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(September 15, 2006)

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

OFFICE OF THE ADMINISTRATOR  
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[Date]

**EPA-SAB-ADV-06-xxx**

The 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: An Advisory Report of the US EPA Science Advisory Board**

Dear Administrator Johnson:

EPA’s Office of Pesticide Programs (OPP), Office of Water (OW), and Office of Research Development (ORD) have coordinated their effort in developing two scientific documents that address the carcinogenic assessments of Dimethylarsinic Acid or DMA<sup>V</sup> and inorganic arsenic (iAs). In response to the Agency’s 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) the mode(s) of carcinogenic action; (c) data selection for dose-response; and (d) approaches and methods for low-dose extrapolation for DMA<sup>V</sup> and iAs.

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The SAB Panel supported the Agency's conclusion that on the basis of available data, human exposure to iAs appears to result in a wider spectrum of active metabolites compared to the expected metabolic profile from exposure to DMA<sup>V</sup>. Hence, the Panel agreed with the Agency that, in the absence of human data on DMA<sup>V</sup>, the bladder tumor data from DMA<sup>V</sup> rat bioassays is better suited for 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 cancer risk assessment for DMA<sup>V</sup> due to the observed metabolic differences between rats and humans. In addition, the Panel agreed with the Agency's conclusion that DMA<sup>V</sup>-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. The Panel, however, concluded there are not sufficient data to support a reactive oxygenated species-mediated mode of direct genetic action for DMA<sup>V</sup>. Furthermore, the Panel supported the nonlinear approach for low dose extrapolation of DMA<sup>V</sup> and the use of uncertainty factors to account for interspecies differences and human variability for sensitive populations, but was not able to offer specific values for these factors due to insufficient data.

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With regard to iAs, the Panel agreed with the Agency's conclusion that the carcinogenic effects of iAs is likely to be mediated by multiple modes of action. The Panel also noted that the animal data does not suggest a linear response. However, the dose response for human data in the low dose region does not describe clearly the shape of the curve, but they do fit with a linear model. Therefore, the panel supports the Agency use of a linear model as recommended by the National Research Council in its 2001 report. The Panel made several suggestions on improvements in the model's programming and documentation conventions. 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. At the same time, 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.

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Finally, the Panel believes there is a critical need for a continued research effort to strengthen EPA's cancer risk assessment for DMA<sup>V</sup> and iAs. The scientific bases for the

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1 Panel’s conclusions and research recommendations are detailed in the report. We look forward  
2 to receiving your response to this review and appreciate the opportunity to provide EPA with  
3 advice on this important subject and stand ready to assist the Agency in any future efforts in  
4 updating the assessment.

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Sincerely,

Dr. M. Granger Morgan, Chair  
EPA Science Advisory Board

Dr. Genevieve Matanoski, Chair  
EPA Science Advisory Board  
Arsenic Review Panel

## NOTICE

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This report has been written as part of the activities of the EPA Science Advisory Board, a public advisory committee providing extramural scientific information and advice to the Administrator and other officials of the Environmental Protection Agency. The Board is structured to provide balanced, expert assessment of scientific matters related to problems facing the Agency. This report has not been reviewed for approval by the Agency and, hence, the contents of this report do not necessarily represent the views and policies of the Environmental Protection Agency, nor of other agencies in the Executive Branch of the Federal government, nor does mention of trade names or commercial products constitute a recommendation for use. Reports of the EPA Science Advisory Board are posted on the EPA Web site at: <http://www.epa.gov/sab>.

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1 **U.S. Environmental Protection Agency**  
2 **Science Advisory Board**  
3 **Arsenic Review Panel**  
4

5 **CHAIR**

6 **Dr. Genevieve Matanoski**, Professor, Department of Epidemiology, Johns Hopkins University,  
7 Baltimore, MD  
8

9 **MEMBERS**

10 **Dr. H. Vasken Aposhian**, Professor, Department of Molecular and Cell Biology , The  
11 University of Arizona, Tucson, AZ  
12

13 **Dr. Aaron Barchowsky**, Associate Professor, Department of Environmental and Occupational  
14 Health, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA  
15

16 **Dr. David Brusick**, Retired, Convance Labs, Vienna, VA  
17

18 **Dr. Kenneth P. Cantor**, Senior Investigator, Occupational and Environmental Epidemiology  
19 Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda,  
20 MD  
21

22 **Dr. John (Jack) Colford**, Associate Professor, Division of Public Health, Biology &  
23 Epidemiology, School of Public Health, University of California, Berkeley, CA  
24

25 **Dr. Yvonne P. Dragan**, Director of the Division of Systems Toxicology (DST) and Chief of the  
26 Center for Hepatotoxicology, National Center for Toxicological Research (NCTR), Food and  
27 Drug Administration's (FDA) , Jefferson, AR  
28

29 **Dr. Sidney Green**, Associate Professor, Department of Pharmacology, College of Medicine,  
30 Howard University, Washington, DC  
31

32 **Dr. Sioban Harlow**, Professor, Department of Epidemiology, School of Public Health,  
33 University of Michigan, Ann Arbor, MI  
34

35 **Dr. Steven Heeringa**, Research Scientist and Director, Statistical Design Group, Institute for  
36 Social Research (ISR) , University of Michigan , Ann Arbor, MI  
37

38 **Dr. Claudia Maria Hopenhayn**, Associate Professor, Department of Epidemiology,  
39

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1 Markey Cancer Control Program, College of Public Health, University of Kentucky, Lexington,  
2 KY

3  
4 **Dr. James E. Klaunig**, Professor and Director, Department of Pharmacology and Toxicology,  
5 School of Medicine, Indiana University, Indianapolis, IN

6  
7 **Dr. X. Chris Le**, Professor, Department of Public Health Sciences, Department of Chemistry &  
8 Department of Laboratory Medicine & Pathology, University of Alberta, Edmonton, Alberta,  
9 Canada

10  
11 **Dr. Michele Medinsky**, Toxicology Consultant, Toxcon, Durham, NC

12  
13 **Dr. Kenneth Portier**, Program Director, Department of Statistics and Evaluation, American  
14 Cancer Society, Atlanta, GA

15  
16 **Dr. Barry Rosen**, Professor and Chairman, Department of Biochemistry and Molecular Biology,  
17 School of Medicine, Wayne State University, Detroit, MI

18  
19 **Dr. Toby Rossman**, Professor, Environmental Medicine, School of Medicine, New York  
20 University, Tuxedo, NY

21  
22 **Dr. Miroslav Styblo**, Research Associate Professor, Department of Nutrition and the Center for  
23 Environmental Medicine, Asthma, and Lung Biology, University of North Carolina, Chapel Hill,  
24 NC

25  
26 **Dr. Justin Teeguarden**, Senior Scientist, Pacific Northwest National Laboratory, Richland, WA

27  
28 **Dr. Michael Waalkes**, Chief, Inorganic Carcinogenesis Section, Laboratory of Comparative  
29 Carcinogenesis, National Cancer Institute, National Institute of Environmental Health Science,  
30 RTP, NC

31  
32 **Dr. Janice Yager**, Research Program Manager, Environment Department, Electric Power  
33 Research Institute, Palo Alto, CA

34  
35 **SCIENCE ADVISORY BOARD STAFF**

36 **Mr. Thomas Miller**, Designated Federal Officer, EPA Science Advisory Board Staff Office.

37  
38

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3  
4

5 **CHAIR**

6 **Dr. M. Granger Morgan**, Professor and Head, Department of Engineering and Public Policy,  
7 Carnegie Mellon University, Pittsburgh, PA  
8

9 **SAB MEMBERS**

10 **Dr. Gregory Biddinger**, Environmental Programs Coordinator, ExxonMobil Biomedical  
11 Sciences, Inc, Houston, TX  
12

13 **Dr. James Bus**, Director of External Technology, Toxicology and Environmental Research and  
14 Consulting, The Dow Chemical Company, Midland, MI  
15

16 **Dr. Trudy Ann Cameron**, Raymond F. Mikesell Professor of Environmental and Resource  
17 Economics, Department of Economics, University of Oregon, Eugene, OR  
18

19 **Dr. Deborah Cory-Slechta**, Director, Environmental and Occupational Health Sciences  
20 Institute, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New  
21 Jersey and Rutgers State University, Piscataway, NJ  
22

23 **Dr. Maureen L. Cropper**, Professor, Department of Economics, University of Maryland,  
24 College Park, MD  
25

26 **Dr. Virginia Dale**, Corporate Fellow, Environmental Sciences Division, Oak Ridge National  
27 Laboratory, Oak Ridge, TN  
28

29 **Dr. Kenneth Dickson**, Professor, Institute of Applied Sciences, University of North Texas,  
30 Denton, TX  
31

32 **Dr. Baruch Fischhoff**, Howard Heinz University Professor, Department of Social and Decision  
33 Sciences, Department of Engineering and Public Policy, Carnegie Mellon University, Pittsburgh,  
34 PA  
35

36 **Dr. A. Myrick Freeman**, William D. Shipman Professor of Economics Emeritus, Department of  
37 Economics, Bowdoin College, Brunswick, ME  
38

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- 1 **Dr. James Galloway**, Professor of Environmental Sciences, Environmental Sciences  
2 Department, University of Virginia, Charlottesville, VA  
3
- 4 **Dr. Lawrence Goulder**, Shuzo Nishihara Professor of Environmental and Resource Economics,  
5 Department of Economics, Stanford University, Stanford, CA  
6
- 7 **Dr. Rogene Henderson**, Scientist Emeritus, Lovelace Respiratory Research Institute,  
8 Albuquerque, NM  
9
- 10 **Dr. Philip Hopke**, Bayard D. Clarkson Distinguished Professor, Department of Chemical  
11 Engineering, Clarkson University, Potsdam, NY  
12
- 13 **Dr. James H. Johnson**, Dean, College of Engineering, Architecture & Computer Sciences,  
14 Howard University, Washington, DC  
15
- 16 **Dr. Meryl Karol**, Associate Dean for Academic Affairs, Graduate School of Public Health,  
17 University of Pittsburgh, Pittsburgh, PA  
18
- 19 **Dr. Catherine Kling**, Professor, Department of Economics, Iowa State University, Ames, IA  
20
- 21 **Dr. George Lambert**, Associate Professor and Director, Center for Child and Reproductive  
22 Environmental Health & Pediatric Clinical Research Center, Department of Pediatrics,  
23 UMDNJ-Robert Wood Johnson Medical School/ University of Medicine and Dentistry of New  
24 Jersey, New Brunswick, NJ  
25
- 26 **Dr. Jill Lipoti**, Director, Division of Environmental Safety and Health, New Jersey Department  
27 of Environmental Protection, Trenton, NJ  
28
- 29 **Dr. Genevieve Matanoski**, Professor, Department of Epidemiology, Johns Hopkins University,  
30 Baltimore, MD  
31
- 32 **Dr. Michael J. McFarland**, Associate Professor, Department of Civil and Environmental  
33 Engineering, Utah State University, Logan, UT  
34
- 35 **Dr. Jana Milford**, Associate Professor, Department of Mechanical Engineering, University of  
36 Colorado, Boulder, CO  
37  
38

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- 1 **Dr. Rebecca Parkin**, Professor and Associate Dean, Environmental and Occupational Health,  
2 School of Public Health and Health Services, The George Washington University, Washington,  
3 DC  
4
- 5 **Mr. David Rejeski**, Foresight and Governance Project Director, Woodrow Wilson International  
6 Center for Scholars, Washington, DC  
7
- 8 **Dr. Joan B. Rose**, Professor and Homer Nowlin Chair for Water Research, Department of  
9 Fisheries and Wildlife, Michigan State University, E. Lansing, MI  
10
- 11 **Dr. Kathleen Segerson**, Professor, Department of Economics, University of Connecticut, Storrs,  
12 CT  
13
- 14 **Dr. Kristin Shrader-Frechette**, O'Neil Professor of Philosophy- Concurrent Professor of  
15 Biological Sciences-and Director of the Center for Environmental Justice and Children's Health,  
16 Department of Biological Sciences and Philosophy Department, University of Notre Dame,  
17 Notre Dame, IN  
18
- 19 **Dr. Robert Stavins**, Albert Pratt Professor of Business and Government, Environment and  
20 Natural Resources Program, John F. Kennedy School of Government, Harvard University,  
21 Cambridge, MA  
22
- 23 **Dr. Deborah Swackhamer**, Professor, Division of Environmental Health Sciences, School of  
24 Public Health, University of Minnesota, Minneapolis, MN  
25
- 26 **Dr. Thomas L. Theis**, Professor and Director, Institute for Environmental Science and Policy,  
27 University of Illinois at Chicago, Chicago, IL  
28
- 29 **Dr. Valerie Thomas**, Anderson Interface Associate Professor of Natural Systems, School of  
30 Industrial and Systems Engineering, Georgia Institute of Technology, Atlanta, GA  
31
- 32 **Dr. Barton H. (Buzz) Thompson, Jr.**, Robert E. Paradise Professor of Natural Resources Law,  
33 Stanford Law School, and Director, Woods Institute for the Environment, Stanford University,  
34 Stanford, CA  
35
- 36 **Dr. Robert Twiss**, Professor, University of California-Berkeley, Ross, CA  
37
- 38 **Dr. Terry F. Young**, Consultant, Environmental Defense, Oakland, CA

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**Dr. Lauren Zeise**, Chief, Reproductive and Cancer Hazard Assessment Section, California Environmental Protection Agency, Oakland, CA

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1                   **ADVISORY ON EPA’S ASSESSMENTS OF CARCINOGENIC**  
2                   **EFFECTS OF ORGANIC AND INORGANIC ARSENIC: AN**  
3                   **ADVISORY REPORT OF THE U.S. EPA SCIENCE ADVISORY**  
4                   **BOARD**

5  
6                   **1. EXECUTIVE SUMMARY**

7  
8                   New information has been developed on the metabolism, pharmacokinetics (PK)  
9                   and mode of carcinogenic action of arsenic and its methylated species and new  
10                  epidemiology studies have been conducted on inorganic arsenic since the publication of  
11                  reviews by the National Research Council (NRC, 1999, 2001). EPA considered this new  
12                  science in the development of the Office of Pesticide Programs (OPP)’s *Draft Science*  
13                  *Issue Paper: Mode of Action for Cacodylic Acid (Dimethylarsinic Acid) and*  
14                  *Recommendations for Dose Response Extrapolation* (USEPA OPP, 2005) and Office of  
15                  Water (OW)’s *Draft Toxicologic Review of Inorganic Arsenic* (USEPA OW 2005).  
16                  EPA’s Office of Research Development (ORD) further captured key scientific issues to  
17                  be considered in its Issue Paper *Cancer Risk Assessment for Organic Arsenical*  
18                  *Herbicides: Comments on Mode of Action, Human Relevance and Implications for*  
19                  *Quantitative Dose-Response Assessment* (Appendix E of USEPA OPP, 2005). The  
20                  Science Advisory Board (SAB) was asked to review these documents and offer advice on  
21                  four main topical areas including the metabolism, mode of action, dose-response, and  
22                  approaches to low-dose extrapolation of cancer risk for Dimethylarsinic Acid (DMA<sup>V</sup>) and  
23                  inorganic acid (iAs). The full charge to the SAB is in Appendix B to this document.  
24

25                  In response to the Agency’s request, the SAB convened an expert Panel to provide advice  
26                  to the Agency on these scientific issues. A summary of the SAB Panel’s response to the  
27                  charge questions is provided below.  
28

29                  **1.1 Metabolism and Toxic Responses of Arsenic Species**

30                  *Charge Question A1*

31                  EPA concluded that available *in vivo* and *in vitro* metabolism and  
32                  pharmacokinetic studies in humans and laboratory animals suggest that the efficiency of  
33                  methylation reactions and cellular uptake varies with the arsenic compound administered  
34                  exogenously. Most studies suggest a predominantly one-way process in mammals and  
35                  that after DMA<sup>V</sup> exposure, significant amounts of iAs<sup>III</sup>, iAs<sup>V</sup>, methylarsonous acid  
36                  and methylarsonic acid are formed.  
37

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1 (MMA<sup>III</sup>), or methylarsinic acid (MMA<sup>V</sup>) are not expected at target tissues. EPA asked  
2 the SAB to comment on how best to consider the PK processes in cancer risk assessment  
3 based on data derived from direct dimethylarsinic acid (DMA<sup>V</sup>) exposure versus direct  
4 inorganic arsenic (iAs) exposure.

