiles observed following direct exposures to iAs versus MMA^V and DMA^V, and the differences in human and rodent toxicologic responses to arsenicals, please comment on the use of data derived from rodent exposures to the organic arsenicals versus use of data derived from direct iAs human exposure, in the DMA^V assessment."*

The Panel believes that neither rodent laboratory data on organic arsenicals nor data from studies of the results of human exposures to inorganic arsenic provide an optimal basis for the assessment of DMA^V exposures in humans. This is because of the differences between the metabolic profiles for inorganic arsenic and DMA, and because of interspecies differences in the metabolism of both arsenicals. The panel agrees that using the data from rodent exposures to DMA^V may, at this time, be the most reasonable approach for the DMA^V assessment.

The reasoning behind this response is linked to the answer to charge question A1 above (see section 3.2.1). The metabolism of iAs yields a wide spectrum of metabolites (Figure 1) some of which ($iAs^{III/V}$, $MMA^{III/V}$) are apparently not produced during the metabolism of exogenous DMA^V. The production of iAs and MMA metabolites may be associated with specific toxic or cancerous endpoints that are absent in DMA^V exposure in rats or humans except when there is a significant co-exposure to iAs as is often found in U.S. drinking water supplies, or in food or the environment. The Panel notes that there are no published data on toxicological responses to DMA^V in humans. The toxic and carcinogenic effects of DMA^V have been examined only in rodents, mainly in rats.

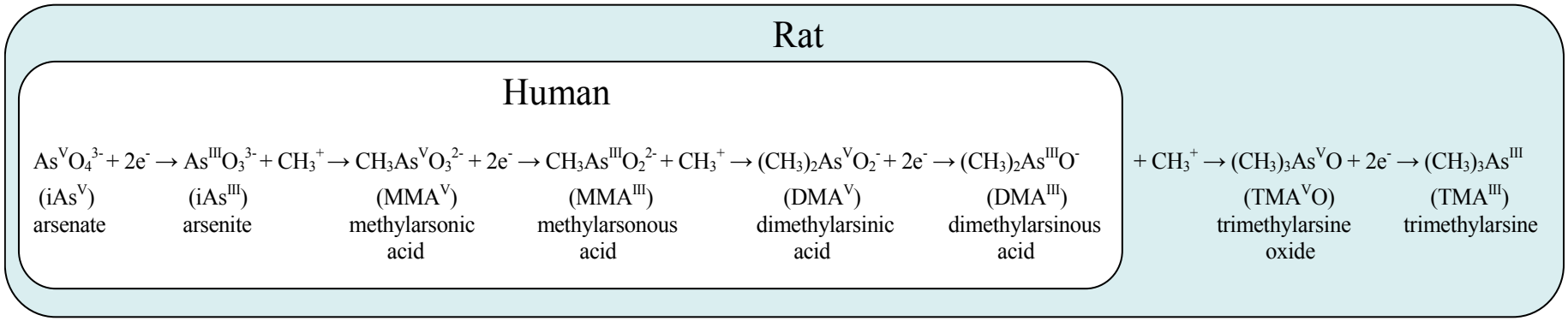

Figure 1. Schema of Inorganic Arsenic Metabolism in the Rat and Human: The metabolic pathway for inorganic arsenic in the rat and human involves a stepwise addition of methyl groups to yield methylarsenic (MMA), dimethylarsenic (DMA), and trimethylarsenic (TMA) metabolites that contain trivalent arsenic (As^{III}) or pentavalent arsenic (As^{V}). Results of epidemiological and laboratory studies suggest that while MMA and DMA are products of this metabolic pathway in both rats and humans, only rats excrete significant amounts of $TMA^{V}O$ in urine when exposed to inorganic arsenic, MMA or DMA. In addition, *in vitro* methylation of inorganic arsenic by recombinant rat, but not human arsenic (+3 oxidation state) methyltransferase produces TMA^{III}**.** Although alternative pathways have been suggested for inorganic arsenic metabolism and additional methylated metabolites were found in the urine of rats and humans exposed to arsenicals, more research is needed to determine the significance of these pathways or metabolites for inorganic arsenic or DMA metabolism in both species.

However, a significant degree of uncertainty is associated with this approach due to the metabolic differences between rats and humans and due to other factors, including those listed in the response to charge question A1 above. The differences in the production and urinary excretion of $TMA^{III/V}$ species that could affect the toxic and cancer outcomes of DMA^{V} exposure are of a particular concern to this panel. $TMA^{V}O$ is a hepatocarcinogen in rats (Shen et al., 2003). TMA^{III} is apparently more potent than DMA^{III} in damaging purified DNA in *in vitro* systems (Andrews, et al., 2003). On the other hand, both $TMA^{V}O$ and TMA^{III} are less acutely toxic or cytotoxic than DMA^{III} (Yamauchi et al., 1990; Cullen, 2005; Sakurai et al., 1998; Ochi et al., 1994). The contribution of these two metabolites to cytotoxicity and carcinogenesis in the urinary bladder of rats exposed to DMA^{V} remains unclear. This uncertainty should be properly addressed in the risk assessment for DMA^{V} exposure in humans.

3.3 Modes of Carcinogenic Action for DMA^{V} and Inorganic Arsenic

3.3.1. Mode of Action of DMA^{V} (Charge Question B1)

EPA's Charge stated that, "When relying on laboratory animal data, two critical assumptions are made: (i) data on animal tumors are predictive of human cancer, and (ii) animal tumor effects found at high experimental doses predict human risk at lower exposures. An understanding of a chemical mode of carcinogenic action can help inform the above assumptions. In the case of DMA^{V}, mode of action (MOA) data are available and were evaluated using the framework described in EPA's cancer guidelines" (USEPA, 2005a). Charge Question B1 *asks the SAB to "… comment on the sufficiency of evidence to establish the animal mode of carcinogenic action for DMA^{V}. Are the scientific conclusions sound and consistent with the available evidence on DMA^{V} and the current state of knowledge for chemical carcinogenesis?"* In addition, the Charge asks the SAB to *"…comment on whether the key events in DMA's mode of action are supported by the available data. Specifically comment on the role of: a) reactive oxygen species in producing chromosomal damage and the strength of the evidence supporting oxidative damage as a causal key event in DMA^{V}/DMA^{III}'s mode of carcinogenic action versus an associative event or a secondary consequence of cytotoxicity; b) cell proliferation and cytotoxicity and the strength of the evidence as causal key events in DMA^{V}/DMA^{III}'s mode of carcinogenic action versus associative or secondary events, and c) other potential modes of action that have substantial scientific support that may be contributing to the carcinogenicity of DMA.*

The Panel concluded that there are adequate data to support a MOA for bladder carcinogenesis induced by high doses of DMA^{V} in the rat that involves cytotoxicity to the bladder epithelium and increased, sustained regenerative proliferation as key events. The urine of DMA^{V}-treated rats contains DMA^{III} at levels that cause necrotic cytotoxicity in vitro, so it is reasonable to postulate that DMA^{III} might mediate the necrotic cytotoxicity in the rat bladder. However, the rat (unlike the human) metabolizes a significant fraction of exogenous DMA^{V} to $TMA^{V}O$ (Cohen et al., 2002; Yoshida et al., 1997, 1998) and

possibly to TMA^{III} (Waters et al., 2004). Thus, these compounds cannot be excluded as additional mediators of the necrotic cytotoxicity in the bladder of rats exposed to DMA^{V}.

The Panel thought that there are not sufficient data to invoke reactive oxygen species (ROS)-induced DNA damage as a key event in the carcinogenic process associated with exposures to DMA^{V} or DMA^{III} for the reasons discussed in the following paragraphs.

Chemically, neither oxidation of As^{III} to As^{V} nor reduction of As^{V} to As^{III} can produce an oxygen radical in the absence of other reactants. Arsenic can only undergo two-electron reduction (no free electron or radical to donate to oxygen). Although there are indirect sources of ROS that can participate in arsenite-stimulated cell signaling (e.g. stimulation of NADPH oxidase; Smith et al., 2001) or arsenic trioxide-mediated apoptosis via mitochondrial collapse (Jing et al., 1999), these have not been demonstrated for DMA. Arsenic compounds could also increase ROS by promoting an inflammatory response in many tissues. This may contribute to carcinogenesis, but it is not likely to be the primary MOA for carcinogenesis.

Much of the argument suggesting that the mode of action of DMA^{V}-induced bladder cancer involves ROS-induced chromosome damage derives from studies on DMA^{III}-induced DNA damage. Very high cytotoxic concentrations of DMA^{III} have been shown to induce DNA damage in cell-free systems and in intact cells (Mass et al., 2001), possibly via an ROS-mediated mechanism or by dimethylarsine (Yamanaka et al., 2003; Kitchin and Ahmad, 2003; Nesnow et al, 2002; Andrews et al., 2003). However, cellular genetic toxicology assays of DMA do not support an ROS-dependent mechanism. Neither DMA^{V} nor DMA^{III} is significantly mutagenic at loci which are known to detect oxidant DNA damage. Neither compound was mutagenic in the Ames *Salmonella* strain TA104, a strain developed primarily to aid in the detection of oxidative mutagens, nor were **8** prophage induced (Kligerman et al., 2003). Prophage induction (which depends on the *E. coli* SOS system, a system responsive to DNA damage) was readily detectable after treatment with other agents acting by oxidant mechanisms, such as bleomycin, carbon tetrachloride (+S9), hydrogen peroxide, and iron compounds (Rossman et al., 1991).

DMA^{V} was either negative or very weakly positive at extremely high doses in a number of other test systems which can detect oxidative mutagens (Moore et al., 1997, Kligerman et al., 2003; Oya-Ohta et al., 1996). Treatment of Muta^{TM}mouse with DMA^{V} (10.6 mg/kg per day) IP for 5 days caused only a (not significant) 1.3-fold increase in lacZ mutations in the lung, but not in the bladder or bone marrow (Noda et al., 2002). DMA^{V} presumably could be converted to DMA^{III} in mouse liver. (Arsenite also gave negative results in this assay.)

The single study of DMA^{III} in the mouse lymphoma assay (Kligerman et al., 2003), which detects clastogens as well as point mutagens at the TK locus, showed no significant effect on mutant fraction at concentrations <1.5 μM (38% survival) in the agar assay. "Significant" was defined as at least a 2-fold increase over control, but no

statistical analysis was done, only a single assay is shown, and there is only one "significant" response, at 1.51 μM. (This assumes a background mutant fraction of between 38 and 50 X 10^{-6}). A microwell assay using the same cells showed a "significant" effect at 2.56 μM (9% survival).

Kligerman et al. (2003) argued that DMA^{III} (and MMA^{III}) are basically clastogens and not point mutagens. The clastogenesis studies suffer from the same problems as the mutagenesis studies: no statistical analysis and single data points at some doses. "Significant" (defined as a 2-fold increase) effects are seen only at toxic doses (toxicity data are not given but can be inferred from other mammalian cell data). In addition, since cytogenetic assays do not require cell survival for scoring, when clastogenic effects are seen in a population of cells with low survival, it is not possible to determine whether those cells with chromosome aberrations would be among the survivors, and thus capable of resulting in a tumor. Examination of chromosomes in tumor and pre-tumor tissues in the rat bladder model might establish whether specific chromosome aberrations are associated with DMA^{V}-induced bladder cancers.

Even model clastogens such as ionizing radiation can also be detected as mutagens at single gene loci such as HPRT, a useful locus for studying mutations *in vitro* as well as *in vivo* (unlike TK). Thus, if DMA^{III} was really a clastogen acting by ROS, it should cause increased deletion mutagenesis at the HPRT locus. More research on the ability of arsenic metabolites to induce gene mutations *in vivo* should be carried out.

The fact that (some) antioxidants blocked DMA^{V}-induced bladder cancer in the rat does not provide evidence as to the origin of the oxidants nor where they act. Activation of NADPH oxidase (Smith et al., 2001) or inhibition of GSH reductase (Styblo et al., 1997) could increase oxidants. Tissues subjected to continuing cellular assault produce a number of cytokines whose signaling may be modulated by oxidants (even in the absence of frank inflammation). Nuclear factor-kappaB (NFkB), which is activated by low-dose arsenite via oxidants (Barchowsky et al., 1996), is thought to provide a link between inflammatory signaling and carcinogenesis, as well as providing survival signaling to block apoptosis in damaged cells that might otherwise die. NFkB is a transcriptional regulator of genes including cyclooxygenase-2 (COX-2), which is also induced by arsenite (Trouba and Germolec, 2004). Research is needed to determine whether bladder cells undergoing stress-related proliferation in the rat DMA^{V} carcinogenesis model show effects on NFkB and other signaling pathways, similar to those seen with arsenite.

Given the preponderance of scientific evidence to date (which is reviewed above in this section), the principal MOA for DMA^{V} does not appear to be mediated via the ROS-induced DNA damage pathway. Rather, the MOA is likely to be sustained cytotoxicity followed by genomic instability as a result of stress-related proliferation. Permanent genetic change is necessary for carcinogenesis, and it is unlikely that increased proliferation alone in the absence of increased genomic instability will result in the multiple changes needed to transform a normal cell into a tumor cell. The mechanism of cell killing by DMA^{V} or DMA^{III} is not known. Regardless of how the cells die, there

is substantial evidence supporting the hypothesis that continual proliferation of surviving cells under conditions of stress results in genomic instability (Karpinets and Foy, 2005). For example, in the case of arsenite this would involve such factors as:

a) Inducing intracellular proliferative signals and over-riding cell cycle checkpoints (reviewed in Rossman, 2003),
b) Blocking DNA repair (reviewed in Rossman, 2003),
c) Inhibiting GSH reductase (Styblo et al., 1997) and thioredoxin reductase (Lin et al., 2001),
d) Inducing stress-related survival signals to block apoptosis (Pi et al., 2005; Wu et al. 2005),
e) Effects on thiols in tubulin and cytoskeletal proteins, interfering with microfilament function and cytoskeletal changes (Li et al., 1992; Ling et al., 2002; Ochi et al., 1999),
f) Affecting DNA methylation levels, (Chen et al., 2004),
g) Inducing oxidant signaling (Barchowsky et al., 1999), and
h) Effects on hormone function (Bodwell et al., 2004).