5  
6 *Summary Response*

7  
8 The Panel agreed that:

- 9
- 10 i) Metabolism of iAs appears to be a one-way process in which iAs is  
11 converted to monomethylarsenic (MMA), dimethylarsenic (DMA), and  
12 in some species to trimethylarsenic (TMA) metabolites with arsenic in  
13 +3 or +5 oxidation states. Thus, significant amounts of MMA or iAs are  
14 not expected to be found in tissues or urine of rats or humans as a result  
15 of exposure to DMA<sup>V</sup>, although iAs may be present in human tissues or  
16 urine from other sources.
  - 17 ii) In contrast, exposure to iAs may result in production, tissue retention,  
18 and urinary excretion of a variety of tri- and pentavalent iAs and  
19 methylated arsenic species.
  - 20 iii) The uptake and reduction of DMA<sup>V</sup> to dimethylarsinous acid (DMA<sup>III</sup>)  
21 are apparently critical steps in activation of DMA<sup>V</sup> – though it is not  
22 clear if, where and to what extent these processes occur in humans  
23 exposed to DMA<sup>V</sup>.
  - 24 iv) The capacity to reduce DMA<sup>V</sup> to DMA<sup>III</sup> seems to exist in human tissues  
25 and the conversion of even a small amount of exogenous DMA<sup>V</sup> to  
26 DMA<sup>III</sup> is of toxicological concern.
  - 27 v) Given the differences in the metabolic pattern for iAs and DMA<sup>V</sup>, the  
28 Panel believes data derived from DMA<sup>V</sup> exposure, not from iAs  
29 exposure, is better suited for cancer risk assessment of DMA<sup>V</sup>.
  - 30 vi) Significant uncertainties are associated with this approach. The  
31 toxicologic data on DMA<sup>V</sup> is all from rat studies, and considering several key  
32 differences between rats and humans in the metabolism of arsenic, these  
33 uncertainties should be considered in the assessment of DMA<sup>V</sup> cancer  
34 risk.
  - 35 vii) The physiologically based pharmacokinetic (PBPK) model under  
36 development by EPA may be a useful approach but it is not yet sufficiently  
37 robust to conduct interspecies extrapolations.

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- 1           viii) EPA should continue developing the arsenic PBPK model and to  
2           conduct research to obtain kinetic constants needed to describe rates of  
3           uptake, efflux, metabolism, and elimination of DMA<sup>V</sup> in rats and  
4           humans.  
5           ix) There is a need to validate such models for predicting tissue  
6           concentrations of active species regardless of the source of arsenic  
7           exposure.  
8

9           *Charge Question A2*

10  
11           EPA concluded that direct exposure to iAs<sup>III</sup> or iAs<sup>V</sup> is expected to result in a  
12           more complex mixture of toxic metabolites than with DMA<sup>V</sup> exposure given that  
13           mixtures of metabolites vary based on which chemical is administered exogenously.  
14           EPA expects a less complex mixture of metabolites following DMA<sup>V</sup> exposure than  
15           following iAs exposure. EPA further expects that the tumorigenic profiles vary with the  
16           arsenical compound administered. For its DMA<sup>V</sup> assessment, EPA asked the SAB to  
17           comment on the use of data derived from rodent exposures to organic arsenicals versus  
18           data derived from direct human exposure to iAs.  
19

20           *Summary Response*

21  
22           The Panel agreed that:

- 23  
24           i) The metabolism of iAs yields a wide spectrum of metabolites which are  
25           apparently not produced during the metabolism of DMA<sup>V</sup>.  
26           ii) Production of iAs and MMA metabolites may be associated with specific  
27           toxic or cancerous endpoints that are absent in DMA<sup>V</sup> exposure to rats or  
28           humans.  
29           iii) All published data on toxicological responses to DMA<sup>V</sup> are from studies  
30           in rodents, mainly rats; no human data are available. As noted in the  
31           response to A1 above, these differences raise concerns for risk  
32           assessments based on these data.  
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## 1.2. Modes of Carcinogenic Action for DMA<sup>V</sup> and iAs

### *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 extrapolations. EPA asked the SAB to comment on the scientific soundness of the postulated MOA for DMA<sup>V</sup> 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<sup>V</sup> 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<sup>V</sup> to trimethylarsine oxide (TMA<sup>VO</sup>) and possibly trimethylarsine (TMA<sup>III</sup>) 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<sup>V</sup> and DMA<sup>III</sup>.
- iv) The Panel's postulated MOA for DMA<sup>V</sup> is:
  - a. Reductive metabolism of DMA<sup>V</sup> to DMA<sup>III</sup>,
  - b. High concentrations of DMA<sup>III</sup> (and possibly DMA<sup>V</sup>) 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.

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1            *Charge question B2*

2  
3            EPA concluded that their postulated MOA for DMA<sup>V</sup> induced bladder  
4 carcinogenesis in the rat would be relevant to humans as there are little or no data to  
5 suggest that key precursor events and ultimately tumor formation would not occur in  
6 exposed humans if sufficient DMA<sup>III</sup> were present. EPA asked the SAB to comment on  
7 the relevance of the postulated key events to tumors in humans and how differences in  
8 humans and experimental animals should be accounted for in DMA<sup>V</sup> risk assessments  
9

10           *Summary Response:*

11  
12           The Panel concluded that:

- 13  
14           i)        If high enough concentrations of DMA<sup>V</sup> or DMA<sup>III</sup> were present in human  
15 urine or the bladder after exposure to DMA<sup>V</sup> it is plausible that a similar  
16 response would take place; however, no data are available to support or  
17 reject this assumption.  
18           ii)        The suggested greater conversion of DMA<sup>V</sup> to TMA<sup>VO</sup> or possibly TMA<sup>III</sup>  
19 in rats vs. in humans, may contribute to induction of bladder cancer in rats  
20 however the extent of the contribution is unknown.  
21           iii)       No studies have been conducted to determine whether the DMA<sup>V</sup>  
22 carcinogenic risk differs by life stage, e.g., among the young, or elderly.  
23

24           *Charge Question B3.*

25  
26           EPA concluded that iAs causes human cancer most likely by many different  
27 modes of action. This is based on the observed findings that iAs undergoes successive  
28 methylation steps in humans and results in the production of a number of intermediate  
29 metabolic products and that each has its own toxicity. EPA asked the SAB to comment  
30 on the soundness of its conclusion.  
31

32           *Summary Response*

33  
34           The Panel concluded that:

- 35  
36           i)        Multiple modes of action may operate in carcinogenesis induced by iAs  
37 because there is simultaneous exposure to multiple metabolic products as

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1 well as multiple target organs and the composition of metabolites can  
2 differ in different organs.

- 3 ii) Each arsenic metabolite has its own cytotoxic and genotoxic capability.  
4 iii) Inorganic arsenic (iAs<sup>III</sup>) and its metabolites are not direct genotoxicants  
5 because these compounds do not react with DNA. However, iAs<sup>III</sup> and  
6 some of its metabolites can exhibit indirect genotoxicity, induce  
7 aneuploidy, cause changes in DNA methylation, and alter signaling and  
8 hormone action. In addition, iAs can act as a transplacental carcinogen  
9 and a cocarcinogen. Arsenic essentiality and the possibility of hormetic  
10 effects are in need of additional research to determine if they are relevant  
11 to arsenic's role in inducing cancers and to clarify their significance in  
12 assessing arsenic risk.

### 13 14 **1.3. Selection of Data for Dose-Response Assessment**

#### 15 *Charge Question C1*

16  
17  
18 In the absence of human data, EPA proposed to use the bladder tumor data from  
19 the DMA<sup>V</sup> rat bioassay for quantifying potential human cancer risk to DMA<sup>V</sup>. EPA asked  
20 the SAB to comment on the appropriateness of this approach. The SAB was also asked  
21 to comment on whether the iAs epidemiology data can be used to inform the DMA<sup>V</sup>  
22 dose-response assessment which is now based on data derived from studies in rats dosed  
23 with DMA<sup>V</sup>.

#### 24 *Summary Response*

25  
26  
27 The Panel agreed that:

- 28  
29 i) Given the lack of human data, the bladder tumor data from DMA<sup>V</sup> rat  
30 bioassays are the most suitable data set for quantifying potential human  
31 cancer risk to DMA<sup>V</sup>.  
32 ii) A discussion of the key uncertainties in using data from studies in rats to  
33 conduct human risk assessment should be presented. Panel responses to  
34 charge questions A1 and C1 discuss issues that members considered  
35 important to discuss in EPA's Science Issue Paper. These issues relate to  
36 the pharmacokinetic and pharmacodynamic similarities and differences  
37 between rats and humans in response to arsenic exposure, the use of

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1 rodent bladder tumor models in general, and issues in the use of rodent  
2 data for human risk assessment.

- 3 iii) The panel concluded that without more detailed information on target  
4 tissue dosimetry for arsenic species, the iAs epidemiology data would be  
5 of limited use to inform the DMA<sup>V</sup> dose-response assessment derived  
6 from rat data with DMA<sup>V</sup>. Additional details are contained in the Panel's  
7 response to charge question C1.

8  
9 *Charge Question C2.*

10  
11 EPA reviewed the available epidemiologic studies including those published since  
12 the NRC 2001 review for U.S. populations exposed to inorganic arsenic via drinking  
13 water. EPA concluded that the Taiwanese dataset remains the most appropriate choice  
14 for estimating cancer risk in humans. The SAB was asked to comment on the soundness  
15 of this conclusion and to comment on whether these data provide adequate  
16 characterization of the impact of childhood exposure to iAs.

17  
18 *Summary Response*

19  
20 The Panel concluded that:

- 21  
22 i) Because of various factors (e.g., size and statistical stability of the  
23 Taiwanese database relative to other studies, the reliability of the  
24 population and mortality counts, the stability of residential patterns, and  
25 the inclusion of long-term exposures), this database remains, at this time,  
26 the most appropriate choice for estimating bladder cancer risk among  
27 humans, though the data has considerable limitations that should be described  
28 qualitatively or quantitatively to help inform risk managers about the strength of  
29 the conclusions.
- 30 ii) There are other epidemiologic databases from studies of populations also  
31 exposed at high levels of arsenic, and the Panel recommends that these be  
32 used to compare the unit risks at the higher exposure levels that have  
33 emerged from the Taiwan data. The Panel suggests that results on bladder  
34 cancer risk from published epidemiology studies of US and other  
35 populations chronically exposed from 0.5 to 160 µg/L inorganic arsenic in  
36 drinking water be critically evaluated, using a uniform set of criteria. If,  
37 after this evaluation, one or more of these studies are shown to be of  
38 potential utility, the low-level studies and Taiwan data may be compared

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1 for concordance. Much of the US and many other populations differ from  
2 the Taiwanese population of interest in factors that might influence the  
3 application of that data to the assessment of U.S. bladder cancer risks  
4 associated with inorganic arsenic. Comparative analyses could lead to  
5 further insights into the possible influence of these differences on  
6 population responses to arsenic in drinking water. Regarding childhood  
7 exposure to iAs, it was the Panel's view that, based on available data, it is  
8 not clear whether children differ from adults with regard to their  
9 sensitivity to the carcinogenic effects of arsenic in drinking water.  
10 However, the possibility of a different response in degree or kind should not be  
11 ignored and needs to be investigated.  
12

#### 13 **1.4. Approaches to Low-Dose Extrapolation for iAs and DMA<sup>V</sup>**

##### 14 ***Charge Question D1.***

15  
16  
17 EPA's Guidelines for Carcinogen Risk Assessment underscore the importance of  
18 understanding the MOA as the basis for making judgments on how to best extrapolate  
19 cancer risk at lower exposures. EPA concluded that available data on DMA<sup>V</sup> are not  
20 sufficient to support development of biologically-based models and therefore opted to  
21 use a default nonlinear low-dose extrapolation method. The SAB was asked to comment  
22 on the Agency's scientific rationale in support of this approach and how uncertainty  
23 should be incorporated into low-dose extrapolation.  
24

##### 25 *Summary Response*

26  
27 The Panel concluded that:

- 28
- 29 i) Neither the MOA postulated by the Panel, nor those postulated by ORD or  
30 OPP contain key events expected to be a linear function of dose of DMA<sup>V</sup>.
  - 31 ii) Several processes important to some postulated key events would have  
32 non-linear components or are non-linear (e.g., saturable metabolic  
33 processes, cytotoxicity, formation of heritable alterations in DNA by ROS,  
34 cell proliferation, repair of ROS-induced DNA damage).
  - 35 iii) The linear approach would be consistent with evidence for direct  
36 genotoxicity of DMA<sup>III/V</sup>; however, it is generally accepted that DMA<sup>V</sup> is  
37 not directly genotoxic and neither DMA<sup>III</sup> nor DMA<sup>V</sup> react directly with  
38 DNA.

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- 1           iv)     There are insufficient data to invoke ROS-induced DNA damage as a key  
2                     event in the carcinogenic process associated with exposures to DMA<sup>V</sup> or  
3                     DMA<sup>III</sup>.
- 4           v)     The nonlinear approach is more consistent with available DMA<sup>V</sup> data and  
5                     current concepts of chemical carcinogenesis.
- 6           vi)    Uncertainty is best incorporated through the use of uncertainty factors that  
7                     capture differences in pharmacokinetic and pharmacodynamic differences  
8                     across species and differences associated with sensitive populations.
- 9           vii)   There are not sufficient data on comparative dosimetry in rats and humans  
10                    to make any conclusive statements about species differences in pharmaco-  
11                    kinetics, though available data on uroepithelial cell cytotoxicity could  
12                    allow EPA to assemble a case for pharmacodynamic equivalency. There  
13                    is no information which may be used to determine the choice of  
14                    uncertainty factors for sensitive human populations.

15  
16           ***Charge Question D2***

17  
18           EPA determined that the most prudent approach for modeling cancer risk from  
19           iAs is to use a linear model because of the remaining uncertainties regarding the ultimate  
20           carcinogenic metabolites and whether mixtures of toxic metabolites interact at the site(s)  
21           of action. EPA asked the SAB if it concurred with the selection of a linear model  
22           following the recommendations of the NRC (2001) to estimate cancer risk in light of the  
23           multiple modes of carcinogenic action for iAs.

24  
25           ***Summary Response:***

26  
27           The Panel concluded that:

- 28  
29           i)     Inorganic arsenic has the potential for a highly complex mode of action.
- 30           ii)    Until more is learned about the complex PK and PD properties of iAs and  
31                    its metabolites there is not sufficient justification for the choice of a  
32                    specific nonlinear form of the dose-response relationship.
- 33           iii)   The NRC (2001) recommendation to base risk assessments on a linear  
34                    dose response model that includes the SW Taiwan population as a  
35                    comparison group seems the most appropriate approach.
- 36           iv)    The Panel also recommends that EPA perform a sensitivity analysis of the  
37                    Taiwanese data with different exposure metrics, with the subgroup of

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1 villages with more than one well measurement, and using a multiplicative  
2 model that includes a quadratic term for dose.

3  
4 ***Charge Question D3***

5  
6 EPA employed a Microsoft Excel software that was previously used by the NRC  
7 (2001) to project estimated cancer risks from iAs exposure. The SAB was asked to  
8 comment on the precision and accuracy of this program.

9  
10 ***Summary Response:***

11  
12 The panel concluded:

- 13  
14 i) That the EPA program conformed to the NRC (2001) recommendation for  
15 modeling cancer hazard as a function of age and the average daily dose of  
16 exposure to arsenic through drinking water sources.  
17 ii) The panel did, however, identify and report to the EPA on two potential  
18 discrepancies in the data inputs and one computational error in the portion  
19 of the program that employs the BEIR-IV formula to evaluate excess  
20 lifetime cancer risk from arsenic exposure.  
21 iii) The panel made several suggestions on improvements in the model's  
22 programming and documentation conventions as well as recommendations  
23 for specific sensitivity analyses designed to test the robustness of the  
24 model to alternative formulations of the hazard function and aggregate  
25 population data inputs.  
26

27 ***Charge Question D4***

28  
29 In calculating estimated cancer risk to the US general population from drinking  
30 water exposure to iAs, the EPA utilized epidemiologic data from Taiwan. EPA followed  
31 the NRC (2001) recommendations to account for the differences in the drinking water  
32 consumption rates for the Taiwanese population and U.S. populations. On the basis of  
33 more recent data (noted in USEPA, 2005b), EPA utilized water intake adjustments for 2  
34 to 3.5 liters/day. EPA asked the SAB to recommend a drinking water value.

35  
36 ***Summary Response***

37  
38 The Panel recommended that:

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- 1  
2 i) EPA should incorporate variability parameters for individual water  
3 consumption into their analysis for the Taiwanese population.  
4 ii) Because assumptions about water consumption are an important source of  
5 variability in the risk estimates, EPA should conduct sensitivity analyses  
6 of the impact of using a range of consumption values for the Taiwanese  
7 population.  
8 iii) A better justification for assuming different consumption levels by sex is  
9 needed and in the absence of such a justification, the panel recommends  
10 an additional sensitivity analysis to examine the impact of equalizing the  
11 sex-specific consumption level.  
12 iv) The source of data for intake from other beverages and cooking water  
13 needs to be more fully discussed and documented.  
14

15 ***Charge Question D5***

16  
17 As recommended by the NRC (2001) EPA considered the background dietary  
18 intake of iAs and incorporated adjustment values of 0, 10, 30, and 50 micrograms per day  
19 into the cancer modeling based on available new data. The SAB was asked to  
20 recommend a value for the background dietary intake of iAs for both the control  
21 population and study population of Southwestern Taiwan.  
22

23 **Summary Response:**

24  
25 **The Panel concluded:**

- 26  
27 i) That sensitivity analyses be conducted using a range of total arsenic food  
28 intake values from at least 50 to 100  $\mu\text{g}$  per day and up to perhaps as high  
29 as 200  $\mu\text{g}$  per day to assess the impact of this range of dietary intakes on  
30 risk of lung and bladder cancer from exposure *via* drinking water in the  
31 Taiwan cohort.  
32 ii) That it cannot be assumed that the control population has an intake of zero  
33 arsenic from food.

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## 2. INTRODUCTION

### 2.1. Background

EPA officials from the Office of Research and Development (ORD), the Office of Water and the EPA Office of Pesticide Programs, requested that the EPA Science Advisory Board (SAB) evaluate certain components of the EPA draft assessment of potential human carcinogenicity associated with arsenic, and arsenic containing compounds (USEPA, 2005a). 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 Office of Pesticide Programs (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<sup>V</sup>, and the monosodium, disodium, and calcium salts of methanearsonate acid --MSMA, DSMA, and CAMA, collectively as referred as MMA<sup>V</sup>).

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 (EPA/SAB, 2000; EPA/SAB, 2001).

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1 Since the 2001 NAS review, new information has been developed on the mode of  
2 carcinogenic action, metabolism and pharmacokinetics (PK) of arsenic and its methylated  
3 species, and new epidemiology studies have been conducted on inorganic arsenic. EPA  
4 considered this new information in its hazard characterization for tolerance assessment of  
5 dimethylarsinic acid (DMA<sup>V</sup>) and methylarsonic acid (MMA<sup>V</sup>)(USEPA OPP, 2005 and  
6 USEPA ORD, 2005). EPA also developed a revised hazard and dose response assessment  
7 for inorganic Arsenic (USEPA OW, 2005) which relies on the two NRC reviews and  
8 provides an updated human health effects and dose-response assessment for inorganic  
9 arsenic. In its Charge to the SAB (USEPA, 2005a), EPA asked for advice on the  
10 soundness of the major science conclusions in these two documents. These documents  
11 focus on the assessment of DMA<sup>V</sup> and inorganic arsenic carcinogenicity (more  
12 specifically, metabolism, mode of action, dose-response, and approaches to low-dose  
13 extrapolation of cancer risk (see the specific Charge questions in subsections 2.1.1  
14 through 2.1.4 of this report).

### 15 16 **2.1.1. Metabolism and Toxic Responses of Arsenic Species**

17  
18 Charge Question A1. Metabolism and pharmacokinetics: Please comment on  
19 how pharmacokinetic processes are best considered regarding the use of data derived  
20 from direct DMA<sup>V</sup> exposure versus direct iAs exposure for cancer risk assessment.

21  
22 Charge Question A2. Response to mixtures of metabolites: Given the  
23 toxicological response profiles observed following direct exposures to iAs versus MMA<sup>V</sup>  
24 and DMA<sup>V</sup>, and the differences in human and rodent toxicologic responses to arsenicals,  
25 please comment on the use of data derived from rodent exposures to the organic  
26 arsenicals versus use of data derived from direct iAs human exposure, in the DMA<sup>V</sup>  
27 assessment.

### 28 29 **2.1.2. Modes of Carcinogenic Action for DMA<sup>V</sup> and Inorganic 30 Arsenic**

31  
32 Charge Question B1. Mode of action of DMA<sup>V</sup>: Please comment on the  
33 sufficiency of evidence to establish the animal mode of carcinogenic action for DMA<sup>V</sup>.  
34 Are the scientific conclusions sound and consistent with the available evidence on DMA<sup>V</sup>  
35 and the current state of knowledge for chemical carcinogenesis. Please comment on  
36 whether the key events in DMA's mode of action are supported by the available data.  
37 Specifically comment on the role of: a) reactive oxygen species in producing  
38 chromosomal damage and the strength of the evidence supporting oxidative damage as a

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1 causal key event in DMA<sup>V</sup>/DMA<sup>III</sup>'s mode of carcinogenic action versus an associative  
2 event or a secondary consequence of cytotoxicity; b) cell proliferation and cytotoxicity  
3 and the strength of the evidence as causal key events in DMA<sup>V</sup>/DMA<sup>III</sup>'s mode of  
4 carcinogenic action versus associative or secondary events, and c) other potential modes  
5 of action that have substantial scientific support that may be contributing to the  
6 carcinogenicity of DMA.

7  
8 Charge Question B2. Human relevance of animal DMA<sup>V</sup> MOA: Please  
9 comment on the relevance of the postulated key events (see B1) to tumors in humans.  
10 Please comment on how, if at all, differences in the human population vs. experimental  
11 animals should be accounted for in the risk assessment for DMA<sup>V</sup>. Please comment on  
12 the Agency's conclusion that the young are likely to respond like the adult to the  
13 formation of bladder tumors following exposure to DMA.

14  
15 Charge Question B3. Modes of carcinogenic action from exposure to inorganic  
16 arsenic: Please comment on the conclusion that the available data support the hypothesis  
17 that multiple modes of action may be operational following exposure to inorganic  
18 arsenic.

### 19 20 **2.1.3. Selection of Data for Dose-Response Assessment**

21  
22 Charge Question C1. Use of animal data for DMA<sup>V</sup>: Please comment on the use  
23 of the bladder tumor data from the DMA<sup>V</sup> rat bioassay as the most suitable dataset for  
24 quantifying potential human cancer risk to DMA<sup>V</sup>, including the weight of evidence to  
25 support this conclusion. Please comment on whether the iAs epidemiology data can be  
26 used to inform the DMA<sup>V</sup> dose-response assessment derived from rat data with DMA<sup>V</sup>.  
27 If so, please discuss how such information might be used (See Appendix B).

28  
29 Charge Question C2. Use of human epidemiological data from direct iAs  
30 exposure: Does the SAB agree that the Taiwanese dataset remains the most appropriate  
31 choice for estimating cancer risk in humans? Please discuss the rationale for your  
32 response. Do these data provide adequate characterization of the impact of childhood  
33 exposure to iAs? Please discuss the rationale for your response.

34  
35  
36  
37

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#### 2.1.4. Approaches to Low-Dose Extrapolation for Inorganic Arsenic and DMA<sup>V</sup>

Charge Question D1. Mode of carcinogenic action understanding for DMA<sup>V/III</sup> 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<sup>V</sup> 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. 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?

#### 2.2. Process for Developing this Report and the Structure of this Report

This advisory was conducted by the Science Advisory Board *Ad Hoc* Arsenic Review Panel (ARP) composed of members of the chartered SAB and its committees, members of the FIFRA Scientific Advisory Panel, and invited outside experts. A *Federal Register* notice on February 23, 2005 requested nominations of candidates for membership on the Arsenic Review Panel (see GPO, 2005a). Panel Members were selected following procedures for panel formation at the EPA Science Advisory Board

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1 (USEPA SAB 2005a). The Arsenic Review Panel held a public telephone conference  
2 meeting to plan for the review on August 11, 2005 (see GPO 2005b). The Panel’s review  
3 meeting was held on September 12-13, 2005 and concluded with the articulation of a  
4 series of recommendations in response to each of the EPA Charge questions. These  
5 recommendations became the core of this report. The Arsenic Review Panel discussed its  
6 draft report during telephone conference meetings on January 24, 2006, February 23,  
7 2006 and February 28, 2006 (see GPO, 2005c; GPO, 2005d; GPO, 2005e; and GPO,  
8 2005f).

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### 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<sup>V</sup> 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 ARP's advice is contained in report 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<sup>V</sup> significant amounts of [arsenite] (iAs<sup>III</sup>), [arsenate] (iAs<sup>V</sup>), [methylarsonous acid] (MMA<sup>III</sup>), or [methylarsonic acid] (MMA<sup>V</sup>) 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<sup>V</sup> exposure versus direct iAs exposure for cancer risk assessment."*

Charge questions A1 and A2 address exposure to and the metabolic fate of DMA<sup>V</sup> associated organoarsenic-containing herbicides. DMA<sup>V</sup> 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<sup>V</sup> in the environment. This is because EPA representatives stated during the September, 2005 Arsenic Review Panel meeting that the environmental conversion of DMA<sup>V</sup> from organoarsenic pesticides, and the risk associated with exposures to these conversion products, will be addressed later by EPA in a separate document.

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1 The panel agrees with the Agency's reasoning behind this question which is  
2 summarized at the beginning of this subsection (3.2.1). In mammalian tissues/cells  
3 (including human), the metabolism of inorganic arsenic (iAs) appears to be a one-way  
4 process in which iAs is converted to MMA, DMA and in some species to TMA  
5 metabolites containing arsenic in +3 or +5 oxidation states (Vahter, 1999; Thomas, et al.,  
6 2001). There is no evidence for demethylation of methylated arsenic species in either  
7 animal or human tissues, though as noted in the preceding paragraph and in point 4 below  
8 microbial transformation is possible in the intestine. While the step-wise addition of  
9 methyl groups is likely a one-way process, a cycling between +3 and +5 arsenic species  
10 may occur at each of the methylation steps due to a spontaneous oxidation of +3 species  
11 (Gong, et al., 2001; Aposhian, et al., 2003) and non-enzymatic (Delnomdedieu, et al.,  
12 1994; Scott et al., 1993) or enzymatic (Zakharayn and Aposhian, 1999; Radabaugh and  
13 Aposhian, 2000; Waters et al., 2004) reduction of +5 species. Given the one-way  
14 character of arsenic methylation, we do not expect to find significant amounts of MMA  
15 or iAs as products of DMA<sup>V</sup> metabolism in either rat or human tissues or urine.  
16

17 In contrast, exposure to iAs may result in the production, tissue retention, and  
18 urinary excretion of all the above iAs and methylated arsenic species. Based on data from  
19 rodent studies, both the uptake and reduction of DMA<sup>V</sup> to DMA<sup>III</sup> are apparently critical  
20 steps in the activation of exogenous DMA<sup>V</sup>. It is not clear, where and to what extent (if  
21 at all) these processes occur in humans exposed to DMA<sup>V</sup>, although it appears that uptake  
22 may be rate limiting for further metabolism of DMA<sup>V</sup>. DMA<sup>III</sup> is a urinary metabolite in  
23 individuals chronically exposed to iAs (Le et al., 2000; Aposhian, et al., 2000; Mandal,  
24 Ogra and Suzuki, 2001; Del Razo et al., 2001; Valenzuela, 2005), indicating that the  
25 capacity to reduce DMA<sup>V</sup> to DMA<sup>III</sup> exists in human tissues. The Panel pointed out that  
26 even the conversion of a small amount/fraction of exogenous DMA<sup>V</sup> to DMA<sup>III</sup> is of  
27 toxicological significance due to the significant toxicity of DMA<sup>III</sup>. Thus, strictly from  
28 the point of view of the metabolic pattern, data derived from DMA<sup>V</sup> exposure (in the rat),  
29 not from iAs exposure in humans, is better suited for cancer risk assessment of DMA<sup>V</sup>.  
30 However, this approach is uncertain because of specific metabolic differences between  
31 rats and humans, and other factors, including:  
32

33 a) The uptake pathway or pathways for DMA<sup>V</sup> in humans is/are unidentified.  
34 The expression or properties of DMA<sup>V</sup> transporters may differ in rats and  
35 humans, leading to differences in uptake of DMA<sup>V</sup> in tissues and organs.  
36

37 b) Results of laboratory and epidemiological studies suggest that the pattern for  
38 DMA<sup>V</sup> metabolism in rats is different from that in humans (Figure 1). Rats

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1 metabolize DMA<sup>V</sup> to DMA<sup>III</sup>, trimethylarsine oxide (TMA<sup>VO</sup>) (Yoshida et al.,  
2 1997; Yoshida et al., 1998; Cohen et al., 2002), and possibly, trimethylarsine  
3 (TMA<sup>III</sup>) (Waters et al., 2004). DMA<sup>V</sup>, DMA<sup>III</sup>, and TMA<sup>VO</sup> are urinary  
4 metabolites of DMA<sup>V</sup> in the rat. In addition, TMA<sup>VO</sup> was also detected in urine  
5 of rats chronically exposed to iAs (Yoshida et al., 1998). In contrast, little or no  
6 TMA<sup>VO</sup> was found in human urine after a single dose of DMA<sup>V</sup> (Marafante et al.,  
7 1987; Buchet et al., 1981) or after acute (Mahieu, et al., 1981; Apostoli et al.,  
8 1997; Benramdane et al., 1999) or chronic exposures to iAs (Vahter, 1999;  
9 Thomas et al., 2001). These data suggest that the capacity to produce TMA<sup>VO</sup>  
10 from iAs or DMA<sup>V</sup> or to excrete TMA<sup>VO</sup> in urine is limited in humans as  
11 compared to rats. Thus, while it is possible that the urinary TMA<sup>V/III</sup> metabolites  
12 significantly affect the overall toxic or cancerous outcomes in the bladder of rats  
13 exposed to DMA<sup>V</sup>, the relative lack of these metabolites in human urine would  
14 suggest that the outcome in humans would not be as severe as in rats. More  
15 research is needed to characterize the role of TMA<sup>V/III</sup> metabolites in bladder  
16 carcinogenesis induced in rats by chronic exposures to DMA<sup>V</sup> and to determine  
17 whether the apparent absence of these metabolites in humans is associated with a  
18 decreased susceptibility to the carcinogenic effects of DMA<sup>V</sup>.

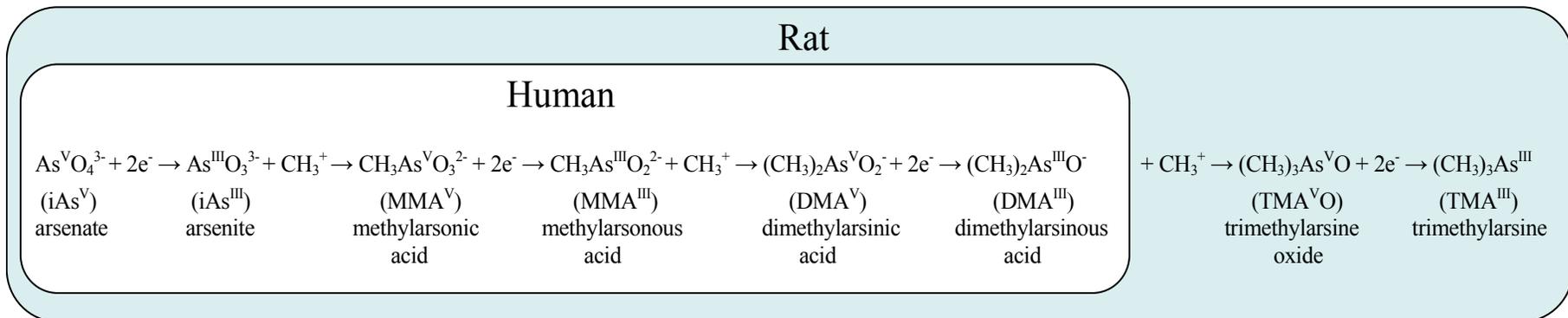
19  
20 c) Accumulation of DMA<sup>III</sup> in rat erythrocytes, due to a high-affinity for binding  
21 to hemoglobin (Lu et al., 2004) contributes to a specific kinetic pattern for DMA<sup>V</sup>  
22 in rats. It is not clear how and to what extent this factor affects the yield and  
23 concentration of the active arsenic species (e.g., DMA<sup>III</sup>, TMA<sup>VO</sup>, or TMA<sup>sIII</sup>) in  
24 urine or in target tissues of rats and how lower accumulation in human  
25 erythrocytes would alter the kinetic pattern for DMA<sup>V</sup> and toxic/cancerous  
26 outcomes of DMA<sup>V</sup> exposure in humans.

27  
28 d) Microorganisms, including intestinal bacteria, have a capacity to either  
29 methylate or demethylate arsenicals (Hall et al., 1997; Cullen et al., 1984; Cullen  
30 et al, 1989; Lehr et al., 2003; Bently and Chasten, 2002; Tamaki and  
31 Frankenberger, 1992; Mukhopadhyay et al, 2002; Ridley et al., 1977; Qin, et al.,  
32 2006). Although the patterns and extent of DMA<sup>V</sup> metabolism by human  
33 intestinal microflora are not known, it is possible that oral exposure to DMA<sup>V</sup>  
34 results in the absorption of a wide spectrum of arsenic metabolites produced by  
35 bacteria in the gastrointestinal tract of exposed individuals. In contrast, bacterial  
36 metabolism would not affect the absorption of DMA<sup>V</sup> after inhalation or dermal

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**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<sup>III</sup>) or pentavalent arsenic (As<sup>V</sup>). 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<sup>V</sup>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<sup>III</sup>. 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.

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1  
2 exposures. Thus, arsenic species found in tissues may differ with different routes  
3 of exposure. Interspecies differences in endogenous intestinal bacteria may further  
4 complicate extrapolation from rats to humans.

5  
6 e) Additional factors may affect the metabolic profiles for DMA<sup>V</sup> in humans,  
7 including co-exposures to other environmental contaminants, deficiencies of  
8 specific nutrients (e.g., selenium) or malnutrition. For example, poor nutrition has  
9 been shown to induce expression of aquaglyceroporin-9 (AQP9), an iAs<sup>III</sup>/MMA<sup>III</sup>  
10 transporter (Liu et al., 2002; Liu et al., 2004; Liu et al., 2006), 20-fold (Carbrey et  
11 al., 2003).

12  
13 All the above concerns should be considered in the risk assessment of DMA<sup>V</sup>  
14 exposure.