More research should be carried out on cells undergoing stress-related proliferation in the rat bladder model to determine whether these same programs have come into play for DMA^V. Changes in DNA methylation patterns have just been demonstrated in arsenic-associated human bladder cancers (Marsit et al. 2006), making this a priority for study in the rat model.

Live cells exposed to the contents of necrotic cells may experience additional stress signals similar to that seen in the "bystander effect" after ionizing radiation (Iyer and Lehnert, 2000) or via cytokines from inflammatory cells. Although there is no direct evidence to support this mechanism in the rat bladder cancer model, it is of interest that heat-killed *E. coli* instilled into the bladder was found to increase bladder carcinogenesis by N-methyl-N-Nitrosourea (Yamamoto et al., 1992), presumably by an inflammatory mechanism. Research on this topic should be carried out both *in vivo* and *in vitro*. Further, generation of low levels of oxidants from enzymatic sources (Smith et al., 2001) or possibly by uncoupling of mitochondrial oxidations (if DMA^V can act in a manner similar to arsenate) may contribute to effects on cell signaling and transcriptional activation, as well as increase oxidant DNA damage.

In summary, the Panel postulates that the mode of action for DMA^V involves the following key events:

a) Reductive metabolism of DMA^V to DMA^{III}.
b) High concentrations of DMA^{III} in urine cause urothelial cytotoxicity. Some toxicity may also be caused by DMA^V itself.
c) Continuous exposure and persistent stress associated regenerative cell proliferation leads to genomic instability, acquisition of genetic alterations, clonal expansion of altered cells and eventually tumors.

This MOA, as well as the original MOA suggested by EPA, depends upon prolonged extensive cytotoxicity in the bladder. Without the continual cytotoxicity, sustained stress-associated proliferation would not occur. The tumor response in the rat bladder system is non-linear, as is the key event (i.e. the necessity for necrotic cytotoxicity). Since the MOA involves cytotoxicity, doses below those causing cytotoxicity would not be expected to cause tumors. The other events mentioned above would not be sufficient to cause tumors in the absence of the cytotoxicity and the resulting proliferative response.

3.3.2 Human relevance of animal DMA^V MOA (Charge Question B2)

EPA states that, "There are little or no scientific data to suggest that if sufficient DMA^{III} were present, key precursor events and ultimately tumor formation would not occur in humans directly exposed to DMA^V." *Charge Question B2* asks the SAB to *"…comment on the relevance of the postulated key events (see B1) to tumors in humans…"and to comment on how, if at all, differences in the human population vs. experimental animals should be accounted for in the risk assessment for* DMA^V.

If high enough (cytotoxic) concentrations of DMA^V or DMA^{III} were present in the human urine or bladder after exposure to DMA^V, it is plausible that a similar response (necrosis followed by regenerative, stress-associated proliferation) would take place. However, no data are available to support or reject this assumption. No studies have been carried out on DMA^V-induced bladder cancer in humans, so it is not known at this time whether there have been any cases. Concentrations high enough to cause necrosis in the bladder might be achievable in an industrial accident or deliberate poisoning. It is not clear whether a repeated or chronic exposure to DMA^V from the environment could produce cytotoxic concentrations of critical metabolites in human urine. Even in the case of high exposure, the exposures would probably have to be repeated often enough to produce persistent necrosis and regeneration in order to cause cancer.

As already mentioned in Charge Question A1 above, DMA^V is converted to TMA^VO, and possibly TMA^{III}, more efficiently by rats than by humans. TMA^VO is a hepatocarcinogen in rats (Shen et al., 2003). TMA^{III} is more potent than DMA^{III} in damaging DNA in *in vitro* systems (Andrews et al, 2003). Thus, although acute toxicities of TMA^VO and TMA^{III} are lower than that of DMA^{III} (Ochi et al., 1994; Sakurai and Kaise, 1998; Yamauchi et al., 1990), these metabolites could contribute to the MOA for DMA^V-induced bladder cancer in rats. The extent of this contribution is unknown. However, it is possible that the rat data overestimates the human risk for bladder cancers from DMA^V.

No studies have been done in either animal models or in human populations to determine whether the young are at greater or lesser risk with regard to DMA^V-induced carcinogenesis, or whether there is greater or lesser risk during any other life stages.

3.3.3 Modes of carcinogenic action from exposures to inorganic arsenic (Charge Question B3)

EPA stated that, "Inorganic arsenic (iAs) undergoes successive methylation steps in humans, resulting in the intermediate production of iAs^{III}, MMA^{V}, MMA^{III}, DMA^{V}, and DMA^{III}. Each arsenical metabolite exhibits its own toxicity." *Charge Question B3* asks the SAB to *"…comment on the conclusion that the available data support the hypothesis that multiple modes of action may be operational following exposure to inorganic arsenic."*

The Panel agrees that multiple modes of action may operate in carcinogenesis induced by inorganic arsenic. This is because there is simultaneous exposure to multiple metabolic products as well as multiple target organs. There are differences in metabolic capability and probably transport into and out of different organs for different metabolic products, so that the composition of the metabolites can differ in different organs as well. Each of the metabolites has its own cytotoxic and genotoxic capability. In general, the pentavalent compounds are less cytotoxic and genotoxic than are the trivalent compounds.

In the remainder of this section of the report, the 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. However, the Panel does not identify what the threshold might be, nor does it describe the shape of the dose-response curve, rather it leaves that to be addressed by EPA in its final assessment based on the outcome of EPA's evaluation of the relevant literature. The Panel identifies this as an extremely important area for research attention.

In the strictest (and original) definition, genotoxic carcinogens, i.e., direct carcinogens (or their metabolites) damage DNA by covalently binding to DNA or intercalating into the DNA-helix. Indirect genotoxicity occurs through interactions with non-DNA targets leading to genotoxic effects. Non-DNA targets, (e.g. proteins) exist in many copies per cell, thus a single event is unlikely to have any significant consequences. This suggests a threshold for effects associated with such events. The modes of action of "non-genotoxic" carcinogens are numerous, and can include regenerative cell growth following cytotoxic effects, modulation of metabolizing enzymes, inhibition of DNA repair, induction of peroxisome proliferation, stimulation of oxidative stress or other signaling resulting in suppression of apoptosis, loss of cell cycle control, and stimulation of proliferation. A number of indirect genotoxic events are listed in Table 1.

The genetic toxicology of iAs^{III} has been previously reviewed (Rossman, 1998, 2003; Basu et al., 2001). The interpretation of the genotoxicity of arsenic compounds is difficult because of the very high cytotoxic concentrations used in many studies and the lack of analysis for statistical significance in many studies. Another part of the problem

Table 1. Some potential mechanisms for indirect genotoxicity of Inorganic Arsenic (iAs)

Potential Mechanisms
Interference with DNA repair
Interference with cell cycle control proteins
Interference with DNA replication
Blocking apoptosis of cells with DNA damage
Interaction with nuclear proteins such as topoisomerases or spindle proteins
Nuclease and protease release from lysosomes or dead cells
Protein denaturation leading to genomic instability
Production of or change in reactive oxygen species leading to altered signaling
Other changes in gene expression (e.g., COX-2; Trouba and Germolec, 2004)
Interference with oxidative phosphorylation
Changes in ionic concentration, pH, or osmolarity
Altered DNA methylation

stems from inappropriate (or absent) assessment of toxicity of arsenic compounds (Komissarova et al., 2005). Low concentrations (even 1 μM) of iAs^{III} can cause apoptosis in human cells that can only be detected 48 hours (or more) after exposure and cytotoxicity assays (other than clonal survival) are usually performed too soon after exposure to enable identification of apoptotic cells after arsenite exposure. Thus, many "positive" genotoxicity results (especially in cytogenetic assays) could have been conducted too soon and thus they could be reported for dead or dying cells. In such cases, they would only be useful in describing a MOA if the cells continued to live and if the genotoxic effect noticed was consistent with effects seen in tumorigenesis studies in animals and in human tumors. In the absence of such tumorigenesis data, cell transformation studies can yield some insight into MOA.

Arsenite, i.e., iAs^{III} (and other arsenicals) does not exhibit direct DNA binding and the inability of iAs^{III} to induce the SOS system in *E. coli* is consistent with its lack of reaction with DNA (Rossman et al., 1984). In mammalian cells, highly toxic concentrations of both inorganic and organic arsenic compounds *in vitro* cause chromosome breakage, which some have attributed to ROS-induced DNA strand breaks but which might be caused directly by other free radical species (Yamanaka and Okada, 1994; Andrews, et al. 2003). Cellular DNA strand breakage and clastogenicity are limited almost exclusively to trivalent species. Unlike many other carcinogens, iAs^{III} is

an extremely weak (or insignificant) mutagen at single gene loci such as HPRT or TK in mammalian cells (Rossman, 1998, 2003). However, iAs^{III} (but not MMA^{III}) at low (nontoxic) concentrations can induce delayed indirect mutagenesis at the HPRT locus after >15 generations as a secondary result of genomic instability (Mure et al., 2003).

The argument has been made that arsenite is a clastogen that causes predominantly multilocus deletions, and that such deletions near the HPRT locus (which is located on a single active X chromosome) may be lethal, accounting for the lack of (or extremely weak) mutagenesis by arsenicals at the HPRT locus (Hei et al., 1998). However, molecular analysis of mutations in the HPRT gene shows that large deletions (up to ~3.5 Mb) can be tolerated in the HPRT region of the human X chromosome (Nelson et al., 1995; Lippert et al., 1995). Despite this, attempts have been made to find genetic markers more likely to detect large deletions. Also, iAs^{III} was an insignificant mutagen, and only at toxic doses, in transgenic Chinese hamster G12 cells (a derivative of V79 cells) (Li and Rossman, 1991). These cells can detect clastogens causing deletions in the single copy of the *E. coli gpt* gene inserted into Chinese hamster chromosome 1. Similar results (extremely weak mutagenesis at toxic arsenite concentrations) are seen in mouse lymphoma cells, which can tolerate deletions at the TK locus due to its autosomal location (Moore et al., 1997), at the transgenic *gpt* locus of AS52 Chinese hamster ovary cells (Meng and Hsie, 1996) and in A_L cells, which are CHO-K1 cells containing a single copy of human chromosome 11 (Hei et al., 1998).

In vivo, iAs^{III} induced micronuclei (MN) in mouse peripheral blood lymphocytes and in mouse bone marrow (Tinwell et al., 1991; Noda et al., 2002). Humans exposed to iAs^{III} show increased MN and sometimes chromosome aberrations in lymphocytes, exfoliated bladder epithelial cells, and buccal epithelial cells (reviewed in Basu et al., 2001). *In vivo* studies on genotoxic activity of methylated arsenic species are limited to a small number of studies in rodents. IP injections of high doses of DMA^V induced a slight but insignificant increase in mutagenesis in the Muta^{TM}Mouse lung, but not in bladder or bone marrow. Also, iAs^{III} was negative in this assay (Noda et al., 2002). High concentrations of DMA^V administered orally to mice caused oxidative damage and DNA strand breaks in the lung (not a target organ for DMA^V carcinogenesis). The strand breaks were attributed to dimethylarsine, via the dimethylarsenic peroxy radical $(CH_3)_2AsOO\cdot$ (Yamanaka and S. Okada, 1994). DMA^V also induced aneuploidy in mouse bone marrow cells (Kashiwada et al., 1998).

As noted in the previous paragraph, micronuclei are induced by iAs^{III} *in vivo*, and MN frequency is increased in humans exposed to iAs^{III} in drinking water. Because of this, it is important to consider the significance of MN for MOA. MN are defined as small, round, DNA-containing cytoplasmic bodies formed during cell division by loss of either acentric chromatin fragments or whole chromosomes. The two basic phenomena leading to the formation of MN are chromosome breakage (double strand breaks associated with clastogenesis) and dysfunction of the mitotic apparatus leading to aneuploidy (change in chromosome number from the normal diploid or haploid number other than an exact multiple). MN as a result of clastogenesis contain acentric chromosome or chromatid fragments while MN associated with aneuploidy contain

whole chromosomes. Currently, the most widespread and reliable assay to identify whole chromosomes in MN is by fluorescent label of their kinetochores (with antibodies) or their centromeres (with DNA probes). However, only a few laboratories routinely use these techniques because they are very costly. In most studies, there is not enough information to determine whether the MN result from: 1) toxicity, 2) clastogenicity, or 3) non-dysjunction (leading to aneuplody). Also, MN in cells trigger apoptosis, so many cells with MN will have no progeny.