15  
16 EPA's briefing documents presented information on a physiologically based  
17 pharmacokinetic (PBPK) model for arsenic disposition and metabolism that is under  
18 development. PBPK modeling might be a useful approach for integrating tissue and  
19 excreta concentrations of arsenic metabolites resulting from exposure to the various  
20 forms of arsenic, including DMA<sup>V</sup>, in laboratory animals and humans. For now, the  
21 modeling work described by EPA is in the developmental stage and is not considered  
22 sufficiently robust to conduct interspecies extrapolations. However, the Panel strongly  
23 encourages the Agency to proceed with PBPK model development, including laboratory  
24 studies to obtain the kinetic constants needed to describe rates of uptake, efflux,  
25 metabolism, and elimination of DMA<sup>V</sup> in both rats and humans. When sufficiently  
26 validated, this model could simulate concentrations of active (toxic or carcinogenic)  
27 metabolites in urine and bladder tissue following exposure to DMA<sup>V</sup>. This approach  
28 could be used for dose response analysis in cancer risk assessment. Such models must be  
29 validated for predicting tissue concentrations of active species regardless of the source of  
30 arsenic exposure.

### 31 32 **3.2.2. Response to mixtures of metabolites (Charge Question A2)**

33  
34 EPA's Charge stated that, "Tumorigenic profiles vary based on which arsenical  
35 compound is administered exogenously. *In vivo* and *in vitro* studies indicate that each of  
36 the arsenical compounds exhibit similarities and differences in their profiles of biological  
37 activities. Direct exposure to iAs<sup>III</sup> or iAs<sup>V</sup> is expected to result in more of a mixture of  
38 toxic metabolites than for direct exposure to DMA<sup>V</sup>; the mixture of metabolites is

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1 expected to vary based on which chemical is administered exogenously. The potential  
2 mixture of metabolites following direct exposure to DMA<sup>V</sup> appears less complex as  
3 compared to iAs” (USEPA, 2005a). *Charge Question A2* asks, “Given the toxicological  
4 response profiles observed following direct exposures to iAs versus MMA<sup>V</sup> and DMA<sup>V</sup>,  
5 and the differences in human and rodent toxicologic responses to arsenicals, please  
6 comment on the use of data derived from rodent exposures to the organic arsenicals  
7 versus use of data derived from direct iAs human exposure, in the DMA<sup>V</sup> assessment.”  
8

9 The answer to this charge question is linked to the answer to question in section  
10 3.2.1 above. The metabolism of iAs yields a wide spectrum of metabolites (Figure 1)  
11 some of which (iAs<sup>III/V</sup>, MMA<sup>III/V</sup>) are apparently not produced during the metabolism of  
12 exogenous DMA<sup>V</sup>. The production of iAs and MMA metabolites may be associated with  
13 specific toxic or cancerous endpoints that are absent in DMA<sup>V</sup> exposure in rats or humans  
14 except when there is a significant co-exposure to iAs as is often found in U.S. drinking  
15 water supplies, or in food or the environment. The Panel notes that there are no  
16 published data on toxicological responses to DMA<sup>V</sup> in humans. The toxic and  
17 carcinogenic effects of DMA<sup>V</sup> have been examined only in rodents, mainly in rats.  
18 Because of the differences between the metabolic profiles for inorganic arsenic and  
19 DMA, and because of the interspecies differences in the metabolism of both arsenicals,  
20 neither data derived from rodent exposures to the organic arsenicals nor data derived  
21 from human exposures to inorganic arsenic provide optimal bases for the assessment of  
22 DMA<sup>V</sup> exposures in humans. In order to provide the best possible answer to EPA’s  
23 questions, the panel agrees that using the data from rodent exposures to DMA<sup>V</sup> may be  
24 the most reasonable approach.  
25

26 However, a significant degree of uncertainty is associated with this approach due  
27 to the metabolic differences between rats and humans and due to other factors, including  
28 those listed in the response to charge question A1 above. The differences in the  
29 production and urinary excretion of TMA<sup>III/V</sup> species that could affect the toxic and  
30 cancerous outcomes of DMA<sup>V</sup> exposure are of a particular concern to this panel.  
31 TMA<sup>V</sup>O is a hepatocarcinogen in rats (Shen et al., 2003). TMA<sup>III</sup> is apparently more  
32 potent than DMA<sup>III</sup> in damaging purified DNA in *in vitro* systems (Andrews, et al.,  
33 2003). On the other hand, both TMA<sup>V</sup>O and TMA<sup>III</sup> are less acutely toxic or cytotoxic  
34 than DMA<sup>III</sup> (Yamauchi et al., 1990; Cullen, 2005; Sakurai et al., 1998; Oochi et al.,  
35 1994). The contribution of these two metabolites to cytotoxicity and carcinogenesis in  
36 the urinary bladder of rats exposed to DMA<sup>V</sup> remains unclear. This uncertainty should be  
37 properly addressed in the risk assessment for DMA<sup>V</sup> exposure in humans.  
38

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2

### 3.3. Modes of Carcinogenic Action for DMA<sup>V</sup> and Inorganic Arsenic

3

4

#### 3.3.1. Mode of Action of DMA<sup>V</sup> (Charge Question B1)

5

6

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<sup>V</sup>, 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<sup>V</sup>. Are the scientific conclusions sound and consistent with the available evidence on DMA<sup>V</sup> 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<sup>V</sup>/DMA<sup>III</sup>'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<sup>V</sup>/DMA<sup>III</sup>'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.

25

26

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

35

36

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

37

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1 associated with exposures to DMA<sup>V</sup> or DMA<sup>III</sup> for the reasons discussed in the following  
2 paragraphs.

3  
4 Chemically, neither oxidation of As<sup>III</sup> to As<sup>V</sup> nor reduction of As<sup>V</sup> to As<sup>III</sup> can  
5 produce an oxygen radical in the absence of other reactants. Arsenic is not a metal and  
6 can only undergo two-electron reduction (no free electron or radical to donate to oxygen).  
7 Although there are indirect sources of ROS that can participate in arsenite-stimulated cell  
8 signaling (e.g. stimulation of NADPH oxidase; Smith et al., 2001) or arsenic trioxide-  
9 mediated apoptosis via mitochondrial collapse (Jing et al., 1999), these have not been  
10 demonstrated for DMA. Arsenic compounds could also increase ROS by promoting an  
11 inflammatory response in many tissues. This may contribute to carcinogenesis, but it is  
12 not the MOA for carcinogenesis.

13  
14 Much of the argument suggesting that the mode of action of DMA<sup>V</sup>-induced  
15 bladder cancer involves ROS-induced chromosome damage derives from studies on  
16 DMA<sup>III</sup>-induced DNA damage. Very high cytotoxic concentrations of DMA<sup>III</sup> have been  
17 shown to induce DNA damage in cell-free systems and in intact cells (Mass et al., 2001),  
18 possibly via an ROS-mediated mechanism or by dimethylarsine (Yamanaka et al., 2003;  
19 Kitchin and Ahmad, 2003; Nesnow et al, 2002; Andrews et al., 2003). However, cellular  
20 genetic toxicology assays of DMA do not support an ROS-dependent mechanism.  
21 Neither DMA<sup>V</sup> nor DMA<sup>III</sup> is significantly mutagenic at loci which are known to detect  
22 oxidant DNA damage. Neither compound was mutagenic in the Ames *Salmonella* strain  
23 TA104, a strain developed primarily to aid in the detection of oxidative mutagens, nor  
24 were  $\lambda$  prophage induced (Kligerman et al., 2003). Prophage induction (which depends  
25 on the *E. coli* SOS system, a system responsive to DNA damage) was readily detectable  
26 after treatment with other agents acting by oxidant mechanisms, such as bleomycin,  
27 carbon tetrachloride (+S9), hydrogen peroxide, and iron compounds (Rossman et al.,  
28 1991).

29  
30 DMA<sup>V</sup> was either negative or very weakly positive at extremely high dose in a  
31 number of other test systems which can detect oxidative mutagens (Moore et al., 1997,  
32 Kligerman et al., 2003; Oya-Ohta et al., 1996). Treatment of Muta<sup>TM</sup> mouse with DMA<sup>V</sup>  
33 (10.6 mg/kg per day) IP for 5 days caused only a (not significant) 1.3-fold increase in  
34 lacZ mutations in the lung, but not in the bladder or bone marrow (Noda et al., 2002).  
35 DMA<sup>V</sup> presumably could be converted to DMA<sup>III</sup> in mouse liver. (Arsenite also gave  
36 negative results in this assay.)  
37

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1 The single study of DMA<sup>III</sup> in the mouse lymphoma assay (Kligerman et al.,  
2 2003), which detects clastogens as well as point mutagens at the TK locus, showed no  
3 significant effect on mutant fraction at concentrations <1.5 μM (38% survival) in the agar  
4 assay. “Significant” was defined as at least a 2-fold increase over control, but no  
5 statistical analysis was done, only a single assay is shown, and there is only one  
6 “significant” response, at 1.51 μM. (This assumes a background mutant fraction of  
7 between 38 and 50 X 10<sup>-6</sup>). A microwell assay using the same cells showed a  
8 “significant” affect at 2.56 μM (9% survival).

9  
10 Kligerman et al. (2003) argued that DMA<sup>III</sup> (and MMA<sup>III</sup>) are basically clastogens  
11 and not point mutagens. The clastogenesis studies suffer from the same problems as the  
12 mutagenesis studies: no statistical analysis and single data points at some doses.  
13 “Significant” (defined as a 2-fold increase) effects are seen only at toxic doses (toxicity  
14 data are not given but can be inferred from other mammalian cell data). In addition, since  
15 cytogenetic assays do not require cell survival for scoring, when clastogenic effects are  
16 seen in a population of cells with low survival, it is not possible to determine whether  
17 those cells with chromosome aberrations would be among the survivors, and thus capable  
18 of resulting in a tumor. Examination of chromosomes in tumor and pre-tumor tissues in  
19 the rat bladder model might establish whether specific chromosome aberrations are  
20 associated with DMA<sup>V</sup>-induced bladder cancers.

21  
22 Even model clastogens such as ionizing radiation can also be detected as  
23 mutagens at single gene loci such as HPRT, a useful locus for studying mutations *in vitro*  
24 as well as *in vivo* (unlike TK). Thus, if DMA<sup>III</sup> was really a clastogen acting by ROS, it  
25 should cause increased deletion mutagenesis at the HPRT locus. More research on the  
26 ability of arsenic metabolites to induce gene mutations *in vivo* should be carried out.

27  
28 The fact that (some) antioxidants blocked DMA<sup>V</sup>-induced bladder cancer in the rat  
29 does not provide evidence as to the origin of the oxidants nor where they act. Activation  
30 of NADPH oxidase (Smith et al., 2001) or inhibition of GSH reductase (Styblo et al.,  
31 1997) could increase oxidants. Tissues subjected to continuing cellular assault produce a  
32 number of cytokines whose signaling may be modulated by oxidants (even in the absence  
33 of frank inflammation). Nuclear factor-kappaB (NFkB), which is activated by low-dose  
34 arsenite via oxidants (Barchowsky et al., 1996), is thought to provide a link between  
35 inflammatory signaling and carcinogenesis, as well as providing survival signaling to  
36 block apoptosis in damaged cells that might otherwise die. NFkB is a transcriptional  
37 regulator of genes including cyclooxygenase-2 (COX-2), which is also induced by  
38 arsenite (Trouba and Germolec, 2004). Research is needed to determine whether bladder

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1 cells undergoing stress-related proliferation in the rat DMA<sup>V</sup> carcinogenesis model show  
2 effects on NFkB and other signaling pathways, similar to those seen with arsenite.

3  
4 Given the preponderance of scientific evidence to date (which is reviewed above in  
5 this section), the principal MOA for DMA<sup>V</sup> is not mediated via the ROS-induced DNA  
6 damage pathway. Rather, the MOA is likely to be sustained cytotoxicity followed by  
7 genomic instability as a result of stress-related proliferation. Permanent genetic change is  
8 necessary for carcinogenesis, and it is unlikely that increased proliferation alone in the  
9 absence of increased genomic instability will result in the 3 or more changes needed to  
10 transform a normal cell into a tumor cell. The mechanism of cell killing by DMA<sup>V</sup> or  
11 DMA<sup>III</sup> is not known. Regardless of how the cells die, there is substantial evidence  
12 supporting the hypothesis that continual proliferation of surviving cells under conditions  
13 of stress results in genomic instability (Karpinets and Foy, 2005). In the case of arsenite,  
14 this would involve such factors as (See also Section 3.3.3):

- 15  
16 a) Inducing intracellular proliferative signals and over-riding cell cycle checkpoints  
17 (reviewed in Rossman, 2003)  
18  
19 b) Blocking DNA repair (reviewed in Rossman, 2003)  
20  
21 c) Inhibiting GSH reductase (Styblo et al., 1997) and thioredoxin reductase (Lin et  
22 al., 2001)  
23  
24 d) Inducing stress-related survival signals to block apoptosis (Pi et al., 2005; Wu et  
25 al. 2005)  
26  
27 e) Effects on thiols in tubulin and cytoskeletal proteins, interfering with  
28 microfilament function and cytoskeletal changes (Li et al., 1992; Ling et al., 2002;  
29 Ochi et al., 1999).  
30  
31 f) Affecting DNA methylation levels, (Chen et al., 2004)  
32  
33 g) Inducing oxidant signaling (Barchowsky et al., 1999).  
34  
35 h) Effects on hormone function (Bodwell et al., 2004)  
36

37 More research should be carried out on cells undergoing stress-related proliferation  
38 in the rat bladder model to determine whether these same programs have come into play.

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1 Changes in DNA methylation patterns have just been demonstrated in arsenic-associated  
2 human bladder cancers (Marsit et al. 2006), making this a priority for study in the rat  
3 model.

4  
5 Live cells exposed to the contents of necrotic cells may experience additional  
6 stress signals similar to that seen in the “bystander effect” after ionizing radiation (Iyer  
7 and Lehnert, 2000) or via cytokines from inflammatory cells. Although there is no direct  
8 evidence to support this mechanism in the rat bladder cancer model, it is of interest that  
9 heat-killed *E. coli* instilled into the bladder was found to increase bladder carcinogenesis  
10 by N-methyl-N-Nitrosourea (Yamamoto et al., 1992), presumably by an inflammatory  
11 mechanism. Research on this topic should be carried out both *in vivo* and *in vitro*.  
12 Further, generation of low levels of oxidants from enzymatic sources (Smith et al., 2001)  
13 or possibly by uncoupling of mitochondrial oxidations (if DMA<sup>V</sup> can act in a manner  
14 similar to arsenate) may contribute to effects on cell signaling and transcriptional  
15 activation, as well as increase oxidant DNA damage.

16  
17 The MOA outlined above, as well as the original MOA suggested by EPA, both  
18 depend upon prolonged extensive cytotoxicity in the bladder. Without the continual  
19 cytotoxicity, sustained stress-associated proliferation would not occur. The tumor  
20 response in the rat bladder system is non-linear, as is the key event (i.e. the necessity for  
21 necrotic cytotoxicity). Since the MOA involves cytotoxicity, doses below those causing  
22 cytotoxicity would not be expected to cause tumors. The other events mentioned above  
23 would not be sufficient to cause tumors in the absence of the cytotoxicity and the  
24 resulting proliferative response.

25  
26 In summary, the postulated MOA for DMA<sup>V</sup> is:

27  
28 a) Reductive metabolism of DMA<sup>V</sup> to DMA<sup>III</sup>.

29  
30 b) High concentrations of DMA<sup>III</sup> in urine cause urothelial cytotoxicity. Some  
31 toxicity may also be caused by DMA<sup>V</sup> itself.

32  
33 c) Continuous exposure and persistent stress associated regenerative cell  
34 proliferation leads to genomic instability, acquisition of genetic alterations, clonal  
35 expansion of altered cells and eventually tumors.

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### 3.3.2 Human relevance of animal DMA<sup>V</sup> MOA (Charge Question B2):

EPA states that, “There are little or no scientific data to suggest that if sufficient DMA<sup>III</sup> were present, key precursor events and ultimately tumor formation would not occur in humans directly exposed to DMA<sup>V</sup>” *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<sup>V</sup>.*

If high enough (cytotoxic) concentrations of DMA<sup>V</sup> or DMA<sup>III</sup> were present in the human urine or bladder after exposure to DMA<sup>V</sup>, 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<sup>V</sup>-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<sup>V</sup> 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<sup>V</sup> is converted to TMA<sup>VO</sup>, and possibly TMA<sup>III</sup>, more efficiently by rats than by humans. TMA<sup>VO</sup> is a hepatocarcinogen in rats (Shen et al., 2003). TMA<sup>III</sup> is more potent than DMA<sup>III</sup> in damaging DNA in *in vitro* systems (Andrews et al, 2003). Thus, although acute toxicities of TMA<sup>VO</sup> and TMA<sup>III</sup> are lower than that of DMA<sup>III</sup> (Ochi et al., 1994; Sakurai and Kaise, 1998; Yamauchi et al., 1990), these metabolites could contribute to the MOA for DMA<sup>V</sup>-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<sup>V</sup>.

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<sup>V</sup>-induced carcinogenesis, or whether there is greater or lesser risk during any other life stages.