In a study of 18 arsenic-exposed individuals (average 1,312 μg arsenic/L drinking water) and 18 matched controls (16 μg/L), the exposed group had a 1.65-fold increase in MN with acentric fragments ($p=0.07$) and a 1.37-fold increase in MN with whole chromosomes ($p=0.15$) (Moore et al., 1996). The combined difference (1.8-fold) was significant. Thus, exposure to iAs^{III} induces MN by multiple mechanisms. In normal human fibroblasts, low dose, long term exposure to iAs^{III} is aneugenic, inducing MN with whole chromosomes, but high dose, short term exposure is clastogenic, inducing MN with chromosome fragments (Yih and Lee, 1999). Evidence supports an aneugenic role for iAs^{III} in many other cells at concentrations lower than those causing chromosome aberrations (Kochhar et al., 1996; Vega et al., 1995; Ramirez et al., 1997; Huang and Lee, 1998; Moore et al., 1997; Sciandrello et al., 2002). Of importance is the association of aneuploidy with malignant transformation induced by arsenite and DMA^{III} (Ochi et al., 2004; Chien et al., 2004). Aneuploidy is an event that has a threshold (Kirsch-Volders et al., 2002), whereas many people assume that clastogenesis does not (at least for ionizing radiation) even though repair of radiation-induced DNA damage exists. The development of aneuploidy is a marker of genomic instability and is typical of many tumors. IP injection of DMA^{V} in mice induced aneuploidy, but no chromosome aberrations, in bone marrow (Kashiwada et al., 1998). (DMA^{V} would be converted to DMA^{III} in the mouse, so the active agent may be DMA^{III}). Bladder tumors in patients with high iAs^{III} exposure showed higher levels of aneuploidy compared with other bladder tumors (Moore et al., 2002).

Genomic instability can result from changes in DNA methylation in iAs^{III}-treated cells. The first report of arsenite inducing DNA methylation changes was the increased cytosine methylation in the p53 promoter in human adenocarcinoma A549 cells (Mass and Wang, 1997). Later it was found that there was both hypo- and hypermethylation (of different genes) in human kidney UOK cells treated with iAs^{III} (Zhong and Mass, 2001). When SHE cells or rat liver TRL1215 cells were transformed by iAs^{III}, specific oncogenes were more highly expressed due to hypomethylation (Zhao et al., 1997; Takahashi et al., 2002) and there was evidence of decreased DNA methyltransferase activity (Zhao et al., 1997). These findings are consistent with the usual DNA methylation changes observed in cancer, in which global methylation is reduced but some gene specific promoter methylation is increased (Baylin and Herman, 2000). Arsenite (iAs^{III})-induced DNA hypomethylation and altered gene expression has been demonstrated in mouse liver (Chen et al., 2004), in hepatocellular carcinoma derived from transplacental iAs^{III} exposure (Waalkes et al., 2003), and in prostate epithelium where the hypomethylation was shown to activate K-ras (Benbrahim-Tallaa, et al., 2005). In a study of iAs^{III} exposed individuals in India, increased levels of hypermethylated p16

and p53 gene promoters were seen in blood DNA (Chanda et al., 2006). Methylated CpG sites are mutational hotspots (e.g. by a second agent), methylation changes affect gene expression, and hypomethylation leads to genomic instability. Low concentrations of iAs^{III} induce delayed mutagenesis and chromosome aberrations that might be mediated by hypomethylation (Mure et al. 2003; Sciandrello et al., 2004). This is a mechanism that might also explain transplacental carcinogenesis.

There are a number of non-genotoxic actions of arsenite (and perhaps MMA^{III} and DMA^{III}) that can contribute to the carcinogenic process. The role of ROS in (low dose) arsenic carcinogenesis is probably via signaling changes rather than as a genotoxicant (otherwise, one would expect more mutagenesis). This may contribute to carcinogenesis, but it is not likely the primary MOA for arsenic carcinogenesis. Cell signaling can be affected by arsenite via low levels of oxidants that do not cause DNA damage (reviewed in Simeonova and Luster, 2004). Low iAs^{III} concentrations increased oxidant signaling and oxidant-dependent activation of nuclear factor kappaB (NFkB) in the absence of DNA damage in human endothelial cells (Barchowsky et al., 1999). The increased oxidants appear to result from activation of membrane-bound NAD(P)H oxidase. Arsenite (iAs^{III})-induced signaling results in expression of inflammatory cytokines such as IL-8 that can mediate atherogenesis (Simeonova and Luster, 2004). Arsenite (iAs^{III}) also increases ROS by promoting an inflammatory response in many tissues. In addition, there is redox chemistry involved in iAs^{III}-dependent signaling in that arsenite binds to protein thiols (particularly vicinal thiols) to stimulate signaling cascades or affect DNA repair. Arsenite (iAs^{III}) interaction with thiols is a redox reaction, but oxygen radicals are not involved.

Exposure to low, non-toxic doses of iAs^{III} enhances positive growth signaling (reviewed in Rossman 2003), which can readily contribute to hyperplastic pre-cancerous skin growth. Arsenite (iAs^{III}) can disrupt glucocorticoid receptor (GR) and other steroid signaling at very low doses, and it has been suggested that these effects on GR may affect carcinogenesis (Kaltreider et al., 2001). This may also affect other disease processes, such as cardiovascular disease, diabetes and other diseases that have been associated with iAs exposure, since GR and other steroid receptors have been shown to be important in these diseases as well.

Animal studies indicate that for some organs, transplacental carcinogenesis after maternal exposure to iAs^{III} occurs. This includes the formation in C3H mice of tumors of the lung and liver, target sites of potential human relevance, after exposure to arsenic *in utero*. In addition, *in utero* arsenic induces tumors of the ovary and adrenal, sites not observed in humans to date. The C3H mouse was selected in these studies because it is, in general, sensitive to chemical carcinogenesis, although this strain shows spontaneous tumor formation in several tissues. Recent work has shown that with gestational exposure to CD1 mice, inorganic arsenic is a complete carcinogen in the female offspring (Waalkes, *et. al*., 2006). The CD1 mouse strain is noteworthy as having a well defined, low rate of spontaneous tumors. EPA's revised assessment document should take note of this important development. Other studies indicate that in skin, neither iAs^{III} nor iAs^{V} is a complete carcinogen, but they act as enhancers (cocarcinogens, sometimes mistakenly

called "promoters") with other agents. Arsenite (iAs^{III}) acts as a cocarcinogen with solar UV light (Rossman et al. 2001; Burns et al., 2004) and arsenate is cocarcinogenic with 9,10 dimethyl1-2-benzanthracene (Motiwale et al., 2005). This leaves open the possibility that a cocarcinogenic MOA may also operate for other organs, but this remains to be investigated.

Arsenite (iAs^{III})-enhanced UV carcinogenesis could result from acquired resistance to UV-induced apoptosis. Such resistance was recently demonstrated in human keratinocytes (HaCaT) treated with 0.1 μM arsenite for 28 weeks (Pi et al., 2005). In mouse keratinocytes, the repair of UV-induced 6-4 photoproducts was slowed by acute 5.0 μM (24 hr) arsenite exposure, which also inhibited the UV-induced apoptosis as indicated by TUNEL flow cytometry and by reduction of caspase 3/7 activities (Wu, et al., 2005). Arsenite (iAs^{III}) also blocked UVB-induced apoptosis in human keratinocytes (Chen et al., 2005). One mechanism by which iAs^{III} may perturb apoptotic pathways is by PI3K-mediated phosphorylation of PKB (Akt). When PI3K activity was inhibited by Wortmannin or LY294002, arsenic-induced apoptotic resistance was also blocked (Pi et al., 2005).

Table 2 lists some activities of iAs^{III} (or its metabolites) that might explain how iAs^{III} can act as a transplacental carcinogen and a cocarcinogen but not a complete carcinogen in neonatal and older animals.

In the future, it will be important to determine whether the trivalent arsenic metabolites can induce tumors *in vivo* or transform or mutate keratinocytes and other major target cells of arsenic at biologically relevant concentrations. The mechanism of iAs-induced carcinogenesis is likely to be different in different tissues, with contributions from all the various arsenic species present in that tissue.

There have been a number of reports claiming an essential role for iAs in various animal species (chick, goat, hamster, pig, rat), but many of these reports exist only in abstracts or in meeting reports (reviewed in Uthus, 1992; Nielson, 1996). Reports in the peer-reviewed literature follow. A study of arsenic-deprived goats found muscle atrophy, reduction in oxidative enzymes and abnormal mitochondria in muscle, possibly via disturbance of calcium metabolism (Schmidt et al., 1984). Studies by Uthus (1990, 1992) suggest that iAs has a role in methionine/methyl metabolism. Arsenic (iAs)-deprived rats had decreased plasma taurine, hepatic polyamines, and S-adenosylmethionine decarboxylase (needed for polyamine synthesis). Arsenic (iAs)-deprivation as well as iAs excess caused DNA hypomethylation in rat liver. The same effect was seen in Caco-2 cells. Arsenic (iAs) deprivation or excess also increased the formation of aberrant colon crypts in rats treated with dimethyl hydrazine (Uthus and Davis, 2005), suggesting a cocarcinogenic effect. So far, no exact biochemical mechanism linking any iAs species with methionine/methyl metabolism has been found, but the fact that many laboratories have reported effects of iAs^{III} on DNA methylation makes this an important area of study.

Table 2. Activities of iAS^{III} that may contribute to its cocarcinogenic and/or transplacental carcinogenic action

Activities
DNA repair inhibition
Increased oxidants (signaling changes)
Gene dosage effects (aneuploidy, amplification)
Altered DNA methylation
Proliferative response
Increased angiogenesis
Effects on immune system (not discussed; see Vega *et al*., 2004)
Inhibition of apoptosis
Hormonal effects
Delayed mutagenesis (not enough generations *in vivo* but maybe enough if transplacental or added to a genotoxic agent?)

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. In cell culture, subtoxic concentrations of iAs^{III} reduced the levels of ROS in keratinocytes and fibroblasts by upregulating thioredoxin and glutathione reductase (Snow et al., 2005). Some DNA repair proteins are also upregulated. The same paper also describes protection of mice from skin tumors induced by dimethylbenzanthracine + phorbol 12-tetradecanoate 13-acetate if the mice were given drinking water containing 0.2-2 ppb arsenate. Inorganic arsenic (iAs) has both positive and negative effects on the growth and function of blood vessels (Soucy et al., 2003, 2005; Kamat et al., 2005). Low concentrations fuel angiogenesis, while higher concentrations injure endothelial cells and promote the vessel dysfunctions seen in ischemic diseases and peripheral vascular diseases. Thus, iAs may provide improved vascularization and growth of normal tissues, which could reduce cardiovascular risks. However, this process could also pose risks for iAs increasing the vascularization and growth of both atherosclerotic lesions (Simeonova and Luster, 2004) and tumors from a secondary source (Kamat et al., 2005). Mice drinking 10-250 ppb iAs^{III} had increased metastases from transplacental tumors (Kamat et al., 2005). However, iAs at high doses has been used to destroy the tumor vasculature (Griffin et al., 2003).

While mechanistic studies suggest that there should be a threshold for iAs bladder cancer, available data, and data from epidemiological studies, are lacking or problematic with regard to low-dose effects. This critically important issue should be the subject of additional mechanistic and epidemiologic research. This research should attempt to determine whether the suggested hormetic or beneficial effects of arsenic, for several endpoints at very low doses, exist more broadly and whether they apply to all life stages. If iAs is shown to be essential, i.e., necessary for certain life-sustaining functions or processes, then a threshold would not be at zero. If iAs would be shown to have hormetic effects, then the issue of a threshold would be less clear and while a threshold might be

possible for one health endpoint, it might not exist for another iAs associated health endpoint. Research should also further illuminate the shape of the dose response curve at low doses for the biological effects of arsenic.

3.4 Selection of Data for Dose-Response Assessment

3.4.1 Use of animal data for DMA^V (Charge Question C1)

EPA's Charge stated that, "A number of different rodent bioassays (standard bioassay, transgenic animals, susceptible rodent strains, initiation and promotion studies) are available on DMA^V." *Charge Question C1* asks the SAB to *"…comment on the use of the bladder tumor data from the DMA^V rat bioassay as the most suitable dataset for quantifying potential human cancer risk to DMA^V, including the weight of evidence to support this conclusion.*

The consensus of the panel is that, given the lack of human data, the bladder tumor data from the DMA^V rat bioassay is the most suitable data set for quantifying potential human cancer risk to DMA^V. Given the differences in metabolic fates of DMA^V and iAs, the use of human data from iAs exposure to predict risk from DMA^V is not recommended. In this case, reliance on interspecies extrapolation using the rat bioassay data is the best alternative.

This question indirectly raises the issue as to the largest source of uncertainty for DMA^V risk assessment—conventional interspecies extrapolation or extrapolation across various forms of arsenic. The available material suggests that extrapolation across various forms of arsenic would lead to the greatest degree of uncertainty in a risk assessment. Although the panel agreed that use of the rat DMA^V bioassay data is the preferred method, the panel also felt strongly that a discussion of the key uncertainties with using data from testing in rats to conduct human risk assessment should be included in EPA's Office of Pesticide Programs report "*Science Issue Paper: Model of Carcinogenic Action for Cacodylic Acid (Dimethylarsinic Acid, DMA^V) and Recommendations for Dose Response Extrapolation*." Issues that panel members consider important to discuss in EPA's Science Issue Paper are discussed in more detail below and in Section 3.2.1. These issues include the pharmacokinetic and pharmacodynamic similarities and differences between rats and humans in response to arsenic exposure (e.g., the role of $TMA^{III/V}$ species); the use of rodent bladder tumor models in general; shared MOAs with iAs due to the possibility of DMA^V demethylation by intestinal bacteria (see section 3.2.1); and issues related to in the use of rodent data for human risk assessment. The panel also recommends that the EPA consider applying the Human Relevance Framework (HRF) proposed by the International Life Sciences Institute-Risk Science Institute (ILSI-RSI) (Seed, et al, 2005) to the mode of action. Application of the framework or its elements may assist EPA in evaluating the human relevance of the DMA^V rat data. This framework has been used to assess the relevance of rodent liver tumors to human cancer risk (Holsapple et al, 2006).

Several pharmacokinetic differences between rats and humans have been reported after arsenic exposure. For example, arsenic methylation in rat hepatocytes proceeds at a

faster rate than in human hepatocytes (Styblo, et al., 1999). Additionally, rats have a considerably slower whole body clearance of DMA^V compared with humans. This slower whole body clearance in rats results from a significant portion of DMA being retained in the erythrocytes of rats (Vahter, et al., 1984). The affinity of rat hemoglobin to bind DMA^{III} is 15 to 20 fold higher than that of human hemoglobin (Lu, et al, 2004). These differences in metabolism and pharmacokinetics may be consistent with a greater sensitivity of the rat to induction of bladder tumors by DMA^V. However, without a more complete data set demonstrating that exposure of the bladder epithelium (urothelial cells) to DMA^V metabolites (particularly DMA^{III}) is greater in rats than in humans for a given dose, the data are not sufficient to support reduction of interspecies uncertainty factors based on differences in pharmacokinetics. Clearly, this is a high priority area of research with the potential to reduce uncertainty in the risk assessment of DMA^V.

In the EPA Science Issue Paper consideration should be given to the pharmacodynamic similarities and differences between rats and humans and the relevance of the rat response to human risk assessment. Although data illustrating the mode of action for DMA^V as a bladder carcinogen in rats seem quite convincing, it should be noted that rats are more sensitive to DMA^V in carcinogenicity testing than are mice (Rossman, 2003; Arnold, et al., 2003). While the relative *in vivo* sensitivities of rats and humans to DMA^{III} are unknown, it has been shown that *in vitro* rat and human urothelial cell lines are equally sensitive in terms of acute toxicity to DMA^{III} in the micromolar range (Cohen et al., 2002). For arsenite, however, the rat MYP3 urothelial cell line showed toxicity at about one tenth (LC_{50} of 0.4 μM) the concentration as did the human 1T1 urothelial cell line (LC_{50} of 4.8 μM). As a result of the Panel's analysis of the information on this key pharmacodynamic response, urothelial cell cytotoxicity, the consensus was the EPA could explore a case for pharmacodynamic equivalency between the test species, rats, and humans from existing experimental data. Pharmacodynamic equivalency could be incorporated into the assessment as a reduction of the pharmacodynamic component of the interspecies uncertainty factor, which is 3, to a value of one. However, as discussed in the response to question D1, there remains considerable uncertainty due to limited comparative in vivo data across species. The final EPA risk assessment should fully discuss the interspecies similarities and differences and the implications for risk assessment as well as explore opportunities to reduce uncertainty factors.

EPA's Science Issue Paper should discuss similarities and differences between rats and humans in the development of bladder tumors and how these differences impact interspecies extrapolation. Studies suggest that in rats it takes two or more years of continuous high dose exposure to DMA^V to induce these tumors. Human bladder tumors are also late occurring. The Science Issue Paper should specifically discuss the similarities and differences in the time for induction of DMA^V related tumors in rats with the pattern observed with humans and arsenic associated urinary bladder cancer.

EPA's Science Issue Paper should also discuss general issues associated with rat urinary bladder cancer. One such issue is the relationship between the induction of tumors and high concentrations of arsenic in the urine. Also, there is a need to address

evidence that simple enhancement of proliferation is not associated with carcinogenesis in many tissues. Studies by Gur et al. (cited on page 97 of the DMA MOA Science Issue Paper, US EPA OPP, 2005) on the carcinogenicity of DMA^V were never published and thus cannot be critically evaluated by the Panel. The Science Issue Paper notes that the Gur studies in rats and mice are key bioassay studies. Reliance on these studies would be stronger if the studies had the benefit of peer review.

The EPA's Science Issue Paper expresses concern with the mouse transplacental model for inorganic arsenic because the strain of mice used (namely C3H) in the original two studies had a significant rate of spontaneous tumors in several tissues that are also targets of arsenic. Recent follow-up work has shown that gestational exposure to inorganic arsenic in CD1 mice is a complete carcinogen in the female offspring (Waalkes, *et al*., 2006). The CD1 mouse strain is noteworthy as having a well defined, low rate of spontaneous tumors. The Science Issue Paper should take note of this important development.

Charge Question C1.B also asks the SAB to "…*comment on whether the iAs epidemiology data can be used to inform the DMA^V dose-response assessment derived from rat data with DMA^V. If so, please discuss how such information might be used.*" (See Appendix A).

The panel consensus was that without more detailed information on target tissue dosimetry of arsenic species the iAs epidemiology data would be of limited use to inform the DMA^V dose-response assessment derived from rat data with DMA^V. Direct exposure to iAs elicits a different cascade of metabolite concentrations with related differential kinetics compared to direct exposure to DMA^V, therefore the iAs epidemiology data cannot reasonably be used to inform the DMA^V dose-response assessment derived from rat data with DMA^V. In the absence of specific information on target tissue levels, assumptions would have to be made regarding the proportion of the iAs for human and DMA^V for rodents that reaches the bladder tissue as the toxic DMA species.

In principle, epidemiology data from iAs exposed humans could be used to inform the DMA assessment to the extent that the data might be able to address the appropriateness of interspecies extrapolation, specifically the relative sensitivities of rat and human to bladder cancer following arsenic exposure. However, as noted above, in order to be useful some information on target tissue dose of DMA following human exposure to iAs and rodent exposure to DMA^V would be necessary. With both *in vivo* tumor indices (human and rodent) expressed in terms of the same tissue dose of relevant metabolites, rather than iAs or DMA^V exposure levels, the relative sensitivities of the human and rodent could be assessed.

3.4.2. Use of human epidemiological data from direct iAs exposure (Charge Question C2)

EPA's Charge states that, **"**Since the NRC (2001) report on iAs, an additional body of literature has developed describing epidemiology data from populations in the

U.S. exposed to iAs in drinking water" (USEPA, 2005a). *Charge Question 2 asks, "Does the SAB agree that the Taiwanese dataset remains the most appropriate choice for estimating cancer risk in humans? Please discuss the rationale for your response."*

For reasons noted below in this section, it is the Panel's view that, at this time, the Taiwanese database remains the most appropriate choice for EPA's use in deriving the cancer unit risk for iAs. However, the Panel suggests that EPA also conduct adjunct analyses to test the robustness of results against their assumptions, determine the impact of variability in some parameters, compare the results against those from other data sets, and provide a transparent assessment of the available epidemiological data using a consistent set of criteria.

The Taiwanese dataset consists of population and mortality data from 42 villages in southwest Taiwan for the years 1973-1986. Arsenic levels in wells from these villages were measured in 1964-1966. The database is one of the largest that has been evaluated for cancer risk relative to arsenic exposures. A total of almost 900,000 person years of follow-up were included, with 1,152 cancer deaths (637 males, 515 females). Among the cancer deaths were 181 due to bladder cancer (85 males, 96 females), 268 lung cancer (147 males, 121 females), and several hundred due to other types of cancer. These data have been subject to several ecologic analyses, starting with the original publications by Chen et al. (1988) and Wu et al. (1989), followed by further analyses by Morales et al. (2000) and by the National Research Council (1999 and 2001).

Among the 42 villages, the arsenic concentration ranged from 10 to 934 ppb (μg/L). Twenty of these 42 villages used a single well. Among many of the 22 villages with multiple wells, many had wide variability in the measured arsenic level in their wells. Analyses using the full dataset give results comparable to results from a reduced dataset including only the villages with single wells, providing some confidence in the stability of the overall results (National Research Council, 1999). The Panel recognizes the limitations of the southwest Taiwan database, including its ecologic character, lack of smoking information, limited precision of exposure estimates, especially among villages with multiple wells, and the possible issue of compromised nutrition among segments of the exposed population. However, in view of the size and statistical stability of the database relative to other studies, the reliability of the population and mortality counts, the stability of residential patterns, and the inclusion of long-term exposures, it is the Panel's view that this database remains, at this time, the most appropriate choice for estimating cancer risk among humans. Supporting this view is the fact that the datasets from Taiwan have been subjected to many years of peer review as part of published studies.

Given the concerns regarding the use of the median well water concentrations in some of the 42 villages in southwest Taiwan that have more than a single measurement, the Panel recommends that EPA conduct a sensitivity analysis. This should include the range of exposures in said villages to provide a range of risk estimates. One alternative (suggested in response to D-3) is a full Monte Carlo analysis in which the individual well concentrations for 22 villages with multiple wells are taken into account. The Panel recognizes the difficulties with this approach including the issue of how to allocate cases to wells within villages. A simpler, but useful first approach would be to test the

sensitivity of the model fitting when arsenic concentrations for multiple-well villages are set to: 1) a low level concentration from the range for the village (10^{th} percentile, 20^{th} percentile); 2) the median (current procedure); and 3) a high level concentration from the village range (90^{th} percentile, 80^{th} percentile).

New studies have been published since the NRC report in 2001 and the Panel considered some of these (and additional information provided by the public during this review process) in its evaluation regarding the Taiwanese data sets that are the focus of this charge question. To be clear, the panel did not do an exhaustive review of all possible toxicologic and epidemiologic literature during its review. That was beyond the scope of the Panel's charge. The Panel recognizes that this must be done in EPA's final assessment and calls on the Agency to do so.

In view of the limitations of this database, the Panel recommends that the other relevant epidemiologic 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. In addition, some populations likely differed from the Taiwanese population with regard to their nutritional status. The accuracy and precision of exposure assessment is a major issue in all environmental epidemiologic studies, and in particular, in studies of arsenic in drinking water. Misclassification of exposure in such studies (when non-differential) can have a profound effect in attenuating the magnitude of the observed risk. The excellence of exposure assessment is an especially strong aspect of several studies from northern Chile, and the Panel recommends that the findings of Smith et al. (1998) and of Ferreccio et al. (2000) be included by EPA in evaluation of other datasets as described below. In addition, arsenic exposures appear to be well characterized in cohort studies of Chiou et al.(2001) of transitional cell carcinoma (mostly bladder cancers) and Chen et al. (2004) of lung cancer, from arsenic-exposed cohorts in southwest and northeast Taiwan. The latter study also provides data on the joint effects of arsenic and cigarette smoking in the Taiwanese population.

The accuracy of estimated long-term exposures to arsenic is of concern for recent studies with water concentrations below 100 ppb. Misclassification of exposure may compromise their overall utility in assessing concordance with risk estimates obtained from the Taiwan study. The Panel suggests that results on bladder cancer risk from published epidemiology studies of U.S. and other populations chronically exposed to arsenic levels ranging from 0.5 to 160 μg/L inorganic arsenic in drinking water, be critically evaluated. A sensitivity analysis to evaluate the potential impact of sources of bias in the low level case control and cohort studies could be informative. Several arsenic epidemiology studies have the advantage of data with drinking water arsenic exposure levels ostensibly most relevant to the U.S. population [Bates, et al., 1995; Karagas et al., 2004; Lewis et al., 1999; Kurttio et al., 1999; Steinmaus, et al., 2003; Bates et al., 2004; Michaud et al., 2004; Chiou et al, 2001; Ferreccio et al., 2000]. Most of these populations have a nutritional and genetic background similar to that of the U.S. or the studies were conducted in a U.S. population. EPA should determine the potential utility of these studies in exploring overall concordance of the cancer risk estimates

derived from their data with risk estimates obtained from extrapolation of the Taiwan data. The Panel suggests 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.

When reviewing these "low-level" studies (and the "high level" studies as well), EPA should consider at least the following issues: estimates of the level of exposure misclassification; temporal variability in assigning past arsenic levels from recent measurements; the extent of reliance on imputed exposure levels; the number of persons exposed at various estimated levels of waterborne arsenic; study response/participation rates; estimates of exposure variability; control selection methods in case-control studies; and the resulting influence of these factors on the magnitude and statistical stability of risk estimates. Most populations in the U.S. and many other countries differ from the Taiwanese population of interest in genetic background (e.g., genetic polymorphisms), dietary intake, and background exposure concentrations to inorganic arsenic, and if one or more of these studies are shown to be of potential utility, comparative analyses of the U.S. and Taiwan data may lead to further insights into the possible influence of these differences on population responses to arsenic in drinking water. For compounds such as arsenic for which there are human data beyond the Taiwanese study on which human cancer risk has been based, data from the other investigations at high exposure levels (>150 μg/L) can be used to gauge the Taiwanese findings [Smith et al.(1998), Ferreccio et al.(2000), Chen et al.(2004), Chiou et al.(2001)].

All of these studies, including those from Taiwan, Chile, Argentina and the U.S. as described above, should be judged by the same set of criteria, with the comparative assessment of those criteria across studies clearly laid out in a tabular format. Some of the criteria have been listed in the previous paragraph. The relative strengths and weaknesses of each study need to be described in relation to each criterion. The caveats and assumptions used should be presented so that they are apparent to anyone who uses these data. Included in the risk assessment background document should be a complete and transparent treatment of variability within and among studies and how it affects risk estimates. The present lack of transparency in the application of the criteria in the process of study selection was pointed out by several panel members.