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### 3.3.3. Modes of carcinogenic action from exposure 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 strictest (and original) definition, genotoxic carcinogens (or their metabolites) damage DNA by covalently binding to DNA or intercalating into the DNA-helix. 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. However, some of these events, as well as others, might also be classified as “indirect genotoxicity” (Table 1).

Indirect genotoxicity can be defined as interactions with non-DNA targets leading to genotoxic effects. Unlike DNA, non-DNA targets such as proteins exist in many copies per cell. Thus, a single “hit” is unlikely to cause a significant biological effect. Indirectly genotoxic agents should therefore have a threshold concentration below which there is no effect. A minimal concentration of agent would be needed to significantly impact non-DNA targets.

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1

**Table 1. Some potential mechanisms for indirect genotoxicity**

Potential Mechanisms
*Interference with DNA repair
*Interference with cell cycle control proteins
Interference with DNA replication
*Blocking apoptosis of cells with DNA damage
*Change in reactive oxygen species leading to altered signaling
*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

\*Effects found with exposure to iAs.

2

3

4

The genetic toxicology of iAs<sup>III</sup> has been previously reviewed (Rossman, 1998, 2003; Basu et al., 2001). However, the interpretation of the genotoxicity of arsenic compounds is problematic owing to the unrealistically high cytotoxic concentrations used in many studies and the lack of analysis for statistical significance. Part of the problem stems from inappropriate (or absent) assessment of toxicity of arsenic compounds (Komissarova et al., 2005). Low concentrations (even 1  $\mu$ M) of iAs<sup>III</sup> can cause apoptosis in human cells that can only be detected 48 hours (or more) after exposure. 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) are reported for dead or dying cells. A genotoxic effect can only be a MOA if it occurs in living cells and if the genotoxic effect is 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.

18

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1           Arsenite, i.e.,  $iAs^{III}$  (and other arsenicals) do not exhibit direct DNA binding. The  
2 inability of  $iAs^{III}$  to induce the SOS system in *E. coli* is consistent with its lack of reaction  
3 with DNA (Rossman et al., 1984). In mammalian cells, toxic concentrations of both  
4 inorganic and organic arsenic compounds *in vitro* cause chromosome breakage, possibly  
5 mediated by ROS-induced DNA strand breaks. Cellular DNA strand breakage and  
6 clastogenicity are limited almost exclusively to trivalent species. Unlike many other  
7 carcinogens,  $iAs^{III}$  is an extremely weak (or insignificant) mutagen at single gene loci  
8 such as HPRT or TK in mammalian cells (Rossman, 1998, 2003). However,  $iAs^{III}$  (but  
9 not  $MMA^{III}$ ) at low (nontoxic) concentrations can induce delayed indirect mutagenesis at  
10 the HPRT locus after >15 generations as a secondary result of genomic instability (Mure  
11 et al., 2003).

12  
13           The argument has been made that arsenite is a clastogen that causes  
14 predominantly multilocus deletions, and that such deletions near the HPRT locus (which  
15 is located on a single active X chromosome) may be lethal, accounting for the lack (or  
16 extremely weak) mutagenesis by arsenicals at the HPRT locus (Hei et al., 1998).  
17 However, molecular analysis of mutations in the HPRT gene show that large deletions  
18 (up to ~3.5 Mb) can be tolerated in the HPRT region of the human X chromosome  
19 (Nelson et al., 1995; Lippert et al., 1995). Despite this, attempts have been made to find  
20 genetic markers more likely to detect large deletions. Also,  $iAs^{III}$  was an insignificant  
21 mutagen, and only at toxic doses, in transgenic Chinese hamster G12 cells (Li and  
22 Rossman, 1991), which can detect clastogens causing deletions in the single copy of the  
23 *E. coli gpt* gene inserted into a Chinese chromosome 1 in V79 cells. Similar results  
24 (extremely weak mutagenesis at toxic arsenite concentrations) are seen in mouse  
25 lymphoma cells, which can tolerate deletions at the TK locus due to its autosomal  
26 location (Moore et al., 1997), at the transgenic *gpt* locus of AS52 Chinese hamster ovary  
27 cells (Meng and Hsie, 1996) and in AL cells, which are CHO-K1 cells containing a single  
28 copy of human chromosome 11 (Hei et al., 1998).

29  
30           *In vivo*,  $iAs^{III}$  induced micronuclei (MN) in mouse peripheral blood lymphocytes  
31 and in mouse bone marrow (Tinwell et al., 1991; Noda et al., 2002). Humans exposed to  
32  $iAs^{III}$  show increased MN and sometimes chromosome aberrations in lymphocytes,  
33 exfoliated bladder epithelial cells, and buccal epithelial cells (reviewed in Basu et al.,  
34 2001). *In vivo* studies on genotoxic activity of methylated arsenic species are limited to a  
35 small number of studies in rodents. IP injections of high doses of  $DMA^V$  induced a slight  
36 but insignificant increase in mutagenesis in the Muta<sup>TM</sup> Mouse lung, but not in bladder or  
37 bone marrow. Also,  $iAs^{III}$  was negative in this assay (Noda et al., 2002). High  
38 concentrations of  $DMA^V$  administered orally to mice caused oxidative damage and DNA

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1 strand breaks in the lung (not a target organ for DMA<sup>V</sup> carcinogenesis). The strand  
2 breaks were attributed to dimethylarsine, via the dimethylarsenic peroxy radical  
3 (CH<sub>3</sub>)<sub>2</sub>AsOO· (Yamanaka and S. Okada, 1994). DMA<sup>V</sup> also induced aneuploidy in  
4 mouse bone marrow cells (Kashiwada et al., 1998).

5  
6 Micronuclei are induced by iAs<sup>III</sup> *in vivo*, and MN frequency is increased in  
7 humans exposed to iAs<sup>III</sup> in drinking water. Because of this, it is important to consider  
8 the significance of MN for MOA. MN are defined as small, round, DNA-containing  
9 cytoplasmic bodies formed during cell division by loss of either acentric chromatin  
10 fragments or whole chromosomes. The two basic phenomena leading to the formation of  
11 MN are chromosome breakage (double strand breaks associated with clastogenesis) and  
12 dysfunction of the mitotic apparatus leading to aneuploidy (change in chromosome  
13 number from the normal diploid or haploid number other than an exact multiple). MN as  
14 a result of clastogenesis contain acentric chromosome or chromatid fragments while MN  
15 associated with aneuploidy contain whole chromosomes. Currently, the most widespread  
16 and reliable assay to identify whole chromosomes in MN is by fluorescent label of their  
17 kinetochores (with antibodies) or their centromeres (with DNA probes). However, only a  
18 few laboratories routinely use these techniques because they are very costly. In most  
19 studies, there is not enough information to determine whether the MN result from: 1)  
20 toxicity, 2) clastogenicity, or 3) non-dysjunction (leading to aneuploidy). Also, MN in  
21 cells trigger apoptosis, so many cells with MN will have no progeny.

22  
23 In a study of 18 arsenic-exposed individuals (average 1,312 µg arsenic/L  
24 drinking water) and 18 matched controls (16 µg/L), the exposed group had a 1.65-fold  
25 increase in MN with acentric fragments (p=0.07) and a 1.37-fold increase in MN with  
26 whole chromosomes (p=0.15) (Moore et al., 1996). The combined difference (1.8-fold)  
27 was significant. Thus, exposure to iAs<sup>III</sup> induces MN by multiple mechanisms. In  
28 normal human fibroblasts, low dose, long term exposure to iAs<sup>III</sup> is aneugenic, inducing  
29 MN with whole chromosomes, but high dose, short term exposure is clastogenic,  
30 inducing MN with chromosome fragments (Yih and Lee, 1999). Evidence supports an  
31 aneugenic role for iAs<sup>III</sup> in many other cells at concentrations lower than those causing  
32 chromosome aberrations (Kochhar et al., 1996; Vega et al., 1995; Ramirez et al., 1997;  
33 Huang and Lee, 1998; Moore et al., 1997; Sciandrello et al., 2002). Of importance is the  
34 association of aneuploidy with malignant transformation induced by arsenite and DMA<sup>III</sup>  
35 (Ochi et al., 2004; Chien et al., 2004). Aneuploidy is an event that has a threshold  
36 (Kirsch-Volders et al., 2002), whereas many people assume that clastogenesis does not  
37 (at least for ionizing radiation) even though repair of radiation-induced DNA damage  
38 exists. The development of aneuploidy is a marker of genomic instability and is typical

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1 of many tumors. IP injection of DMA<sup>V</sup> in mice induced aneuploidy, but no chromosome  
2 aberrations, in bone marrow (Kashiwada et al., 1998). (DMA<sup>V</sup> would be converted to  
3 DMA<sup>III</sup> in the mouse, so the active agent may be DMA<sup>III</sup>). Bladder tumors in patients  
4 with high iAs<sup>III</sup> exposure showed higher levels of aneuploidy compared with other  
5 bladder tumors (Moore et al., 2002).

6  
7 Genomic instability can result from changes in DNA methylation in iAs<sup>III</sup>-treated  
8 cells. The first report of arsenite inducing DNA methylation changes was the increased  
9 cytosine methylation in the p53 promoter in human adenocarcinoma A549 cells (Mass  
10 and Wang, 1997). Later it was found that there was both hypo- and hypermethylation (of  
11 different genes) in human kidney UOK cells treated with iAs<sup>III</sup> (Zhong and Mass, 2001).  
12 When SHE cells or rat liver TRL1215 cells were transformed by iAs<sup>III</sup>, specific  
13 oncogenes were more highly expressed due to hypomethylation (Zhao et al., 1997;  
14 Takahashi et al., 2002) and there was evidence of decreased DNA methyltransferase  
15 activity (Zhao et al., 1997). These findings are consistent with the usual DNA  
16 methylation changes observed in cancer, in which global methylation is reduced but some  
17 gene specific promoter methylation is increased (Baylin and Herman, 2000). Arsenite  
18 (iAs<sup>III</sup>) -induced DNA hypomethylation and altered gene expression has been  
19 demonstrated in mouse liver (Chen et al., 2004), in hepatocellular carcinoma derived  
20 from transplacental iAs<sup>III</sup> exposure (Waalkes et al., 2003), and in prostate epithelium  
21 where the hypomethylation was shown to activate K-ras (Benbrahim-Tallaa, et al., 2005).  
22 In a study of iAs<sup>III</sup> exposed individuals in India, increased levels of hypermethylated p16  
23 and p53 gene promoters were seen in blood DNA (Chanda et al., 2006). Methylated CpG  
24 sites are mutational hotspots (e.g. by a second agent), methylation changes affect gene  
25 expression, and hypomethylation leads to genomic instability. Low concentrations of  
26 iAs<sup>III</sup> induce delayed mutagenesis and chromosome aberrations that might be mediated  
27 by hypomethylation (Mure et al. 2003; Sciandrello et al., 2004). This is a mechanism  
28 that might also explain transplacental carcinogenesis.

29  
30 There are a number of non-genotoxic actions of arsenite (and perhaps MMA<sup>III</sup> and  
31 DMA<sup>III</sup>) that can contribute to the carcinogenic process. The role of ROS in (low dose)  
32 arsenic carcinogenesis is probably via signaling changes rather than as a genotoxicant  
33 (otherwise, one would expect more mutagenesis). This may contribute to carcinogenesis,  
34 but it is not the MOA for carcinogenesis. Cell signaling can be affected by arsenite via  
35 low levels of oxidants that do not cause DNA damage (reviewed in Simeonova and  
36 Luster, 2004). Low iAs<sup>III</sup> concentrations increased oxidant signaling and oxidant-  
37 dependent activation of nuclear factor kB (NFkB) in the absence of DNA damage in  
38 human endothelial cells (Barchowsky et al., 1999). The increased oxidants appear to

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1 result from activation of membrane-bound NAD(P)H oxidase. Arsenite ( $iAs^{III}$ )-induced  
2 signaling results in expression of inflammatory cytokines such as IL-8 that can mediate  
3 atherogenesis (Simeonova and Luster, 2004). Arsenite ( $iAs^{III}$ ) also increases ROS by  
4 promoting an inflammatory response in many tissues. In addition, there is redox  
5 chemistry involved in  $iAs^{III}$ -dependent signaling in that arsenite binds to protein thiols  
6 (particularly vicinal thiols) to stimulate signaling cascades or affect DNA repair.  
7 Arsenite ( $iAs^{III}$ ) interaction with thiols is a redox reaction, but oxygen radicals are not  
8 involved.

9  
10 Exposure to low, non-toxic doses of  $iAs^{III}$  enhances positive growth signaling  
11 (reviewed in Rossman 2003), which can readily contribute to hyperplastic pre-cancerous  
12 skin growth. Arsenite ( $iAs^{III}$ ) can disrupt glucocorticoid receptor (GR) and other steroid  
13 signaling at very low doses, and it has been suggested that these effects on GR may affect  
14 carcinogenesis (Kaltreider et al., 2001). This may also affect other disease processes,  
15 such as cardiovascular disease, diabetes and other diseases that have been associated with  
16  $iAs$  exposure, since GR and other steroid receptors have been shown to be important in  
17 these diseases as well.

18  
19 Animal studies indicate that for some organs, transplacental carcinogenesis after  
20 maternal exposure to  $iAs^{III}$  occurs. This includes the formation in C3H mice of tumors of  
21 the lung and liver, target sites of potential human relevance, after exposure to arsenic *in*  
22 *utero*. In addition, *in utero* arsenic induces tumors of the ovary and adrenal, sites not  
23 observed in humans to date. The C3H mouse was selected in these studies because it is,  
24 in general, sensitive to chemical carcinogenesis, although this strain shows spontaneous  
25 tumor formation in several tissues. Recent work has shown that with gestational exposure  
26 to CD1 mice, inorganic arsenic is a complete carcinogen in the female offspring  
27 (Waalkes, *et. al.*, 2006). The CD1 mouse strain is noteworthy as having a well defined,  
28 low rate of spontaneous tumors. The document should take note of this important  
29 development. Other studies indicate that in skin, neither  $iAs^{III}$  nor  $iAs^V$  are complete  
30 carcinogens, but they act as enhancers (cocarcinogens, sometimes mistakenly called  
31 “promoters”) with other agents. Arsenite ( $iAs^{III}$ ) acts as a cocarcinogen with solar UV  
32 light (Rossman et al. 2001; Burns et al., 2004) and arsenate is cocarcinogenic with 9,10  
33 dimethyl-11-2-benzanthracene (Motiwale et al., 2005). This leaves open the possibility  
34 that a cocarcinogenic MOA may also operate for other organs, but this remains to be  
35 tested.

36  
37 Arsenite ( $iAs^{III}$ )-enhanced UV carcinogenesis could result from acquired  
38 resistance to UV-induced apoptosis. Such resistance was recently demonstrated in

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

10  
 11 Table 2 lists some activities of  $iAs^{III}$  (or its metabolites) that might explain how  
 12  $iAs^{III}$  can act as a transplacental carcinogen and a cocarcinogen but not a complete  
 13 carcinogen in neonatal and older animals.

14  
 15 In the future, it will be important to determine whether the trivalent arsenic  
 16 metabolites can induce tumors *in vivo* or transform or mutate keratinocytes and other  
 17 major target cells of arsenic at biologically relevant concentrations. The mechanism of  
 18  $iAs$ -induced carcinogenesis is likely to be different in different tissues, with contributions  
 19 from all species present in that tissue.

20  
 21 **Table 2: Activities of  $iAs^{III}$  that may contribute to its**  
 22 **cocarcinogenic and/or transplacental carcinogenic action.**  
 23

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 affects  
 Delayed mutagenesis (not enough generations *in vivo* but  
 maybe enough if transplacental or added to a genotoxic  
 agent?)

24  
 25

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1           There have been a number of reports claiming an essential role for iAs in various  
2 animal species (chick, goat, hamster, pig, rat), but many of these reports exist only in  
3 abstracts or in meeting reports (reviewed in Uthus, 1992; Nielson, 1996). Reports in the  
4 peer-reviewed literature follow: A study of arsenic-deprived goats found muscle atrophy,  
5 reduction in oxidative enzymes and abnormal mitochondria in muscle, possibly via  
6 disturbance of calcium metabolism (Schmidt et al., 1984). Studies by Uthus (1990, 1992,  
7 and 2003) suggest that iAs has a role in methionine/methyl metabolism. Arsenic (iAs)-  
8 deprived rats had decreased plasma taurine, hepatic polyamines, and S-  
9 adenosylmethionine decarboxylase (needed for polyamine synthesis). Arsenic (iAs)-  
10 deprivation as well as iAs excess caused DNA hypomethylation in rat liver. The same  
11 effect was seen in Caco-2 cells. Arsenic (iAs) deprivation or excess also increased the  
12 formation of aberrant colon crypts in rats treated with dimethyl hydrazine (Uthus and  
13 Davis, 2005), suggesting a cocarcinogenic effect. So far, no exact biochemical  
14 mechanism linking any iAs species with methionine/methyl metabolism has been found,  
15 but the fact that many laboratories have reported effects of iAs<sup>III</sup> on DNA methylation  
16 makes this an important area of study.