<u>*Charge Question C2*</u> also asks, *"Do these data provide adequate characterization of the impact of childhood exposure to iAs? Please discuss the rationale for your response."*

The Taiwanese data are inadequate to characterize the impact of childhood exposure to inorganic arsenic with respect to carcinogenesis. That is, it is not clear whether children differ from adults with regard to their sensitivity to the carcinogenic effects of arsenic in drinking water. More data are needed to fully characterize the

impact of transplacental exposures. However, data from the studies in southwestern Taiwan which include childhood exposures in the calculation of lifetime dose show that in the population under 30 years of age there were no bladder cancer cases, and only 5 lung cancer cases but few cases are actually expected in that age group. Childhood exposures are included in the lifetime dose estimates. Smith et al (1998) report the highest excessive risk for male lung cancer in the 30-39 year old age group, suggesting the importance of childhood exposure and risk and perhaps smoking behavior as young adults. For 533 women exposed to arsenic in drinking water from tube wells at greater than 50 μg/L compared with those exposed at 50μg/L or less, findings suggest that there are significantly increased odds ratios for spontaneous abortion, stillbirth and neonatal death (Milton et al., 2005). Another reproductive study in Chile, which followed over 800 pregnancies, found that pregnant women drinking water containing 40 μg/L gave birth to infants of lower birth weight than a comparable group drinking water containing very low arsenic concentrations (<1 μg/L) (Hopenhayn et al, 2003). Thus maternal exposure at moderately high levels may have toxic effects; the issue of childhood carcinogenic susceptibility has had only limited study.

3.5 Approaches to Low-Dose Extrapolation for Inorganic Arsenic and DMA^V

3.5.1 Mode of carcinogenic action understanding for $DMA^{V/III}$ and implications for dose-response extrapolation to estimate human cancer risk (Charge Question D1)

EPA's Charge stated that, "The use of mode of action data in the assessment of potential carcinogens is a main focus of EPA's 2005 cancer guidelines. As stated in th[o]se guidelines 'The approach to dose-response assessment for a particular agent is based on the conclusion reached as to its potential mode(s) of action.' Although a biologically-based model is the preferred approach to estimating cancer risk, there are insufficient data on DMA^V to support development of such a model." *Charge Question D1* asks the SAB to *"…comment on the scientific evidence and biological rationale in support of nonlinear versus linear low dose extrapolation approaches, which approach is more consistent with the available data on DMA^V and current concepts of chemical carcinogenesis, and how scientific uncertainty should most appropriately be incorporated into low-dose extrapolation."*

3.5.1.1 *Please comment on the scientific evidence and biological rationale in support of the nonlinear versus linear low dose extrapolation approaches*

The Panel believes, based on the review of EPA's analyses, that there are adequate data to support much of the EPA-postulated MOA for bladder carcinogenesis induced by high doses of DMA^V in the rat. The MOA involves cytotoxicity of the bladder epithelium and increased, sustained regenerative proliferation, as key events. However, the Panel concluded that there are insufficient data to invoke ROS-induced DNA damage as a key event in the carcinogenic process, associated with exposures to DMA^V or DMA^{III} (see Charge Question B1).

The postulated MOA for DMA^V is:

a) Reductive metabolism of DMA^V to DMA^{III}.
b) High concentrations of DMA^{III} in urine cause urothelial cytotoxicity. Some toxicity may also be caused by DMA^V itself.
c) Continuous exposure and persistent, stress associated, regenerative cell proliferation leads to genomic instability, acquisition of genetic alterations, clonal expansion of altered cells and eventually tumors.

Neither the MOA postulated here, nor those postulated by ORD or OPP (USEPA OPP, 2005; USEPA ORD, 2005), contain key events expected to be a linear function of dose. Reductive metabolism of DMA^V is likely to be saturable and therefore non-linear but this does not necessarily imply a threshold-based response. *In vitro* cytotoxicity of uroepithelial cells, using rat (MYP3) and human (1T1) bladder cell lines, occurs only at concentrations greater than ~0.35 μM (rat, 0.38 μM DMA^{III}; human 1T1 0.35 μM DMA^{III}) (unpublished data[1]). *In vivo*, cytotoxicity of the uroepithelium occurred at the lowest tested dietary DMA^V concentration (2 ppm), but the incidence and severity increased, and the latency decreased significantly as a function of dose. Statistically significant increases in regenerative cell proliferation only occur in rats at DMA^V dietary concentrations greater than 40 ppm, again, a nonlinear or apparent threshold response.

Even ROS production, and its interaction with DNA, would be expected to be linear at some low dose, but nonlinear across the larger dose range. Formation of heritable alterations in DNA by ROS is believed to be a nonlinear or curvilinear effect (USEPA ORD, 2005) best represented by a quadratic function with a low-dose linear component (USEPA OPP, 2005). The formation rate of heritable alterations is a function of the rate of DNA damage and the rates the various DNA repair processes and finally the rate of DNA misreplication (USEPA OPP, 2005). The latter being a function of cytotoxicity and regenerative cell proliferation which in the case of DMA^V, are also highly nonlinear functions of dose (USEPA ORD, 2005). With respect to repair of postulated ROS induced DNA damage, highly specific enzymatic systems that exist for

[1] Personal communication from L. Arnold of Dr. Cohen's Lab with the EPA SAB Designated Federal Officer; 4.4.2006. Samuel M. Cohen, M.D., Ph.D. Professor and Chair, Pathology and Microbiology Havlik-Wall Professor of Oncology University of Nebraska Medical Center, Text of the email is provided below: "During the process of determining the LC_{50} for the various arsenicals we did develop some data concerning the no effect level especially in the MYP3 rat bladder cell line. We do not have as much data for the 1T1 human bladder cell line since we used concentrations that we had already determined caused cytotoxicity in the MYP3 cell line. We do have detailed data for DMA^{III} for both cell lines since there was a very sharp drop between concentrations which had no effect on the cell viability and concentrations which were cytotoxic. In the MYP3 rat bladder cell line, DMA^{III} concentrations of 0.38 μM and below had no effect on the viability of MYP3 cells but at a concentration of 0.39 μM DMA^{III} the cell viability dropped to 69%. In the 1T1 human bladder cell line, DMA^{III} concentrations of 0.35 μM and below had no effect on viability but at 0.40 μM the viability dropped to 76%. The following data show doses for other arsenicals at which there was no effect on cell viability however, the no effect dose may be somewhat higher but we do not have enough data points to determine an exact concentration.

MYP3 rat bladder cell line:

Arsenite-0.05 μM; Arsenate-1 μM; MMA^{III}-0.5μM; MMA^V-1mM; DMA^V-0.05mM; TMAO-0.1mM

their repair (Slupphaug et al., 2003) protect the genome, whether from exogenous chemicals or the high levels of endogenous ROS induced DNA damage. These enzymatic repair processes are expected to be nonlinear processes. EPA's position on a linear oxidative stress MOA induced by DMA^V is likely not defensible and should not be used. The state of the science is overwhelmingly in favor of a nonlinear approach for the risk assessment of DMA^V. In summary, the Panel's opinion is that the available data support the nonlinear approach for the low dose extrapolation of DMA^V.

The linear approach would be consistent with evidence for direct genotoxicity of $DMA^{III/V}$. There are no compelling data demonstrating that $DMA^{III/V}$ are directly genotoxic. It is generally accepted that DMA^V is not directly genotoxic (not DNA reactive). This conclusion is well supported by the data presented in the *Science Issue Paper: Model of Carcinogenic Action for Cacodylic Acid (Dimethylarsinic Acid, DMA^V) and Recommendations for Dose Response Extrapolation*, and in section 3.3.1 of this report.

3.5.1.2 Charge Question D1 further asks the SAB, *"Which approach is more consistent with the available data on DMA^V and current concepts of chemical carcinogenesis,"*

The non-linear approach is more consistent with the available data and current concepts of chemical carcinogenesis (see section 3.5.1.1, above).

3.5.1.3 Charge Question D1 asks the SAB, *"How [should] scientific uncertainty most appropriately be incorporated into low-dose extrapolation?"*

After some discussion, the Panel viewed this question from the perspective of the EPA's RfC guidelines (USEPA, 1994). Similar guidelines for the derivation of chemical specific uncertainty factors have been developed by the International Program for Chemical Safety (IPCS 2001). These guidelines provide an approach for incorporating uncertainty into risk assessments in the form of uncertainty factors. Uncertainties in the interspecies extrapolation of the rat dose-response data can be broadly grouped into a) those related to interspecies differences in pharmacokinetics, b) those related to interspecies differences in pharmacodynamics, and c) those associated with sensitive populations such as children and the elderly. The default value for interspecies differences in pharmacokinetics is 3, the default for interspecies differences in pharmacodynamics is 3, and the default for sensitive populations is 10, made up of two factors of 3 each, one for pharmacokinetic differences and one for pharmacodynamic differences.

While it was the opinion of the Panel that rats might deliver a higher dose of the proximate toxicant, DMA^{III} to the bladder for a given dose of DMA^V than humans, the Panel recognized that there was insufficient data on the comparative dosimetry in rats and humans to make any conclusive statements about species differences in pharmacokinetics. The possible role of microbial demethylation in humans is another

potential issue for consideration. Therefore, the uncertainty factor for interspecies differences in pharmacokinetics should be 3, the default value. However, there appears to be emerging data on DMA^V kinetics which might be brought to bear on the question and the agency is encouraged to consider these data with respect to pharmacokinetic differences between the species and the characterization of this component of uncertainty in the dose response assessment.

As a result of the Panel's analysis of the data for the key pharmacodynamic response, uroepithelial cell cytotoxicity, the consensus was the EPA could assemble a case for pharmacodynamic equivalency between the test species, rats, and humans from existing experimental data. Cohen et al. showed LC_{50}s of 0.5 and 0.8 μM DMA^{III} for rat and human bladder epithelial cells lines (Cohen, 2002). In the context of EPA and International Program on Chemical Safety (IPCS) guidelines, this finding could be incorporated in the assessment as a reduction of the pharmacodynamic component of the interspecies uncertainty factor (typically a default value of 3), to a value of one. However, this suggestion is based upon limited comparative in vitro data. The application of uncertainty factors has also been addressed in the Panel's response to question C1. There is presently no arsenic-specific information that can inform the choice of uncertainty factors for sensitive human populations. For now, the choice of these factors must be based on more general considerations, including EPA's science policy judgment of the degree of precaution that it deems appropriate.

3.5.2 Implementation of the recommendations of the NRC (2001)(Charge Question D2)

EPA believes that the most prudent approach for modeling cancer risk from exposure to iAs is to use a linear model because there are significant remaining uncertainties regarding which of the metabolite(s) may be the ultimate carcinogenic moiety and whether or not mixtures of toxic metabolites interact at the site(s) of action" (USEPA, 2005A). *EPA asked if the SAB concurs, for now, with the selection of a linear model to estimate cancer risk for inorganic arsenic [i.e., following the recommendations of the NRC (2001)]. EPA also asked that the SAB discuss its response in light of the highly complex mode of action for iAs.*

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. Therefore, based on information in this section and on the Panel's understanding of the EPA's 2005 Guidelines for Cancer Risk Assessment, the final recommendation of NRC (2001) to base current risk assessments on a linear dose response model that includes the SW Taiwan population as a comparison group, seems to be the most appropriate approach. Below, the Panel suggests that EPA conduct sensitivity analyses to address uncertainties in this issue.

Existing epidemiologic studies have been mentioned in the response to charge question C2. These studies of different populations across different countries seem to

support a possible linear dose-response between exposure from drinking water and internal cancer risks (particularly in Taiwan, Chile and Argentina). These dose-response relationships are observed at higher exposure levels (>100 ppb). The Panel believes that because of limitations in the epidemiologic studies conducted to date, that adequate human data at the lower range of iAs exposure is lacking.

Some recent studies have included populations with exposures in the lower range (<100 ppb), but they tend to be problematic for use in dose-response analysis for lower exposure levels. In particular, when studies are based almost exclusively on low dose exposure populations (Lamm et al, 2004; Bates et al, 2003; Steinmaus et al, 2003), they lack statistical power and the estimations of low dose risk tend to be unstable and to have a high degree of uncertainty. Some of these studies also have problems related to study design. For example, in the Lamm et al. (2004) ecological study, exposure assessment is highly problematic given that a single median county-level exposure value is assigned to all the person-years contributed by each county in the analysis, even though it is not clear that these are the arsenic exposure values for a large number of residents within each county. A recent follow-up of the Taiwanese cohort reports a monotonic trend in lung cancer risk for exposure to arsenic levels ranging from <10 to 700 μg/L, however this study also has limited power to examine the form of the dose-response relationship within the 10-100 μg/L range (Chen et al 2004). There are no human data available that is adequate to characterize the shape of the dose response curve below a given point of departure.

At present the experimental evidence on mode of action 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). In examining the dose-response relationships of arsenicals in inducing direct or indirect mutagenic responses (including effects thought to be clastogenic in nature), it is clear that effects are only seen at doses that induce cytotoxicity. This implies a threshold (Rossman, T.G. 2003). Until more is learned about the complex properties and MOAs of iAs and its metabolites there is insufficient justification for the choice of a specific nonlinear form of the dose-response relationship. Under these circumstances, the EPA's 2005 Guidelines for Cancer Risk Assessment are clear that linear extrapolation below the point of departure is the method to be used.

Although the EPA has chosen a linear model for the arsenic dose component of the hazard model for lung and bladder cancer, the Panel encourages the Agency to test the sensitivity of the assumption of linearity by comparing its corresponding estimate of excess life risk to an alternative hazard model that has a dose contribution that is multiplicative and nonlinear in form (see question D3 for additional information).

In summary, the Panel recognizes the potential for a highly complex mode of action of iAs and its metabolites, but until more is learned about the complex PK and PD properties of iAs and its metabolites there is not sufficient justification for the choice of a specific nonlinear form of the dose-response relationship. Based on this and the EPA's

2005 Guidelines for Cancer Risk Assessment, the final recommendation of NRC (2001) to base current risk assessments on a linear dose response model that includes the southwest Taiwan population as a comparison group seems the most appropriate approach. However, the Panel also recommends performing a sensitivity analysis with different exposure metrics with the subgroup of villages with more than one well measurement (as discussed in responses to charge questions C2 and D3) and using a multiplicative model that includes a quadratic term for dose, as performed by NRC (2001) and as discussed in charge question D3.

3.5.3 EPA Model Re-Implementation (Charge Question D3)

The Charge states that, "EPA re-implemented the model presented in the NRC (2001) in the language R as well as in an Excel spreadsheet format. In addition, extensive testing of the resulting code was conducted" (USEPA, 2005a). *Charge Question D3* asks the SAB to *"… comment upon precision and accuracy of the re-implementation of the model."*

Question D3 suggests that the estimation of the dose-response model and the hazard assessment were originally programmed in the R language. Page 63 of the issue paper indicates that the Poisson hazard model was originally estimated in the R language (optim routine) but neither the main text of the paper nor its appendices provided any additional information. A clarifying question from the panel through the Designated Federal Officer provided clarifying information, stating that:

> "The reference to the implementation in R in question D.3 is outdated, and should have been removed. This was an oversight on EPA's part. The model implementation in Excel is our implementation of record, and was used to prepare the results in the draft toxicological review. We would ask the Panel to please review and comment only on the implementation in Excel. (Background: EPA did originally implement its model in R. However we found that version to be not very transparent, and hard to debug. We then re-implemented the model in Excel, found and corrected some errors, and used that corrected version to prepare the tox review. While Excel may not be the best choice from the standpoint of numerical accuracy, it is greatly superior in the transparency of the implementation, and is powerful enough to perform the entire model calculation from start to finish, even including the nonlinear optimization. Once the Panel is satisfied that the implementation in Excel is correct and appropriate, then the model can be re-implemented in R or some other numerically superior language.)"

The Agency staff is to be commended for deciding to test its original R-language version of the model program through a separate implementation in Excel. The Excel version serves as a check of programming performed in alternative systems (e.g. R, S) and provides transparency for review by non-specialists. For the calculations required in this model of hazard and excess risk, the Excel computations should provide sufficient numerical accuracy. If the EPA returns to another model program, it should begin with

the original model formulas and not simply transcribe the programming from the existing Excel version of the model. As a debugging and error-checking tool, comparisons of intermediate results from the two model implementations should be performed to verify the equivalence of the two model systems.

Overview of the EXCEL spreadsheet implementation of the model: The Excel model implementation is described in Appendix B (pages 105-106) of the Issue Paper. The Issue Paper (page 65) referenced a URL, www.epa.gov/waterscience.sab; however this proved to be not available. EPA staff notified the panel of the correct address, http://epa.gov/waterscience/sab/. The Issue Paper suggests that a listing of the variable and parameter input field is provided in Table B-3 but the current draft of the Issue Paper did not include this table. (The fields in the spreadsheet model were interpreted by the Panel based on the description provided in the text of the Issue Paper and general understanding of the model fitting procedure employed.)

The spreadsheet model requires two Excel files and associated macros. The first of these is MCCancerfit.XLS. This workbook component of the model consists of eight worksheets in four pairs (e.g. fblad and MC fblad for female bladder cancer) that cover the two cancers of interest (lung and bladder) and gender (male, female). The initial worksheet (e.g. fblad) in each of the four cancer/gender pairs contains the input data for fitting the hazard model. The first step in the model fitting algorithm is to employ the Excel Solver to find initial values of a1, a2, a3 and β (Cells G2:G5) that maximize the Poisson likelihood under the following model:

$$\lambda_{i,dose} = \exp(a_1 + a_2 \cdot age_i + a_3 \cdot age_i^2) \cdot (1 + \beta \cdot dose)$$

where:

$age_i =$ the midpoint of a five-year age range, e.g. 22.5 for 20-24;

$dose =$ the arsenic dose in ppb.

This is the model described by the EPA in the Issue Paper and is one of two models that appeared to provide best fit to the data based on the Akaike Information Criterion (NRC, 2001).

The second worksheet in each the four disease/gender pairs (e.g. MC fblad) is used in conjunction with the initial starting values, generated by Solver and stored in Cell N2, to simulate the empirical Bayes posterior distribution of the model parameters based on a set of 1000 random perturbations of the coefficient vector (a1,a2,a3, β) about the maximum likelihood estimates produced by Solver. The perturbation involves independent, random (uniform) dispersion of the coefficient estimates in a relative range of +/- 10% about the point estimates generated by Solver. Parameter draws outside this range are not performed since the posterior likelihood takes on a near zero value outside the boundaries +/- 10% of MLE. The corresponding macro (e.g. mcfblad) is then invoked to apply the observed data and these perturbed coefficient values to establish the value of the posterior log-likelihood for each of the 1000 draws. The empirical Bayes

estimate of the slope parameter and its lower confidence limit are then estimated based on the mean and standard deviation of the simulated posterior distribution:

$$\bar{b} = \frac{\sum_{j=1}^{1000} b_j \cdot \frac{L_j}{L_{\max}}}{\sum_{j=1}^{1000} \frac{L_j}{L_{\max}}}$$

$$sd(b) = sqrt\left\{ \frac{1000}{999} \cdot \frac{\sum_{j=1}^{1000} \left[\frac{L_j}{L_{\max}} \cdot (b_j - \bar{b})^2 \right]}{\sum_{j=1}^{1000} \frac{L_j}{L_{\max}}} \right\}$$

$$and,$$

$$UCL(b) = \bar{b} + 2 \cdot sd(b)$$

The estimated UCL(b) is then carried forward to the BEIR.IV computation of the excess lifetime risk in the BEIR.xls spreadsheet.

Based on its review, the Panel noted that for the given data inputs, the empirical Bayes estimation algorithm programmed in the MCCancerFit.xls spreadsheet does match the form of the model and the general description of the parameter fitting algorithm outlined in the Issue Paper.

As described in the Issue Paper, the EPA data inputs for at risk populations and cancer deaths agree with Morales, et al. (2000). In general, the panel recommends that all tables of inputs for these models be published in appendices to the Issue Paper or final risk assessment so that reviewers can independently reference and verify the critical inputs to the hazard and excess risk analysis.

The MCCancerft.xls spreadsheet includes an adjustment of 50 μg/day of arsenic from food intake. Based on the formula provided on page 103 of the Issue Paper, the current model assumes a combined daily intake of 2 liters/day of cooking and drinking water. The Issue Paper suggests that the current analysis uses 30 μg/day. Although the Issue Paper notes the NRC (2001) finding that dietary intake had no significant effect on the estimated cancer slope factor, the apparent discrepancy between the value of 30 μg/day cited in the Issue Paper and the 50 μg/day value used in the spreadsheet model should be resolved. The model does not allocate a food input of arsenic to the control population. This is a decision that presumes food-based intake of arsenic originates from cooking water only.

The second Excel workbook in the risk assessment model employs estimates of the dose response model parameter, β, and its upper bound to evaluate excess lifetime risk under the BEIR-IV formula. The BEIR.xls workbook includes four worksheets, one

for each cancer type by gender combination (flung, mlung, fblad, mblad). The estimates of the linear dose response parameter and its estimated 95% UCL (see above) are manually pasted from the corresponding worksheet in MCCancerFit.xls. The excess risk is computed in cell T15. Solver can be applied to the dose value in Cell T11 (not U10 as indicated on Page 105 in the Issue Paper) to establish the dose level required to produce a user-specified values of excess risk (i.e., ED_{01}).

The columns of each worksheet in the BEIR.xls spreadsheet incorporate data for a specific age range of the U.S. population. These columns are not labeled with the corresponding age range. Identifying labels should be applied to all rows and columns in these worksheets. By deduction, column 3 applies to individuals age 20-24, column 4 to age 25-29, etc. If this is correct, the Panel recommends that the entry in cell B3 of each of the four BEIR.xls spreadsheets be verified. It appears that this mortality figure may apply to more than just the 20-24 year old population represented in Column 3. Referring to the data inputs for 20-24 year olds in the flung spreadsheet in BEIR.xls, the population value is 9,423,000, all deaths are 18,121 and the baseline hazard is .00192. Moving over one column to the 25-29 year olds, the population is nearly the same at 9,491,000, all deaths are 1580 and the baseline hazard is .00017—less than $1/10^{th}$ that for the previous five year age group.

The BEIR.xls spreadsheet implementation of the BEIR-IV excess risk calculation includes a 3-fold divisor to transform the risk to a U.S. population base (assuming exposure per kg is 3-fold higher in the SW Taiwanese population). This scaling occurs in the calculation of the age-specific cancer hazard (Row 11). It should be documented and also should be a target for future sensitivity studies. Since this is a model parameter it should identified as a distinct input on the spreadsheet instead of simply embedded in the calculations.

The notation for the BEIR-IV formula on Page 102 in the Issue Paper does not distinguish between total survivorship (S_i) and survivorship adjusted for the added risk of cancer. However, the spreadsheet implementation of the model decomposes survival into the product of baseline survival and a survival factor that reflects excess cancer deaths due to the prior age group's exposure to arsenic. Based on a version of the spreadsheet downloaded from the Office of Water website, calculation of cancer-specific survival (Row 13) appears to incorporate mortality through age interval I, not interval I-1 as it should. This should be checked. The calculation of baseline survival appears to be correct – the survival parameter at age interval I includes only mortality through the end of time period I-1. With this exception, calculation of Excess Risk follows the BEIR IV formula.

Following the series of checks and corrections to the model listed above, the Panel encourages the Agency to extend its testing of the model sensitivity to alternative models forms and model assumptions. Specific areas where the Panel felt additional sensitivity testing is warranted include:

a) A Monte Carlo analysis in which the individual well concentrations for 22 villages with multiple wells are taken into account. The Panel recognizes the difficulties with this approach including the issue of how to allocate cases to wells within villages.

b) MCCancerFit.xls:
   a. A test of the sensitivity of the model to the choice of the reference population (SW Taiwan).
   b. A test of the sensitivity of model results to the assumption that the reference population has 0 intake of arsenic via food.
   c. A contrast of results for the linear dose model employed in this program to alternative hazard models that are multiplicative and nonlinear in form. For example, the following multiplicative, quadratic model is one of several that NRC(2001) found to have best fit to the data based on the Akaike Information Criterion (AIC):

$$\lambda_{i,C} = \exp(a_1 + a_2 \cdot age_i + a_3 \cdot age_i^2) \cdot \exp(\beta_0 + \beta_1 \cdot dose + \beta_2 \cdot dose^2)$$

c) BEIR.xls

   a. The Panel recommends a sensitivity analysis be conducted to investigate the effect of the age groupings used to estimate the baseline hazard and excess lifetime risk. In addition to the current practice of using 5-year intervals (e.g. 20-24, 25-29, etc.), a logical choice is to test the sensitivity of the model results to using 10-year groupings (e.g. 20-29, 30-39…).
   b. The exposure/kg parameter used to transfer the dose/response model from the original SW Taiwanese population to a U.S. general population is a major driver in the computation of excess lifetime risk. In preparing its final risk assessment, the EPA should conduct a sensitivity analysis to determine precisely how much the choice of a factor of 3 impacts the final estimates of excess lifetime risk.

3.5.4 Available literature describing drinking water consumption rates for the Southwestern Taiwanese study population (Charge Question D4)

EPA, as well as the NRC (2001) state that the drinking water consumption rate, as well as variability of that rate in both U.S. and Taiwanese populations, are important factors to consider. EPA notes that in calculating risk estimates for U.S. populations exposed to arsenic through drinking water, NRC used a drinking water consumption rate of 1 L/day for the U.S. population and two possible consumption rates for the Taiwanese population: 1 L/day (identical to the U.S. population) and 2.2 L/day with little or no supporting rationale. Since publication of NRC 2001, a number of new studies have become available and are summarized in the Cancer Slope Factor Workgroup Issue Paper. Agency reviews of the relevant literature suggest that the mean drinking water

(for the Taiwanese study population) consumption rate is between 1 to 4.6 L/day. EPA's current cancer modeling includes water intake adjustments for 2.0 and 3.5 L/day" (USEPA, 2005a). *Charge Question D4 asks what drinking water value the panel recommended for use in deriving the cancer slope factor for inorganic arsenic?*

The Panel agrees that assumptions about water consumption levels in the U.S. and in Taiwan have a substantial impact on the risk assessment. Relative to U.S. consumption, overestimating water consumption in Taiwan decreases potency estimates and underestimating consumption increases potency estimates. Evidence for gender differences in consumption is limited, but considerable within-population variability in consumption occurs (NRC, 2001). EPA should evaluate the impact of drinking water consumption rates associated with more highly exposed population groups with potentially different exposures and susceptibilities (e.g., children, pregnant women) in its arsenic exposure estimates as the Agency determines the overall affects of drinking water consumption rates on arsenic risk.

U.S. water consumption data are obtained from comprehensive U.S. surveys including surveys by the U.S. Department of Agriculture (USDA) and as part of the National Health and Nutrition Examination Survey (NHANES) (as cited in USEPA, 2005), among others. These studies provide information on tap water consumption as well as water consumption attributable to other beverage consumption and consumption of food prepared with water containing arsenic. Estimates of mean daily drinking water consumption and total water consumption (including water used in food preparation) range from 1.0 to 2.8 L/day and from 1.2 to 3.2 L/day respectively.