17  
18           Hormetic effects of iAs (beneficial effects at very low doses) have also been  
19 suggested and need further investigation even if arsenic should not be essential. In cell  
20 culture, subtoxic concentrations of iAs<sup>III</sup> reduced the levels of ROS in keratinocytes and  
21 fibroblasts by upregulating thioredoxin and glutathione reductase (Snow et al., 2005).  
22 Some DNA repair proteins are also upregulated. The same paper also describes  
23 protection of mice from skin tumors induced by dimethylbenzanthracene + phorbol 12-  
24 tetradecanoate 13-acetate if the mice were given drinking water containing 0.2-2 ppb  
25 arsenate. Inorganic arsenic (iAs) has both positive and negative effects on the growth  
26 and function of blood vessels (Soucy et al., 2003, 2005; Kamat et al., 2005). Low  
27 concentrations fuel angiogenesis, while higher concentrations injure endothelial cells and  
28 promote the vessel dysfunctions seen in ischemic diseases and peripheral vascular  
29 diseases. Thus at low levels iAs may provide improved vascularization and growth of  
30 normal tissues, which could reduce cardiovascular risks. However, this process could  
31 also pose risks for iAs increasing the vascularization and growth of both atherosclerotic  
32 lesions (Simeonova and Luster, 2004) and tumors from a secondary source (Kamat et al.,  
33 2005). Mice drinking 10-250 ppb iAs<sup>III</sup> had increased metastases from transplacental  
34 tumors (Kamat et al., 2005). However, iAs at high doses has been used to destroy the  
35 tumor vasculature (Griffin et al., 2003).

36  
37           If iAs is essential or hormetic for humans and/or if epidemiological data could be  
38 strengthened at the low-dose range to demonstrate either a low-dose benefit or no effect

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1 at low dose, then a threshold is certain. However, at this time, while the mechanistic  
2 studies suggest that there should be a threshold, the epidemiological data are lacking or  
3 problematic with regard to low-dose effects. This is an extremely important issue and  
4 should be investigated.

### 6 **3.4. Selection of Data for Dose-Response Assessment**

#### 8 **3.4.1. Use of animal data for DMA<sup>V</sup> (Charge Question C1)**

9  
10 EPA's Charge stated that, "A number of different rodent bioassays (standard  
11 bioassay, transgenic animals, susceptible rodent strains, initiation and promotion studies)  
12 are available on DMA<sup>V</sup>." *Charge Question C1 asks the SAB to "...comment on the use of*  
13 *the bladder tumor data from the DMA<sup>V</sup> rat bioassay as the most suitable dataset for quantifying*  
14 *potential human cancer risk to DMA<sup>V</sup>, including the weight of evidence to support this*  
15 *conclusion.*

16  
17 The consensus of the panel is that, given the lack of human data, the bladder  
18 tumor data from the DMA<sup>V</sup> rat bioassay is the most suitable data set for quantifying  
19 potential human cancer risk to DMA<sup>V</sup>. Given the differences in metabolic fates of  
20 DMA<sup>V</sup> and iAs, the use of human data from iAs exposure to predict risk from DMA<sup>V</sup> is  
21 not recommended. In this case, reliance on interspecies extrapolation using the rat  
22 bioassay data is the best alternative.

23  
24 This question indirectly raises the issue as to the largest source of uncertainty for  
25 DMA<sup>V</sup> risk assessment—conventional interspecies extrapolation or extrapolation across  
26 various forms of arsenic. The available material suggests that extrapolation across  
27 various forms of arsenic would lead to the greatest degree of uncertainty in a risk  
28 assessment. Although the panel agreed that use of the rat DMA<sup>V</sup> bioassay data is the  
29 preferred method, the panel also felt strongly that a discussion of the key uncertainties  
30 with using data from testing in rats to conduct human risk assessment should be included  
31 in EPA's Office of Pesticide Programs report "*Science Issue Paper: Model of*  
32 *Carcinogenic Action for Cacodylic Acid (Dimethylarsinic Acid, DMA<sup>V</sup>) and*  
33 *Recommendations for Dose Response Extrapolation.*" Issues that panel members  
34 consider important to discuss in EPA's Science Issue Paper are discussed in more detail  
35 below and in Section 3.2.1. These issues relate to the pharmacokinetic and  
36 pharmacodynamic similarities and differences between rats and humans in response to  
37 arsenic exposure, the use of rodent bladder tumor models in general, and issues in the use  
38 of rodent data for human risk assessment. The panel also recommends that the EPA

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1 consider applying the Human Relevance Framework (HRF) proposed by the International  
2 Life Sciences Institute-Risk Science Institute (ILSI-RSI) (Seed, et al, 2005) to the mode  
3 of action. Application of the framework or its elements may assist EPA in evaluating the  
4 human relevance of the DMA<sup>V</sup> rat data. This framework has been used to assess the  
5 relevance of rodent liver tumors to human cancer risk (Holsapple et al, 2006).

6  
7 Several pharmacokinetic differences between rats and humans have been reported  
8 after arsenic exposure. For example, arsenic methylation in rat hepatocytes proceeds at a  
9 faster rate than in human hepatocytes (Styblo, et al., 1999). Additionally, rats have a  
10 considerably slower whole body clearance of DMA<sup>V</sup> compared with humans. This  
11 slower whole body clearance in rats results from a significant portion of DMA being  
12 retained in the erythrocytes of rats (Vahter, et al., 1984). The affinity of rat hemoglobin  
13 to bind DMA<sup>III</sup> is 15 to 20 fold higher than that of human hemoglobin (Lu, et al, 2004).  
14 These differences in metabolism and pharmacokinetics may be consistent with a greater  
15 sensitivity of the rat to induction of bladder tumors by DMA<sup>V</sup>. However, without a more  
16 complete data set demonstrating that exposure of the bladder epithelium (urothelial cells)  
17 to DMA<sup>V</sup> metabolites (particularly DMA<sup>III</sup>) is greater in rats than in humans for a given  
18 dose, the data are not sufficient to support reduction of interspecies uncertainty factors  
19 based on differences in pharmacokinetics. Clearly, this is a high priority area of research  
20 with the potential to reduce uncertainty in the risk assessment of DMA<sup>V</sup>. Likewise,  
21 laboratory animal studies have shown that DMA<sup>V</sup> is not absorbed well -- approximately  
22 80% of a dose of the parent compound is excreted in a short time after exposure (Buchet,  
23 et al., 1981; Marafante, E., et al., 1987). The extent of DMA<sup>V</sup> absorption by humans  
24 relative to rats is not known.

25  
26 In the EPA Science Issue Paper consideration should be given to the  
27 pharmacodynamic similarities and differences between rats and humans and the  
28 relevance of the rat response to human risk assessment. Although data illustrating the  
29 mode of action for DMA<sup>V</sup> as a bladder carcinogen in rats seem quite convincing, it  
30 should be noted that rats are more sensitive to DMA<sup>V</sup> in carcinogenicity testing than are  
31 mice (Rossman, 2003; Arnold, et al., 2003). While the relative *in vivo* sensitivities of rats  
32 and humans to DMA<sup>III</sup> are unknown, it has been shown that *in vitro* rat and human  
33 urothelial cell lines are equally sensitive in terms of acute toxicity to DMA<sup>III</sup> in the  
34 micromolar range (Cohen et al., 2002). For arsenite, however, the rat MYP3 urothelial  
35 cell line showed toxicity at about one tenth (LC<sub>50</sub> of 0.4 uM) the concentration as did the  
36 human 1T1 urothelial cell line (LC<sub>50</sub> of 4.8 uM). As a result of the Arsenic Review  
37 Panel's analysis of the data for this key pharmacodynamic response, urothelial cell  
38 cytotoxicity, the consensus was the EPA could assemble a case for pharmacodynamic

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1 equivalency between the test species, rats, and humans from existing experimental data.  
2 Pharmacodynamic equivalency could be incorporated in the assessment as a reduction of  
3 the pharmacodynamic component of the interspecies uncertainty factor, which is 3, to a  
4 value of one. The application of uncertainty factors has also been addressed in the  
5 Panel's response to question D1. The final EPA risk assessment should fully discuss the  
6 interspecies similarities and differences and the implications for risk assessment as well  
7 as explore opportunities to reduce uncertainty factors.

8  
9 EPA's Science Issue Paper should discuss similarities and differences between  
10 rats and humans in the development of bladder tumors and how these differences impact  
11 interspecies extrapolation. Studies suggest that in rats it takes two or more years of  
12 continuous high dose exposure to DMA<sup>V</sup> to induce these tumors. Human bladder tumors  
13 are also late occurring. The Science Issue Paper should specifically discuss the  
14 similarities and differences in the time for induction of DMA<sup>V</sup> related tumors in rats with  
15 the pattern observed with humans and arsenic associated urinary bladder cancer.

16  
17 EPA's Science Issue Paper should also discuss general issues associated with rat  
18 urinary bladder cancer. One such issue is the relationship between the induction of  
19 tumors and high concentrations of arsenic in the urine. Also, there is a need to address  
20 evidence that simple enhancement of proliferation is not associated with carcinogenesis  
21 in many tissues. Studies by Gur et al. (listed on page 97 of the DMA MOA Science Issue  
22 Paper) on the carcinogenicity of DMA<sup>V</sup> were never published and thus cannot be  
23 critically evaluated by the Panel. The Science Issue Paper notes that the Gur studies in  
24 rats and mice are key bioassay studies. Reliance on these studies would be stronger if the  
25 studies had the benefit of peer review.

26  
27 The EPA's Science Issue Paper expresses concern with the mouse transplacental  
28 model for inorganic arsenic because the strain of mice used (namely C3H) in the original  
29 two studies had a significant rate of spontaneous tumors in several tissues that are also  
30 targets of arsenic. Recent follow-up work has shown that gestational exposure to  
31 inorganic arsenic in CD1 mice is a complete carcinogen in the female offspring  
32 (Waalkes, *et al.*, 2006). The CD1 mouse strain is noteworthy as having a well defined,  
33 low rate of spontaneous tumors. The Science Issue Paper should take note of this  
34 important development.

35  
36 *Charge Question C1.B also asks the SAB to "...comment on whether the iAs*  
37 *epidemiology data can be used to inform the DMA<sup>V</sup> dose-response assessment derived*

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1 *from rat data with DMA<sup>V</sup>. If so, please discuss how such information might be used.*  
2 *(See Appendix).*  
3

4 The panel consensus was that without more detailed information on target tissue  
5 dosimetry of arsenic species the iAs epidemiology data would be of limited use to inform  
6 the DMA<sup>V</sup> dose-response assessment derived from rat data with DMA<sup>V</sup>. Direct exposure  
7 to iAs elicits a different cascade of metabolite concentrations with related differential  
8 kinetics compared to direct exposure to DMA<sup>V</sup>, therefore the iAs epidemiology data  
9 cannot reasonably be used to inform the DMA<sup>V</sup> dose-response assessment derived from  
10 rat data with DMA<sup>V</sup>. In the absence of specific information on target tissue levels,  
11 assumptions would have to be made regarding the proportion of the iAs for human and  
12 DMA<sup>V</sup> for rodents that reaches the bladder tissue as the toxic DMA species.  
13

14 In principle, epidemiology data from iAs exposed humans could be used to  
15 inform the DMA assessment to the extent that the data might be able to address the  
16 appropriateness of interspecies extrapolation, specifically the relative sensitivities of rat  
17 and human to bladder cancer following arsenic exposure. However, as noted above, in  
18 order to be useful some information on target tissue dose of DMA following human  
19 exposure to iAs and rodent exposure to DMA<sup>V</sup> would be necessary. With both *in vivo*  
20 tumor indices (human and rodent) expressed in terms of the same tissue dose of relevant  
21 metabolites, rather than iAs or DMA<sup>V</sup> exposure levels, the relative sensitivities of the  
22 human and rodent could be assessed.  
23

#### 24 **3.4.2. Use of human epidemiological data from direct iAs exposure** 25

26 Use of human epidemiological data from direct iAs exposure (Charge Question  
27 C2) EPA's Charge states that, "Since the NRC (2001) report on iAs, an additional body  
28 of literature has developed describing epidemiology data from populations in the US  
29 exposed to iAs in drinking water" (USEPA, 2005a). *Charge Question 2 asks, "Does the*  
30 *SAB agree that the Taiwanese dataset remains the most appropriate choice for estimating*  
31 *cancer risk in humans? Please discuss the rationale for your response."*  
32

33 It is the Panel's view that, at this time, the Taiwanese database remains the most  
34 appropriate choice for deriving the cancer unit risk; however, the Panel suggests that EPA  
35 also conduct adjunct analyses to test the robustness of results against their assumptions, determine  
36 the impact of variability in some parameters, compare the results against those from other data  
37 sets, and provide a transparent assessment of the available epidemiological data using a consistent  
38 set of criteria.

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1  
2 The Taiwanese dataset consists of population and mortality data from 42 villages in  
3 southwest Taiwan for the years 1973-1986. Arsenic levels in wells from these villages  
4 were measured in 1964-1966. The database is one of the largest that has been evaluated  
5 for cancer risk relative to arsenic exposures. A total of almost 900,000 person years of  
6 follow-up were included, with 1,152 cancer deaths (637 males, 515 females). Among the  
7 cancer deaths were 181 due to bladder cancer (85 males, 96 females), 268 lung cancer  
8 (147 males, 121 females), and several hundred due to other types of cancer. These data  
9 have been subject to several ecologic analyses, starting with the original publications by  
10 Chen et al. (1988) and Wu et al. (1989), followed by further analyses by Morales et al.  
11 (2000) and by the National Research Council (1999 and 2001).  
12

13 Among the 42 villages, the arsenic concentration ranged from 10 to 934 ppb ( $\mu\text{g/L}$ ).  
14 Twenty of these 42 villages used a single well. Among many of the 22 villages with  
15 multiple wells, many had wide variability in the measured arsenic level in their wells.  
16 Analyses using the full dataset give results comparable to results from a reduced dataset  
17 including only the villages with single wells, providing some confidence in the stability  
18 of the overall results (National Research Council, 1999). The Panel recognizes the  
19 limitations of the southwest Taiwan database, including its ecologic character, lack of  
20 smoking information, limited precision of exposure estimates, especially among villages  
21 with multiple wells, and the possible issue of compromised nutrition among segments of  
22 the exposed population. However, in view of the size and statistical stability of the  
23 database relative to other studies, the reliability of the population and mortality counts,  
24 the stability of residential patterns, and the inclusion of long-term exposures, it is the  
25 Panel's view that this database remains, at this time, the most appropriate choice for  
26 estimating cancer risk among humans. Supporting this view is the fact that the datasets  
27 from Taiwan have been subjected to many years of peer review as part of published  
28 studies.  
29

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

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1 set to: 1) a low level concentration from the range for the village (10<sup>th</sup> percentile, 20<sup>th</sup>  
2 percentile); 2) the median (current procedure); and 3) a high level concentration from the  
3 village range (90<sup>th</sup> percentile, 80<sup>th</sup> percentile).  
4

5 In view of the limitations of this database, the Panel recommends that other  
6 epidemiologic databases from studies of arsenic-exposed populations be used to compare  
7 the unit risks at high exposure levels that emerge from the Taiwan data. Several of these  
8 studies had the advantage of data with excellent exposure assessment. In addition, some  
9 populations likely differed from the Taiwanese population with regard to their nutritional  
10 status. The accuracy and precision of exposure assessment is a major issue in all  
11 environmental epidemiologic studies, and in particular, in studies of arsenic in drinking  
12 water. Misclassification of exposure in such studies (when non-differential) can have a  
13 profound effect in attenuating the magnitude of the observed risk. The excellence of  
14 exposure assessment is an especially strong aspect of several studies from northern Chile,  
15 and the Panel recommends that the findings of Smith et al. (1998) and of Ferreccio et al.  
16 (2000) be included by EPA in evaluation of other datasets as described below. In  
17 addition, arsenic exposures appear to be well characterized in cohort studies of Chiou et  
18 al.(2001) of transitional cell carcinoma (mostly bladder cancers) and Chen et al. (2004) of  
19 lung cancer, from arsenic-exposed cohorts in southwest and northeast Taiwan. The latter  
20 study also provides data on the joint effects of arsenic and cigarette smoking in the  
21 Taiwanese population.  
22

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

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1 data. The Panel suggests that if findings from a critical review of “low-level” studies  
2 indicate that some or all studies are potentially of value in further analyses, that results  
3 from these studies should be explored in secondary analyses, particularly on bladder  
4 cancer risk, and compared with the main analysis for concordance. Analyses integrating  
5 health outcome information from a number of epidemiology studies can result in  
6 improved statistical power and precision of the estimates; these factors represent an  
7 additional advantage of utilizing a larger dataset.

8  
9 When reviewing these “low-level” studies (and the “high level” studies as well),  
10 EPA should consider at least the following issues: Estimates of the level of exposure  
11 misclassification; temporal variability in assigning past arsenic levels from recent  
12 measurements; the extent of reliance on imputed exposure levels; the number of persons  
13 exposed at various estimated levels of waterborne arsenic; study response/participation  
14 rates; estimates of exposure variability; control selection methods in case-control studies;  
15 and the resulting influence of these factors on the magnitude and statistical stability of  
16 risk estimates. Most populations in the U.S. and many other countries differ from the  
17 Taiwanese population of interest in genetic background (e.g., genetic polymorphisms),  
18 dietary intake, and background exposure concentrations to inorganic arsenic, and if one  
19 or more of these studies are shown to be of potential utility, comparative analyses of the  
20 U.S. and Taiwan data may lead to further insights into the possible influence of these  
21 differences on population responses to arsenic in drinking water. For compounds such as  
22 arsenic for which there are human data beyond the Taiwanese study on which human  
23 cancer risk has been based, data from the other investigations at high exposure levels  
24 (>150 ug/l) can be used to gauge the Taiwanese findings [Smith et al.(1998), Ferreccio et  
25 al.(2000), Chen et al.(2004), Chiou et al.(2001)].