In comparison, information on water-consumption in Taiwan derives from a small study by Yang and Blackwell and an EPA informal, anecdotal assessment (as cited in USEPA, 2005) that include only information on drinking water consumption. Information on water consumption in South Asia, another world region with high arsenic levels in the water supply, is available from a large population based survey in India (Chowdhury et al., 2001 cited in EPA 2005) and a small study from Bangladesh (Watanabe et al., 2004). The South Asian studies include information on water consumption associated with food preparation. Although similar in socioeconomic characteristics, the diet and climate differ in Taiwan and South Asia, with temperatures higher in South Asia. These studies report mean daily drinking water intake of 1 to 3.5 L, with an additional 1 L associated with food preparation.

The Panel recommends that:

a) EPA incorporate variability parameters for individual water consumption into their analysis for the Taiwanese population as they have done for the U.S. population estimates as NRC recommended;
b) Because assumptions about water consumption are an important source of variability in the risk estimates, EPA should conduct sensitivity analyses of the impact of using a range of consumption values for the Taiwanese population.

c) Because data on gender differences in consumption in Taiwan are limited, a better justification for assuming different consumption levels by gender is needed, particularly given the lack of sex difference in consumption in U.S. and observed in studies from other countries (Watanabe et al., 2004). In the absence of such a justification, the panel recommends an additional sensitivity analysis to examine the impact of equalizing the gender-specific consumption level.
d) The source of data for intake from other beverages and cooking water needs to be more fully discussed and documented. Specifically, the document should more clearly articulate how different sources of water intake are incorporated into the risk model including beverages other than water (e.g. green tea) and water used in food preparation. Clarification of both the assumed consumption level and how water consumption and consumption variability is introduced within the model is needed.

3.5.5 Selection of an estimate of dietary intake of arsenic from food (Charge Question D5)

EPA stated that, "The issue of intake of arsenic from food (e.g., dry rice, sweet potatoes) has been distinguished from the issue of intake of arsenic from drinking water. The NRC addressed the issue of arsenic in food by determining how sensitive the calculation of ED_{01} was to the consumption rate. NRC found that changing the consumption rate from 50 µg/day to 30 µg/day did not change the calculated ED_{01} significantly (about 1% difference). Since the publication of NRC 2001, a number of new studies have become available, summarized in the Cancer Slope Factor Workgroup Issue Paper. EPA's current cancer modeling includes dietary intake adjustments for 0, 10, 30, and 50 µg/day" (USEPA, 2005a)." *Charge Question D5* asks the SAB*"… what background dietary arsenic intake value it recommends for both the control population and study population of Southwestern Taiwan (which is used in deriving the cancer slope factor for inorganic arsenic?)"*

The Panel did not recommend a specific value for EPA to use; however, it did recommend a sensitivity analysis to assess the impact of a range of dietary intakes on risk from lung and bladder cancer risk associated with arsenic in drinking water used by this population (e.g. 50 to as high as 200 µg/day). The Panel stated that an intake of zero arsenic from food should not be assumed.

Three studies that summarize daily arsenic consumption as derived from food in areas of high arsenic intake are listed in Table 4 (USEPA OPP, 2005). Based on the NRC's recommendations, EPA used a range of 30-50 µg per day total arsenic intake from dry rice (uncooked) and dried yams in the diet of southeastern Taiwan that also was based on the work of Schoof et al. (1998) as listed in this table. In materials presented and submitted to the Panel (Schoof, 2005), Dr. Schoof, stated her belief that the field had not been recently treated given the levels she found (7 to 8 ppm) but that seasonality and recent application of arsenicals should influence the levels of arsenic found in the field

and in plants grown on those fields. Thus the Schoof et al. (1998) data cited in Table 4 may underestimate the dietary arsenic intake from food in this population.

In the following paragraphs, the term total arsenic indicates the sum of all inorganic and organic arsenic species. The term inorganic arsenic as stated in published literature on analysis of arsenic in food generally refers to the sum of the inorganic arsenic species (iAs ^{III} and iAs^{V}). Unless specifically stated otherwise, the term organic arsenic indicates total organic arsenic compounds in food. In reference to seafood, arsenobetaine is generally the major organic arsenic compound present when organic arsenic compounds are specifically identified in the analysis; other minor organic arsenicals may also be present. The methylated arsenic metabolites (MMA^{III} MMA^{V}, DMA^{III}, DMA^{V}) are organic arsenic compounds, however, they are not generally determined in food.

Daily intake of arsenic from food observed by Chowdhury et al. (2001) and Watanabe et al., (2004) suggest total arsenic intakes ranging from a mean of 120 to 285 µg/day from food in Bangladeshi and Indian populations exposed to high levels of naturally occurring arsenic. Mean total arsenic intakes for males were shown to be 214 µg /day and for females 120 µg /day (Watanabe et al., 2004). In studies conducted in West Bengal in which both chemical analysis of food items and interviews for food intake were conducted to assess exposure, Roychowdhury et al., (2002) show daily dietary intakes from food for adults (based on 34 families in 5 villages) ranging from 171-189 µg/day and for children of about 10 years ranging from 91-101 µg/day. These figures are ranges of means for two different geographic areas – standard deviations were not published. Although these data are not derived specifically from the area of Taiwan studied, they indicate along with ancillary information presented here and elsewhere that dietary exposure from food in this geographic area may be higher than previously thought. Raw rice, a staple of the area, has been shown in other studies to contain among the highest iAs values in food (Schoof, et al., 1999) while for vegetables approximately 95% of total arsenic is organic arsenic (Chowdhury et al., 2001). Variation in arsenic concentration and speciation occurs relative to rice cultivar (Williams et al., 2005). Duxbury et al. (2003) estimates that 30-85% of arsenic in rice is inorganic arsenic.

Diet is the largest source of total arsenic exposure in the U.S. relative to water and air exposures. Average intake is about 40 µg/day total arsenic (ATSDR, 2006) compared with the approximately five-fold higher total dietary arsenic intake observed in Asian studies cited in the foregoing paragraph. The estimated range of daily intake of total arsenic from food in the U.S. is reported at 2-92 µg/day (Tao and Bolger, 1999) while U.S. daily total intake of iAs at the 10th and 90th percentiles is estimated to be 1.8 to 11.4 µg/day for males and 1.3 to 9.4 µg/day for females (Meacher, et al, 2002). The U.S. dietary intake of inorganic arsenic is estimated to range from 1 to 20 µg/day (ATSDR, 2006). U.S. shellfish and other marine foods contain the highest total arsenic concentrations and are the largest dietary source (76% - 96%) of arsenic, however, most of the arsenic in seafood is present as the organic arsenic compound arsenobetaine, which is excreted rapidly and unchanged and does not appear to be harmful to humans (ATSDR, 2006). It is known, however, that fish may contain some portion of iAs further

pointing to the need for the sensitivity analysis described below. Certain seafood may also contain DMA that may also contribute to background exposure from food relative to water sources (Huang, et al., 2003).

It is clear that the adjustment for background iAs intake from food is extremely important given that the total exposure dose from all sources does likely matter in terms of toxicity and cancer induction and that the U.S. population likely has a considerably lower total arsenic intake from food than do populations in Asia.

The Panel recommends that a range of values from at least 50 to 100 µg/day and up to perhaps as high as 200 µg/day be run in a sensitivity analysis to assess the impact of this range of dietary intakes on risk of lung and bladder cancer from exposure via drinking water in this population. The cancer risk model needs to be evaluated using a wider range of iAs food values above 50 µg/day to determine if there is a change in the arsenic cancer exposure-response slope as a result. It also cannot be assumed that the control population has an intake of zero arsenic from food.

Such a sensitivity analysis of the impact of dietary arsenic uptake using a range of data from high arsenic-exposed populations is unlikely to introduce larger uncertainty than the myriad dietary differences – protein deficiency, Se, Zn, folate deficiency etc. – between this Taiwanese population and the U.S. population

Much greater rigor needs to be applied in discussing and presenting documented data sources and making clear the basis on which assumptions are being made and the relative strength of those assumptions. Comparisons of the impact of differing levels of iAs intake from food between the exposed and reference population need to be made on the basis of comparative relative risk. Clearer statements are needed on the data limitations of past daily dietary arsenic intake for the Blackfoot endemic area of Taiwan and for the reference population(s).

EPA needs to be aware of and include a discussion of methodological and analytical issues related to reported arsenic concentrations in food, because these values are dependent upon differential extraction processes and analytical procedures applied by diverse laboratories on a variety of food stuffs. Only the arsenic extracted from food can be measured. More importantly, laboratory extraction procedures are not designed to equate with that portion of arsenic in food that is bioavailable. Thus, the arsenic value resulting from extraction and measurement is not necessarily related to the concentration that is bioavailable to humans from specific sample sources. There is an immediate need for thorough research on the bioavailability of arsenic from food.

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APPENDIX A

Charge to EPA Science Advisory Board Arsenic Review Panel
July 25, 2005

Background: There are both natural and anthropogenic sources of arsenic and arsenic containing compounds (or arsenicals). Exposure to arsenicals can be through different environmental media including drinking water, food, soil, and air. EPA assesses and regulates the potential exposure and health risks associated with exposure to arsenic and arsenic containing compounds through several statutory authorities. The Safe Drinking Water Act (SDWA), directs EPA to establish national standards for contaminants including arsenical compounds in public drinking water supplies. EPA's Superfund and Resource Conservation and Recovery Act (RCRA) programs evaluate exposure to arsenic compounds at sites selected for clean up or remediation. Under the Clean Air Act, EPA's Office of Air and Radiation sets emissions standards for sources of arsenic to air. These include standards based on control technology and those based on risks to human health from inhalation of airborne arsenic or ingestion of arsenic arising from air sources. EPA's Office of Pesticide Programs (OPP) evaluates the exposure and health risks associated with arsenicals used as pesticides in the U.S. Under the mandate of the Food Quality Protection Agency (FQPA), EPA must reevaluate all pesticide food tolerances (the legal limits of pesticides on/in food or animal feed) in the U.S. by August, 2006. There are several organic arsenic herbicides that are undergoing reregistration and/or tolerance reassessment including cacodylic acid (referred to as dimethylarsinic acid or DMA^V), monosodium, disodium, and calcium salts of methanearsonate acid (MSMA, DSMA, and CAMA, collectively as referred as MMA^V). In 2003, most residential uses of chromated copper arsenate (CCA) as a wood preservative were cancelled.

The health effects of arsenicals have been the subject of two reviews by the National Research Council (NRC) of the National Academy of Sciences (NAS) (NRC 1999; 2001). Since the 2001 NAS review, there has been substantial new information developed on the mode of carcinogenic action and metabolism and toxicokinetics for arsenic and its methylated species, and new epidemiology on inorganic arsenic. The Agency has considered this new science in regards to the hazard characterization required for tolerance assessment of DMA^V (and MMA^V) as described in the draft OPP Science Issue Paper: Mode of Action for Cacodylic Acid (Dimethylarsinic Acid) and Recommendations for Dose Response Extrapolation, and also in the ORD Issue Paper - Cancer Risk Assessment for Organic Arsenical Herbicides: Comments on Mode of Action, Human Relevance and Implications for Quantitative Dose-Response Assessment (See Appendix E). In addition, the Agency has developed a revised hazard and dose response assessment/characterization of inorganic Arsenic (Toxicological review of inorganic arsenic in Support of Summary Information on the Integrated Risk Information System (IRIS)) which relies on the two NRC reviews and provides an updated human health effects and dose-response assessment for inorganic arsenic. The Agency seeks comment and advice from the SAB on the scientific soundness of major science conclusions drawn in these two documents regarding the carcinogenic assessments of

DMA^V and inorganic arsenic and the appropriateness of the Agency's application of its own Guidelines for Carcinogen Risk Assessment for arsenicals.

Overview of Science and Assessment Issues: Ingestion of inorganic arsenic has been demonstrated to cause cancer of the skin, lung, and urinary bladder in humans. Historically, standard chronic bioassays with exposure to inorganic arsenic in rodents have been negative for increased tumor formation. There are, however, more recent studies at high doses, in transgenic animals, and following transplacental exposures which have demonstrated cancer potential in rodent studies following exposure to inorganic arsenic. The NRC 1999 report advises that the bladder and lung cancer human mortality data, particularly from the southwestern Taiwanese studies provide the best dose-response data for evaluating the long-term effects of ingestion of inorganic arsenic. In the 2001 NRC report, a number of recommendations were made to EPA to revise the oral cancer slope for inorganic arsenic. Given the available database, and recognizing that the mode(s) of action by which inorganic arsenic causes cancer has not been fully established, the draft Toxicological Review of Arsenic, consistent with advice from the NRC uses linear low dose extrapolation to estimate cancer risks from ingestion of arsenic at low dose and has addressed many of the NRC recommendations.

In approaching the cancer assessment on the pesticide cacodylic acid (DMA^V), an organic arsenical, EPA has confronted a number of challenging issues. No human epidemiological information is available for DMA^V. Rodent cancer bioassay data have shown that dietary administration of DMA^V can result in bladder carcinogenesis in the rat. DMA, however, is a key urinary metabolite from exposure to inorganic arsenic. Thus, the question is raised regarding the extent the cancer epidemiology on inorganic arsenic may provide an appropriate dataset or may inform the low dose extrapolation for the cancer risk associated with direct exposure to DMA^V. Available *in vivo* and *in vitro* pharmacokinetic, metabolism studies, and toxicology studies were reviewed to address this issue. The draft OPP Science Issue Paper states that the evidence indicates inorganic arsenic and DMA^V have different pharmacokinetic and pharmacodynamic characteristics, EPA proposes to use the rat bioassay data on DMA^V to estimate its cancer risk. The ORD Issue Paper (Appendix E of the OPP Science Issue Paper: Cancer Mode of Action of Cacodylic Acid (Dimethylarsinic Acid) and Recommendations for Dose Response Extrapolation) provides additional discussion on the MOA issues and perspective on the nexus between science issues for organic and inorganic arsenicals. **T**he use of mode of action data in the assessment of potential carcinogens is a main focus of EPA's 2005 cancer guidelines. Mode of action data are available on DMA and were evaluated to guide the low dose extrapolation.

The Agency seeks comments and advice from the SAB on key science issues concerning (A) the metabolism and toxic responses of arsenic species, (B) the mode of action for carcinogenesis and implications for dose-response extrapolation for DMA^V and inorganic arsenic, (C) the selection of data for dose-response, and (D) approaches to low-dose extrapolation. In addition, the Agency is requesting comment on the implications of newer epidemiology and the incorporation of the 2001 NRC recommendation on modeling the human cancer data for inorganic arsenic.

Issues and Charge Questions

A. Metabolism and Toxic Responses of Arsenic Species

A1. Metabolism and pharmacokinetics: Evidence from *in vivo* and *in vitro* metabolism and pharmacokinetic studies with humans and laboratory animals suggests that the efficiency of the methylation reaction(s) and cellular uptake varies based on which arsenical compound is administered exogenously. Most available studies suggest that the metabolic process in most mammals is primarily a one-way process and that following direct exposure to DMA^V significant amounts of iAs^{III}, iAs^V, MMA^{III}, or MMA^V at the target tissue are not expected.

Please comment on how pharmacokinetic processes are best considered regarding the use of data derived from direct DMA^V exposure versus direct iAs exposure for cancer risk assessment.

A2. Response to mixtures of metabolites: Tumorigenic profiles vary based on which arsenical compound is administered exogenously. *In vivo* and *in vitro* studies indicate that each of the arsenical compounds exhibit similarities and differences in their profiles of biological activities. Direct exposure to iAs^{III} or iAs^V is expected to result in more of a mixture of toxic metabolites than for direct exposure to DMAv; the mixture of metabolites is expected to vary based on which chemical is administered exogenously. The potential mixture of metabolites following direct exposure to DMA^V appears less complex as compared to iAs.

Given the toxicological response profiles observed following direct exposures to iAs versus MMA^v and DMA^V, and the differences in human and rodent toxicologic responses to arsenicals, please comment on the use of data derived from rodent exposures to the organic arsenicals versus use of data derived from direct iAs human exposure, in the DMA^V assessment.

B. Modes of Carcinogenic Action for DMA^V and Inorganic Arsenic

B1. Mode of action of DMA^v: When relying on laboratory animal data, two critical assumptions are made: (i) data on animal tumors are predictive of human cancer, and (ii) animal tumor effects found at high experimental doses predict human risk at lower exposures. An understanding of a chemical mode of carcinogenic action can help inform the above assumptions. In the case of DMA^V, mode of action (MOA) data are available and were evaluated using the framework described in EPA's cancer guidelines.

Please comment on the sufficiency of evidence to establish the animal mode of carcinogenic action for DMA^V. Are the scientific conclusions sound and consistent with the available evidence on DMA^V and the current state of knowledge for chemical carcinogenesis.

Please comment on whether the key events in DMA's mode of action are supported by the available data. Specifically comment on the role of: a) reactive oxygen species in

producing chromosomal damage and the strength of the evidence supporting oxidative damage as a causal key event in DMA^V/DMA^{III}'s mode of carcinogenic action versus an associative event or a secondary consequence of cytotoxicity; b) cell proliferation and cytotoxicity and the strength of the evidence as causal key events in DMA^V/DMA^{III}'s mode of carcinogenic action versus associative or secondary events, and c) other potential modes of action that have substantial scientific support that may be contributing to the carcinogenicity of DMA.

B2. Human relevance of animal DMA^V MOA: There are little or no scientific data to suggest that if sufficient DMA^{III} were present, key precursor events and ultimately tumor formation would not occur in humans directly exposed to DMA^V.

Please comment on the relevance of the postulated key events (see B1) to tumors in humans.

Please comment on how, if at all, differences in the human population vs. experimental animals should be accounted for in the risk assessment for DMA^V.

There are little to no chemical specific data regarding an increased susceptibility of humans for bladder tumor development during different life stages.

Please comment on the Agency's conclusion that the young are likely to respond like the adult to the formation of bladder tumors following exposure to DMA.

B3. Modes of carcinogenic action from exposure to inorganic arsenic: Inorganic arsenic (iAs) undergoes successive methylation steps in humans, resulting in the intermediate production of iAs^{III}, MMA^V, MMA^{III}, DMA^V, and DMA^{III}. Each arsenical metabolite exhibits its own toxicity.

Please comment on the conclusion that the available data support the hypothesis that multiple modes of action may be operational following exposure to inorganic arsenic.

C. Selection of Data for Dose-Response Assessment

C1. Use of animal data for DMA^V : A number of different rodent bioassays (standard bioassay, transgenic animals, susceptible rodent strains, initiation and promotion studies) are available on DMA^V.

Please comment on the use of the bladder tumor data from the DMA^V rat bioassay as the most suitable dataset for quantifying potential human cancer risk to DMA^V, including the weight of evidence to support this conclusion.

Please comment on whether the iAs epidemiology data can be used to inform the DMA^V dose-response assessment derived from rat data with DMA^V. If so, please discuss how such information might be used. (See Appendix).

C2. Use of human epidemiological data from direct iAs exposure: Since the NRC (2001) report on iAs, an additional body of literature has developed describing epidemiology data from populations in the US exposed to iAs in drinking water.

Does the SAB agree that the Taiwanese dataset remains the most appropriate choice for estimating cancer risk in humans? Please discuss the rationale for your response.

Do these data provide adequate characterization of the impact of childhood exposure to iAs? Please discuss the rationale for your response.

D. Approaches to Low-Dose Extrapolation for Inorganic Arsenic and DMA^V

D1. Mode of carcinogenic action understanding for $DMA^{V/III}$ and implications for dose response extrapolation to estimate human cancer risk: The use of mode of action data in the assessment of potential carcinogens is a main focus of EPA's 2005 cancer guidelines. As stated in these guidelines "The approach to dose-response assessment for a particular agent is based on the conclusion reached as to its potential mode(s) of action". Although a biological-based model is the preferred approach to estimating cancer risk, there are insufficient data on DMA^V to support development of such a model.

Please comment on the scientific evidence and biological rationale in support of nonlinear versus linear low dose extrapolation approaches, which approach is more consistent with the available data on DMA^V and current concepts of chemical carcinogenesis, and how scientific uncertainty should most appropriately be incorporated into low-dose extrapolation.

D2. Implementation of the recommendations of the NRC (2001): EPA has determined that the most prudent approach for modeling cancer risk from exposure to iAs is to use a linear model because there are significant remaining uncertainties regarding which of the metabolite(s) may be the ultimate carcinogenic moiety and whether or not mixtures of toxic metabolites interact at the site(s) of action.

Does the panel concur with the selection of a linear model following the recommendations of the NRC (2001) to estimate cancer risk at this time? Please discuss your response in light of the highly complex mode of action for iAs with its metabolites.

D3. EPA re-implemented the model presented in the NRC (2001) in the language R as well as in an Excel spreadsheet format. In addition, extensive testing of the resulting code was conducted.

Please comment upon precision and accuracy of the re-implementation of the model.

D4. Available literature describing drinking water consumption rates for the southwestern Taiwanese study population: NRC (2001) stated that the

drinking water consumption rate, as well as variability of that rate in both US and Taiwanese populations, are important factors to consider. In calculating risk estimates for U.S. populations exposed to arsenic through drinking water, NRC used a drinking water consumption rate of 1 L/day for the US population and two possible consumption rates for the Taiwanese population: 1 L/day (identical to the US population) and 2.2 L/day with little or no supporting rationale. Since publication of NRC 2001, a number of new studies have become available and are summarized in the Cancer Slope Factor Workgroup Issue Paper. Agency reviews of the relevant literature suggests that the mean drinking water (for the Taiwanese study population) consumption rate is between 1 to 4.6 L/day. EPA's current cancer modeling includes water intake adjustments for 2.0 and 3.5 L/day.

What drinking water value does the panel recommend for use in deriving the cancer slope factor for inorganic arsenic?

D5. Selection of an estimate of dietary intake of arsenic from food: The issue of intake of arsenic from food (e.g., dry rice, sweet potatoes) has been distinguished from the issue of intake of arsenic from drinking water. The NRC addressed the issue of arsenic in food by determining how sensitive the calculation of ED_{01} was to the consumption rate. NRC found that changing the consumption rate from 50 µg/day to 30 µg/day did not change the calculated ED_{01} significantly (about 1% difference). Since the publication of NRC 2001, a number of new studies have become available, summarized in the Cancer Slope Factor Workgroup Issue Paper. EPA's current cancer modeling includes dietary intake adjustments for 0, 10, 30, and 50 µg/day.

What background dietary intake (of arsenic) value does the panel recommend for both the control population and study population of Southwestern Taiwan used in deriving the cancer slope factor for inorganic arsenic?

APPENDIX B

Assignments to Charge-Specific Groups (revised on 8/24/05)

Issue A: Metabolism and Toxic Responses of Arsenic Species

Question A1: Metabolism and Pharmacokinetics

Dr. Aposhian	Dr. Rosen
Dr. Medinsky	Dr. Styblo
Dr. Le	Dr. Hopenhayn

Question A2: Response to mixtures of metabolites

Dr. Aposhian	Dr. Rosen
Dr. Medinsky	Dr. Styblo
Dr. Le	Dr. Hopenhayn

Issue B: Modes of Carcinogenic Action for DMA and iAs

Question B1: Mode of Action of DMA^V

Dr. Barchowsky	Dr. Rossman
Dr. Brusick	Dr. Styblo
Dr. Dragan	Dr. Waalkes
Dr. Klaunig	

Question B2: Human relevance of animal DMA^V MOA

Dr. Barchowsky	Dr. Rossman
Dr. Brusick	Dr. Styblo
Dr. Dragan	Dr. Waalkes
Dr. Klaunig	

Question B3: Modes of carcinogenic action from exposure to inorganic arsenic

Dr. Barchowsky	Dr. Klaunig
Dr. Brusick	Dr. Rossman
Dr. Dragan	Dr. Waalkes

Issue C: Selection of Data for Dose-Response Assessment

Question C1: Use of animal data for DMAV

Dr. Cantor	Dr. Teeguarden
Dr. Green	Dr. Waalkes
Dr. Medinsky	Dr. Yager

Question C2: Use of human epidemiological data from direct iAs exposure

Dr. Cantor
Dr. Colford
Dr. Harlow
Dr. Hopenhayn
Dr. Rosen
Dr. Rossman
Dr. Yager

Issue D: Approaches to Low-Dose Extrapolation for iAs and DMA^V

Question D1: Mode of Carcinogenic action understanding for DMAV/III and implications for dose response extrapolation to estimate human cancer risk

Dr. Cantor
Dr. Colford
Dr. Green
Dr. Klaunig
Dr. Medinsky
Dr. Teeguarden
Dr. Waalkes

Question D2: Implementation of the recommendations of the NRC (2001)

Dr. Colford
Dr. Harlow
Dr. Hopenhayn
Dr. Heeringa
Dr. Portier
Dr. Rosen
Dr. Rossman

Question D3: EPA re-implementation of the NRC (2001) model in language R and Excel spreadsheet.

Dr. Heeringa
Dr. Portier
Dr. Teeguarden

Question D4: Literature describing drinking water consumption rates for the southwestern Taiwanese study population

Dr. Barchowsky
Dr. Harlow
Dr. Colford
Dr. Yager

Question D5: Selection of an estimate for dietary intake of arsenic

Dr. Aposhian
Dr. Barchowsky
Dr. Harlow
Dr. Styblo
Dr. Yager

APPENDIX C

ABBREVIATIONS

Abbreviations	Meaning
ARP	US EPA SAB Arsenic Review Panel
As	Arsenic
BEIR	Biological Effects of Ionizing Radiation
CAMA	Calcium salt of MMA^{V}
CCA	Chromated copper arsenate
DMA^{III}	Dimethylarsinous acid
DMA^{V}	Dimethylarsinic acid, Cacodylic Acid
DSMA	Disodium salt of MMA^{V}
EPA	US Environmental Protection Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FQPA	The Food Quality Protection Act
GPO	US Government Printing Office
iAs	Inorganic arsenic
iAs^{III}	Arsenite, Trivalent inorganic arsenic
NFkB	Nuclear factor-kappa B
iAs^{V}	Arsenate, Pentavalent inorganic arsenic
MLE	Maximum Likelihood Estimates
MMA^{III}	Methylarsonous acid
MMA^{V}	Methanearsonate acid, methylarsenic acid
MN	Micronuclei
MOA	Mode of Action
MSMA	Monosodium salt of MMA^{V}
NAS	National Academy of Sciences
NHANES	National Health and Nutrition Examination Survey
NRC	National Research Council of the NAS
OPP	US EPA Office of Pesticide Programs
ORD	US EPA Office of Research & Development
OW	US EPA Office of Water
PBPK	Physiologically Based Pharmacokinetic Models
PD	Pharmacodynamics
PK	Pharmacokinetics
RCRA	Resource Conservation and Recovery Act
ROS	Reactive Oxygen Species
SAB	US EPA Science Advisory Board
TMA^{III}	Trimethylarsine
$TMA^{V}O$	Trimethylarsine oxide