26  
27 All of these studies, including those from Taiwan, Chile, Argentina and the U.S.  
28 as described above, should be judged by the same set of criteria, with the comparative  
29 assessment of those criteria across studies clearly laid out in a tabular format. Some of  
30 the criteria have been listed in the previous paragraph. The relative strengths and  
31 weaknesses of each study need to be described in relation to each criterion. The caveats  
32 and assumptions used should be presented so that they are apparent to anyone who uses  
33 the data. Included in the risk assessment background document should be a complete and  
34 transparent treatment of variability within and among studies and how it affects risk  
35 estimates. The present lack of transparency in the application of the criteria in the  
36 process of study selection was pointed out by several panel members.

37

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1            *Charge Question C2* also asks, “Do these data provide adequate characterization  
2 of the impact of childhood exposure to iAs? Please discuss the rationale for your  
3 response.”  
4

5            The Taiwanese data are inadequate to characterize the impact of childhood  
6 exposure to inorganic arsenic with respect to carcinogenesis. That is, it is not clear  
7 whether children differ from adults with regard to their sensitivity to the carcinogenic  
8 effects of arsenic in drinking water. More data are needed to fully characterize the impact  
9 of transplacental exposures. However, data from the studies in Southwestern Taiwan  
10 which include childhood exposures in the calculation of lifetime dose show that in the  
11 population under 30 years of age there were no bladder cancer cases, and only 5 lung  
12 cancer cases but few cases are actually expected in that age group. Childhood exposures  
13 are included in the lifetime dose estimates. Smith et al (1998) report the highest excessive  
14 risk for male lung cancer in the 30-39 year old age group, suggesting the importance of  
15 childhood exposure and risk and perhaps smoking behavior as young adults. For 533  
16 women exposed to arsenic in drinking water from tube wells at greater than 50 µg/L  
17 compared with those exposed at 50 µg/L, or less, findings suggest that there are  
18 significantly increased odds ratios for spontaneous abortion, stillbirth and neonatal death  
19 (Milton et al., 2005). Another reproductive study in Chile, which followed over 800  
20 pregnancies, found that pregnant women drinking water containing 40 µg/L gave birth to  
21 infants of lower birth weight than a comparable group drinking water containing very low  
22 arsenic concentrations (<1 µg/L) (Hopenhayn et al, 2003). Thus maternal exposure at  
23 moderately high levels may have untoward toxicity effects; the issue of childhood  
24 carcinogenic susceptibility has had only limited study.  
25

### 26            **3.5. Approaches to Low-Dose Extrapolation for Inorganic Arsenic and 27            DMA<sup>V</sup>**

#### 28 29            **3.5.1. Mode of carcinogenic action understanding for DMA<sup>V/III</sup> and 30            implications for dose response extrapolation to estimate human 31            cancer risk (Charge Question D1).** 32

33            EPA’s Charge stated that, “The use of mode of action data in the assessment of  
34 potential carcinogens is a main focus of EPA’s 2005 cancer guidelines. As stated in  
35 th[o]se guidelines ‘The approach to dose-response assessment for a particular agent is  
36 based on the conclusion reached as to its potential mode(s) of action.’ Although a  
37 biologically-based model is the preferred approach to estimating cancer risk, there are  
38 insufficient data on DMA<sup>V</sup> to support development of such a model.” *Charge Question*

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1 *DI asks the SAB to “...comment on the scientific evidence and biological rationale in*  
2 *support of nonlinear versus linear low dose extrapolation approaches, which approach is*  
3 *more consistent with the available data on DMA<sup>V</sup> and current concepts of chemical*  
4 *carcinogenesis, and how scientific uncertainty should most appropriately be*  
5 *incorporated into low-dose extrapolation.”*

6 **3.5.1.1 Please comment on the scientific evidence and biological**  
7 **rationale in support of the nonlinear versus linear low dose**  
8 **extrapolation approaches**

9  
10 The Panel believes that there are adequate data to support a MOA for bladder  
11 carcinogenesis induced by high doses of DMA<sup>V</sup> in the rat (see B1). The MOA involves  
12 cytotoxicity of the bladder epithelium and increased, sustained regenerative proliferation,  
13 as key events. DNA damage produced by ROS is not a key event in the MOA. The  
14 committee felt that there are insufficient data to invoke ROS-induced DNA damage as a  
15 key event in the carcinogenic process, associated with exposures to DMA<sup>V</sup> or DMA<sup>III</sup>.

16  
17 The postulated MOA for DMA<sup>V</sup> is:

- 18  
19 a) Reductive metabolism of DMA<sup>V</sup> to DMA<sup>III</sup>.  
20  
21 b) High concentrations of DMA<sup>III</sup> in urine cause urothelial cytotoxicity. Some  
22 toxicity may also be caused by DMA<sup>V</sup> itself.  
23  
24 c) Continuous exposure and persistent, stress associated, regenerative cell  
25 proliferation leads to genomic instability, acquisition of genetic alterations,  
26 clonal expansion of altered cells and eventually tumors.  
27

28 Neither the MOA postulated here, nor those postulated by ORD or OPP (USEPA  
29 OPP, 2005; USEPA ORD, 2005b), contain key events expected to be a linear function of  
30 dose. Reductive metabolism of DMA<sup>V</sup> is likely to be saturable and therefore non-linear.  
31 *In vitro*, using rat (MYP3) and human (1T1) bladder cell lines, cytotoxicity of  
32 uroepithelial cells occurs only at concentrations greater than ~0.35 μM (rat, 0.38 μM  
33 DMA<sup>III</sup>; human 1T1 0.35 μM DMA<sup>III</sup>) (unpublished data<sup>1</sup>). *In vivo*, cytotoxicity of the

---

<sup>1</sup> 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

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1 uroepithelium occurred at the lowest tested dietary DMA<sup>V</sup> concentration (2 ppm), but the  
2 incidence and severity increased, and the latency decreased significantly as a function of  
3 dose. Statistically significant increases in regenerative cell proliferation only occur in  
4 rats at DMA<sup>V</sup> dietary concentrations greater than 40 ppm, again, a nonlinear or apparent  
5 threshold response.

6  
7 Even the production of ROS and its interaction with DNA, a key event in the  
8 MOA postulated by OPP and ORD, but eliminated as a key event by this SAB Panel (see  
9 B1), would be a nonlinear function of DMA<sup>V</sup> dose. ROS production specifically, if  
10 saturable metabolic processes are involved, would be expected to be linear at some low  
11 dose, but nonlinear across the larger dose range. Formation of heritable alterations in  
12 DNA by ROS is believed to be a nonlinear or curvilinear effect (USEPA ORD, 2005)  
13 best represented by a quadratic function with a low-dose linear component (USEPA OPP,  
14 2005). The formation rate of heritable alterations is a function of the rate of DNA  
15 damage and the rates the various DNA repair processes and finally the rate of DNA  
16 misreplication (USEPA OPP, 2005). The latter being a function of cytotoxicity and  
17 regenerative cell proliferation which in the case of DMA<sup>V</sup>, are also highly nonlinear  
18 functions of dose (USEPA ORD, 2005). With respect to repair of postulated ROS  
19 induced DNA damage, highly specific enzymatic systems that exist for their repair  
20 (Slupphaug et al., 2003) protect the genome, whether from exogenous chemicals or the  
21 high levels of endogenous ROS induced DNA damage. These enzymatic repair processes  
22 are expected to be nonlinear processes. This summary is presented as guidance to the  
23 EPA should it choose not to accept the recommendations of the SAB Panel with regard to  
24 ROS, and instead choose to invoke a role for ROS induced DNA damage by DMA<sup>V</sup>. The  
25 state of the science is overwhelmingly in favor of a nonlinear approach for the risk

---

below: “During the process of determining the LC50 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 DMAIII 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, DMAIII concentrations of 0.38 uM and below had no effect on the viability of MYP3 cells but at a concentration of 0.39 uM DMAIII the cell viability dropped to 69%. In the 1T1 human bladder cell line, DMAIII concentrations of 0.35 uM and below had no effect on viability but at 0.40 uM 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.

Footnote 1 continued: MYP3 rat bladder cell line  
Arsenite-0.05 uM; Arsenate-1 uM; MMAIII-0.5 uM; MMAV-1mM;  
DMAV-0.05mM; TMAO-0.1mM

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1 assessment of DMA<sup>V</sup>. It was therefore the Panel’s consensus opinion that the available  
2 data support the nonlinear approach for the low dose extrapolation of DMA<sup>V</sup>.

3  
4 The linear approach would be consistent with evidence for direct genotoxicity of  
5 DMA<sup>III/V</sup>. There is no compelling data that DMA<sup>III/V</sup> are directly genotoxic. It is generally  
6 accepted that DMA<sup>V</sup> is not directly genotoxic (not DNA reactive). This conclusion is  
7 well supported by the data presented in the “Science Issue Paper: Model of Carcinogenic  
8 Action for Cacodylic Acid (Dimethylarsinic Acid, DMA<sup>V</sup>) and Recommendations for  
9 Dose Response Extrapolation,” and in section 3.3.1 of this report.

10  
11 **3.5.1.2 Charge Question D1 further asks the SAB, “Which**  
12 **approach is more consistent with the available data on DMA<sup>V</sup>**  
13 **and current concepts of chemical carcinogenesis,”**

14  
15 The non-linear approach is more consistent with the available data and current  
16 concepts of chemical carcinogenesis (see section 3.5.1.1, above).

17  
18 **3.5.1.3 Finally, Charge Question D1 asks the SAB, “How**  
19 **[should] scientific uncertainty most appropriately be**  
20 **incorporated into low-dose extrapolation?”**

21  
22 After some discussion, the Panel viewed this question from the perspective of the  
23 EPA’s RfC guidelines (EPA 1994). Similar guidelines for the derivation of chemical  
24 specific uncertainty factors have been developed by the International Program for  
25 Chemical Safety (IPCS 2001). These guidelines provide an approach for incorporating  
26 uncertainty into risk assessments in the form of uncertainty factors. Uncertainties in the  
27 interspecies extrapolation of the rat dose-response data can be broadly grouped into a)  
28 those related to interspecies differences in pharmacokinetics, b) those related to  
29 interspecies differences in pharmacodynamics, and c) those associated with sensitive  
30 populations such as children and the elderly. The default value for interspecies  
31 differences in pharmacokinetics is 3, the default for interspecies differences in  
32 pharmacodynamics is 3, and the default for sensitive populations is 10, made up of two  
33 factors of 3 each, one for pharmacokinetic differences and one for pharmacodynamic  
34 differences.

35 While it was the opinion that rats might deliver a higher dose of the proximate  
36 toxicant, DMA<sup>III</sup> to the bladder for a given dose of DMA<sup>V</sup> than humans, the Panel  
37 recognized that there was insufficient data on the comparative dosimetry in rats and

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1 humans to make any conclusive statements about species differences in  
2 pharmacokinetics. The uncertainty factor for interspecies differences in pharmacokinetics  
3 should be 3, the default value. However, there appears to be emerging data on DMA<sup>V</sup>  
4 kinetics which might be brought to bear on the question and the agency is encouraged to  
5 consider these data with respect to pharmacokinetic differences between the species and  
6 the characterization of this component of uncertainty in the dose response assessment.

7  
8 As a result of the Arsenic Review Panel's analysis of the data for the key  
9 pharmacodynamic response, uroepithelial cell cytotoxicity, the consensus was the EPA  
10 could assemble a case for pharmacodynamic equivalency between the test species, rats,  
11 and humans from existing experimental data. Cohen et al. showed LC<sub>50</sub>s of 0.5 and 0.8  
12  $\mu\text{M}$  DMA<sup>III</sup> for rat and human bladder epithelial cells lines (Cohen, 2002). In the context  
13 of EPA and International Program on Chemical Safety (IPCS) guidelines, this finding  
14 could be incorporated in the assessment as a reduction of the pharmacodynamic  
15 component of the interspecies uncertainty factor, which is 3, to a value of one. The  
16 application of uncertainty factors has also been addressed in the Panel's response to  
17 question C1. There is presently no information which may be used inform the choice of  
18 uncertainty factors for sensitive human populations.

19  
20 **3.5.2. Implementation of the recommendations of the NRC (2001)**  
21 **(Charge Question D2)**  
22

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

31  
32 The Panel believes that adequate human data at the lower range of iAs exposure is  
33 lacking because of limitations in epidemiologic studies conducted to date. Existing  
34 studies have been mentioned in the response to charge question C2. In summary, there  
35 have been a number of studies in different populations across different countries that  
36 seem to support a possible linear dose-response between exposure from drinking water  
37 and internal cancer risks (particularly in Taiwan, Chile and Argentina). These dose-  
38 response relationships are observed at higher exposure levels (>100 ppb).

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1  
2           Some recent studies have included populations with exposures in the lower range  
3 (<100 ppb), but they tend to be problematic for use in dose-response analysis for lower  
4 exposure levels. In particular, when studies are based almost exclusively on low dose  
5 exposure populations (Lamm et al, 2004; Bates et al, 2003; Steinmaus et al, 2003), they  
6 lack statistical power and the estimations of low dose risk tend to be unstable and to have  
7 a high degree of uncertainty. Some of these studies also have problems related to study  
8 design. For example, in the Lamm et al. (2004) ecological study, exposure assessment is  
9 not only highly problematic given that a single median county-level exposure value is  
10 assigned to all the person-years contributed by each county in the analysis, even though it  
11 is not clear that these are the arsenic exposure values for large number of residents within  
12 each county. A recent follow-up of the Taiwanese cohort reports a monotonic trend in  
13 lung cancer risk for exposure to arsenic levels ranging from <10 to 700 µg/L, however  
14 this study also has limited power to examine the form of the dose-response relationship  
15 within the 10-100 µg/L range (Chen et al 2004). There is no human data available that is  
16 adequate to characterize the shape of the dose response curve below a given point of  
17 departure.

18           At present the experimental evidence on mode of action of inorganic arsenic  
19 supports a possible nonlinear dose-response at low exposure levels yet there is no clear  
20 indication of what shape a nonlinear dose-response would take for application to human  
21 cancer risks at low exposures (<50 or <100 ppb). In examining the dose-response  
22 relationships of arsenicals in inducing direct or indirect mutagenic responses (including  
23 effects thought to be clastogenic in nature), it is clear that effects are only seen at doses  
24 that induce cytotoxicity. This implies a threshold (Rossman, T.G. 2003). Until more is  
25 learned about the complex properties and MOAs of iAs and its metabolites there is  
26 insufficient justification for the choice of a specific nonlinear form of the dose-response  
27 relationship. Under these circumstances, the EPA's 2005 Guidelines for Cancer Risk  
28 Assessment are clear that linear extrapolation below the point of departure is the method  
29 to be used.

30  
31           Although the EPA has chosen a linear model for the arsenic dose component of  
32 the hazard model for lung and bladder cancer, the Panel encourages the Agency to test  
33 the sensitivity of the assumption of linearity by comparing its corresponding estimate of  
34 excess life risk to an alternative hazard model that has a dose contribution that is  
35 multiplicative and nonlinear in form (see question D3 for additional information).  
36

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1 In summary, the Panel recognizes the potential for a highly complex mode of  
2 action of iAs and its metabolites, but until more is learned about the complex PK and PD  
3 properties of iAs and its metabolites there not sufficient justification for the choice of a  
4 specific nonlinear form of the dose-response relationship. Based on this and the EPA’s  
5 2005 Guidelines for Cancer Risk Assessment, the final recommendation of NRC (2001)  
6 to base current risk assessments on a linear dose response model that includes the SW  
7 Taiwan population as a comparison group seems the most appropriate approach.  
8 However, the Panel also recommends performing a sensitivity analysis with different  
9 exposure metrics with the subgroup of villages with more than one well measurement (as  
10 discussed in responses to charge questions C2 and D3) and using a multiplicative model  
11 that includes a quadratic term for dose, as performed by NRC (2001) and as discussed in  
12 charge question D3.

### 13 **3.5.3. EPA Model Re-implementation (Charge Question D3)**

14 The Charge states that, “EPA re-implemented the model presented in the NRC  
15 (2001) in the language R as well as in an Excel spreadsheet format. In addition,  
16 extensive testing of the resulting code was conducted” (USEPA, 2005a). *Charge*  
17 *Question D3 asks the SAB to “... comment upon precision and accuracy of the re-*  
18 *implementation of the model.”*

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

25 “The reference to the implementation in R in question D.3 is outdated, and should  
26 have been removed. This was an oversight on EPA's part. The model  
27 implementation in Excel is our implementation of record, and was used to prepare  
28 the results in the draft toxicological review. We would ask the Panel to please  
29 review and comment only on the implementation in Excel. (Background: EPA  
30 did originally implement its model in R. However we found that version to be not  
31 very transparent, and hard to debug. We then re-implemented the model in Excel,  
32 found and corrected some errors, and used that corrected version to prepare the  
33 tox review. While Excel may not be the best choice from the standpoint of  
34 numerical accuracy, it is greatly superior in the transparency of the  
35  
36  
37  
38

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1 implementation, and is powerful enough to perform the entire model calculation  
2 from start to finish, even including the nonlinear optimization. Once the Panel is  
3 satisfied that the implementation in Excel is correct and appropriate, then the  
4 model can be re-implemented in R or some other numerically superior  
5 language.)”

6  
7 The Agency staff is to be commended for deciding to test its original R-language  
8 version of the model program through a separate implementation in Excel. The Excel  
9 version serves as a check of programming performed in alternative systems (e.g. R, S)  
10 and provides transparency for review by non-specialists. For the calculations required in  
11 this model of hazard and excess risk, the Excel computations should provide sufficient  
12 numerical accuracy. If the EPA returns to another model program, it should begin with  
13 the original model formulas and not simply transcribe the programming from the existing  
14 Excel version of the model. As a debugging and error-checking tool, comparisons of  
15 intermediate results from the two model implementations should be performed to verify  
16 the equivalence of the two model systems.

17  
18 Overview of the EXCEL spreadsheet implementation of the model: The Excel  
19 model implementation is described in Appendix B (pages 105-106) of the Issue Paper.  
20 The Issue Paper (page 65) referenced a URL, [www.epa.gov/waterscience.sab](http://www.epa.gov/waterscience.sab); however  
21 this proved to be not available. EPA staff notified the panel of the correct address,  
22 <http://epa.gov/waterscience/sab/>. The Issue Paper suggests that a listing of the variable  
23 and parameter input field is provided in Table B-3 but the current draft of the Issue Paper  
24 did not include this table. (The fields in the spreadsheet model were interpreted by the  
25 Panel based on the description provided in the text of the Issue Paper and general  
26 understanding of the model fitting procedure employed.)

27  
28 The spreadsheet model requires two Excel files and associated macros. The first  
29 of these is MCCancerfit.XLS. This workbook component of the model consists of eight  
30 worksheets in four pairs (e.g. fblad and MC fblad for female bladder cancer) that cover  
31 the two cancers of interest (lung and bladder) and gender (male, female). The initial  
32 worksheet (e.g. fblad) in each of the four cancer/gender pairs contains the input data for  
33 fitting the hazard model. The first step in the model fitting algorithm is to employ the  
34 Excel Solver to find initial values of  $a_1$ ,  $a_2$ ,  $a_3$  and  $\beta$  (Cells G2:G5) that maximize the  
35 Poisson likelihood under the following model:

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

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 ( $a_1, a_2, a_3, \beta$ ) 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:

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

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

and,

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

2  
3 The estimated UCL(b) is then carried forward to the BEIR.IV computation of the  
4 excess lifetime risk in the BEIR.xls spreadsheet.

5  
6 Based on its review, the Panel noted that for the given data inputs, the empirical  
7 Bayes estimation algorithm programmed in the MCCancerFit.xls spreadsheet does match  
8 the form of the model and the general description of the parameter fitting algorithm  
9 outlined in the Issue Paper.

10  
11 As described in the Issue Paper, the EPA data inputs for at risk populations and  
12 cancer deaths agree with Morales, et al. (2000). In general, the panel recommends that  
13 all tables of inputs for these models be published in appendices to the Issue Paper or final  
14 risk assessment so that reviewers can independently reference and verify the critical  
15 inputs to the hazard and excess risk analysis.

16  
17 The MCCancerft.xls spreadsheet includes an adjustment of 50 µg/day of arsenic  
18 from food intake. Based on the formula provided on page 103 of the Issue Paper, the  
19 current model assumes a combined daily intake of 2 liters/day of cooking and drinking  
20 water. The Issue Paper suggests that the current analysis uses 30 µg/day. Although the  
21 Issue Paper notes the NRC (2001) finding that dietary intake had no significant effect on  
22 the estimated cancer slope factor, the apparent discrepancy between the value of 30  
23 µg/day cited in the Issue Paper and the 50 µg/day value used in the spreadsheet model  
24 should be resolved. The model does not allocate a food input of arsenic to the control

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1 population. This is a decision that presumes food-based intake of arsenic originates from  
2 cooking water only.

3  
4 The second Excel workbook in the risk assessment model employs estimates of  
5 the dose response model parameter,  $\beta$ , and its upper bound to evaluate excess lifetime  
6 risk under the BEIR-IV formula. The BEIR.xls workbook includes four worksheets, one  
7 for each cancer type by gender combination ( flung, mlung, fblad, mblad). The estimates  
8 of the linear dose response parameter and its estimated 95% UCL (see above) are  
9 manually pasted from the corresponding worksheet in MCCancerFit.xls. The excess risk  
10 is computed in cell T15. Solver can be applied to the dose value in Cell T11 (not U10 as  
11 indicated on Page 105 in the Issue Paper) to establish the dose level required to produce a  
12 user-specified values of excess risk (i.e., ED<sub>01</sub>).

13  
14 The columns of each worksheet in the BEIR.xls spreadsheet incorporate data for a  
15 specific age range of the U.S. population. These columns are not labeled with the  
16 corresponding age range. Identifying labels should be applied to all rows and columns in  
17 these worksheets. By deduction, column 3 applies to individuals age 20-24, column 4 to  
18 age 25-29, etc. If this is correct, the Panel recommends that the entry in cell B3 of each  
19 of the four BEIR.xls spreadsheets be verified. It appears that this mortality figure may  
20 apply to more than just the 20-24 year old population represented in Column 3. Referring  
21 to the data inputs for 20-24 year olds in the flung spreadsheet in BEIR.xls, the population  
22 value is 9,423,000, all deaths are 18,121 and the baseline hazard is .00192. Moving over  
23 one column to the 25-29 year olds, the population is nearly the same at 9,491,000, all  
24 deaths are 1580 and the baseline hazard is .00017—less than 1/10<sup>th</sup> that for the previous  
25 five year age group.

26  
27 The BEIR.xls spreadsheet implementation of the BEIR-IV excess risk calculation  
28 includes a 3-fold divisor to transform the risk to a U.S. population base (assuming  
29 exposure per kg is 3-fold higher in the SW Taiwanese population). This scaling occurs in  
30 the calculation of the age-specific cancer hazard (Row 11). It should be documented and  
31 also should be a target for future sensitivity studies. Since this is a model parameter it  
32 should identified as a distinct input on the spreadsheet instead of simply embedded in the  
33 calculations.

34  
35 The notation for the BEIR-IV formula on Page 102 in the Issue Paper does not  
36 distinguish between total survivorship ( $S_i$ ) and survivorship adjusted for the added risk of  
37 cancer. However, the spreadsheet implementation of the model decomposes survival into  
38 the product of baseline survival and a survival factor that reflects excess cancer deaths

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1 due to the prior age group's exposure to arsenic. Based on a version of the spreadsheet  
 2 downloaded from the Office of Water website, calculation of cancer-specific survival  
 3 (Row 13) appears to incorporate mortality through age interval I, not interval I-1 as it  
 4 should. This should be checked. The calculation of baseline survival appears to be  
 5 correct – the survival parameter at age interval I includes only mortality through the end  
 6 of time period I-1. With this exception, calculation of Excess Risk follows the BEIR IV  
 7 formula.

8  
 9 Following the series of checks and corrections to the model listed above, the Panel  
 10 encourages the Agency to extend its testing of the model's sensitivity to alternative  
 11 models forms and model assumptions. Specific areas where the Panel felt additional  
 12 sensitivity testing is warranted include:

- 13  
 14 a) A Monte Carlo analysis in which the individual well concentrations for 22  
 15 villages with multiple wells are taken into account. The Panel recognizes the  
 16 difficulties with this approach including the issue of how to allocate cases to  
 17 wells within villages.  
 18  
 19 b) MCCancerFit.xls:
- 20 ○ A test of the sensitivity of the model to the choice of the reference
  - 21 population (SW Taiwan).
  - 22 ○ A test of the sensitivity of model results to the assumption that the
  - 23 reference population has 0 intake of arsenic via food.
  - 24 ○ A contrast of results for the linear dose model employed in this program to
  - 25 alternative hazard models that are multiplicative and nonlinear in form.
  - 26 For example, the following multiplicative, quadratic model is one of
  - 27 several that NRC(2001) found to have best fit to the data based on the
  - 28 Akaike Information Criterion (AIC):

$$30 \lambda_{i,C} = \exp(a_1 + a_2 \cdot age_i + a_3 \cdot age_i^2) \cdot \exp(\beta_0 + \beta_1 \cdot dose + \beta_2 \cdot dose^2)$$

- 31  
 32 c) BEIR.xls

- 33
- 34 ○ The Panel recommends a sensitivity analysis be conducted to investigate
- 35 the effect of the age groupings used to estimate the baseline hazard and
- 36 excess lifetime risk. In addition to the current practice of using 5-year

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1 intervals (e.g. 20-24, 25-29, etc.), a logical choice is to test the sensitivity  
2 of the model results to using 10-year groupings (e.g. 20-29, 30-39...).

- 3  
4 ○ The exposure/kg parameter used to transfer the dose/response model from  
5 the original SW Taiwanese population to a U.S. general population is a  
6 major driver in the computation of excess lifetime risk. In preparing its  
7 final risk assessment, the EPA should conduct a sensitivity analysis to  
8 determine precisely how much the choice of a factor of 3 impacts the final  
9 estimates of excess lifetime risk.

10  
11 **3.5.4. Available literature describing drinking water consumption**  
12 **rates for the southwestern Taiwanese study population (Charge**  
13 **Question D4)**  
14

15 EPA, as well as the NRC (2001) state that the drinking water consumption rate, as  
16 well as variability of that rate in both U.S. and Taiwanese populations, are important  
17 factors to consider. EPA notes that in calculating risk estimates for U.S. populations  
18 exposed to arsenic through drinking water, NRC used a drinking water consumption rate  
19 of 1 L/day for the U.S. population and two possible consumption rates for the Taiwanese  
20 population: 1 L/day (identical to the U.S. population) and 2.2 L/day with little or no  
21 supporting rationale. Since publication of NRC 2001, a number of new studies have  
22 become available and are summarized in the Cancer Slope Factor Workgroup Issue  
23 Paper. Agency reviews of the relevant literature suggest that the mean drinking water  
24 (for the Taiwanese study population) consumption rate is between 1 to 4.6 L/day. EPA's  
25 current cancer modeling includes water intake adjustments for 2.0 and 3.5 L/day"  
26 (USEPA, 2005a).  
27

28 *Charge Question D4 asks what drinking water value the panel recommended for*  
29 *use in deriving the cancer slope factor for inorganic arsenic?*  
30

31 The Panel notes that assumptions about water consumption levels in the U.S. and  
32 in Taiwan have a substantial impact on the risk assessment. Relative to U.S.  
33 consumption, overestimating water consumption in Taiwan decreases risk estimates and  
34 underestimating consumption increases risk estimates. Evidence for sex differences in  
35 consumption is limited, but considerable within-population variability in consumption  
36 occurs (NRC, 2001).  
37

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1 U.S. water consumption data are obtained from comprehensive U.S. surveys  
2 including surveys by the U.S. Department of Agriculture (USDA) and as part of the  
3 National Health and Nutrition Examination Survey (NHANES) (as cited in EPA 2005),  
4 among others. These studies provide information on tap water consumption as well as  
5 water consumption attributable to other beverage consumption and consumption of food  
6 prepared with water containing arsenic. Estimates of mean daily drinking water  
7 consumption and total water consumption (including water used in food preparation)  
8 range from 1.0 to 2.8 L/day and from 1.2 to 3.2 L/day respectively.  
9

10 In comparison, information on water-consumption in Taiwan derives from a small  
11 study by Yang and Blackwell and an EPA informal, anecdotal assessment (as cited in  
12 EPA 2005) that include only information on drinking water consumption. Information on  
13 water consumption in South Asia, another world region with high arsenic levels in the  
14 water supply, is available from a large population based survey in India (Chowdhury et  
15 al., 2001 cited in EPA 2005) and a small study from Bangladesh (Watanabe et al., 2004).  
16 The South Asian studies include information on water consumption associated with food  
17 preparation. Although similar in socioeconomic characteristics, the diet and climate differ  
18 in Taiwan and South Asia, with temperatures higher in South Asia. These studies report  
19 mean daily drinking water intake of 1 to 3.5 L, with an additional 1 L associated with  
20 food preparation.  
21

22 The Panel recommends that:

- 23
- 24 a) EPA incorporate variability parameters for individual water consumption into their  
25 analysis for the Taiwanese population as they have done for the U.S. population as  
26 per NRC recommendation;  
27
  - 28 b) Because assumptions about water consumption are an important source of  
29 variability in the risk estimates, EPA should conduct sensitivity analyses of the  
30 impact of using a range of consumption values for the Taiwanese population.  
31
  - 32 c) Because data on sex differences in consumption in Taiwan are limited, a better  
33 justification for assuming different consumption levels by sex is needed, particularly  
34 given the lack of sex difference in consumption in U.S. and observed in studies from  
35 other countries (Watanabe et al., 2004). In the absence of such a justification, the  
36 panel recommends an additional sensitivity analysis to examine the impact of  
37 equalizing the sex-specific consumption level.  
38

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1 d) The source of data for intake from other beverages and cooking water needs to be  
2 more fully discussed and documented. Specifically, the document should more clearly  
3 articulate how different sources of water intake are incorporated into the risk model  
4 including beverages other than water (e.g. green tea) and water used in food  
5 preparation. Clarification of both the assumed consumption level and how water  
6 consumption and consumption variability is introduced within the model is needed.  
7

### 8 **3.5.5. Selection of an estimate of dietary intake of arsenic from food** 9 **(Charge Question D5)**

10  
11 EPA stated that, “The issue of intake of arsenic from food (e.g., dry rice, sweet  
12 potatoes) has been distinguished from the issue of intake of arsenic from drinking water.  
13 The NRC addressed the issue of arsenic in food by determining how sensitive the  
14 calculation of ED<sub>01</sub> was to the consumption rate. NRC found that changing the  
15 consumption rate from 50 µg/day to 30 µg/day did not change the calculated ED<sub>01</sub>  
16 significantly (about 1% difference). Since the publication of NRC 2001, a number of  
17 new studies have become available, summarized in the Cancer Slope Factor Workgroup  
18 Issue Paper. EPA’s current cancer modeling includes dietary intake adjustments for 0,  
19 10, 30, and 50 µg/day” (USEPA, 2005a).”  
20

21 *Charge Question D5 asks the SAB what background dietary arsenic intake value*  
22 *it recommends for both the control population and study population of Southwestern*  
23 *Taiwan (which is used in deriving the cancer slope factor for inorganic arsenic?)*  
24

25 Three studies that summarize daily arsenic consumption as derived from food in  
26 areas of high arsenic intake are listed in Table 4 (USEPA OPP, 2005). Based on NRC  
27 recommendations, EPA used a range of 30-50 µg per day total arsenic intake from dry  
28 rice (uncooked) and dried yams in the diet of Southeastern Taiwan that also was based on  
29 the work of Schoof et al. (1998) as listed in this table. In materials presented and  
30 submitted to the Panel (Schoof, 2005), Dr. Schoof, however, affirmed that the 1998 data  
31 were obtained during the dry season in Taiwan when arsenical pesticides were not in use.  
32 Findings in the soil (5 ppm) indicated that arsenical pesticides had not been applied at  
33 this time even though it is known that arsenical pesticides were applied to soil (and taken  
34 up in food crops) during the wetter season. Thus the Schoof et al. (1998) data cited in  
35 Table 4 may underestimate the dietary arsenic intake from food in this population.  
36

37 In the following sections, the term total arsenic indicates the sum of all inorganic  
38 and organic arsenic species. The term inorganic arsenic as stated in published literature

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1 on analysis of arsenic in food generally refers to the sum of the inorganic arsenic species  
2 (iAs<sup>III</sup> and iAs<sup>V</sup>). Unless specifically stated otherwise, the term organic arsenic indicates  
3 total organic arsenic compounds in food. In reference to seafood, arsenobetaine is  
4 generally the major organic arsenic compound present when organic arsenic compounds  
5 are specifically identified in the analysis; other minor organic arsenicals may also be  
6 present. The methylated arsenic metabolites (MMA<sup>III</sup>, MMA<sup>V</sup>, DMA<sup>III</sup>, DMA<sup>V</sup>) are  
7 organic arsenic compounds, however, they are not generally determined in food.  
8

9 Daily intake of arsenic from food observed by Chowdhury et al. (2001) and  
10 Watanabe et al., (2004) suggest total arsenic intakes ranging from a mean of 120 to 285  
11 µg/day from food in Bangladeshi and Indian populations exposed to high levels of  
12 naturally occurring arsenic. Mean total arsenic intakes for males were shown to be 214  
13 µg/day and for females 120 µg/day (Watanabe et al., 2004). In studies conducted in  
14 West Bengal in which both chemical analysis of food items and interviews for food  
15 intake were conducted to assess exposure, Roychowdhury et al., (2002) show daily  
16 dietary intakes from food for adults (based on 34 families in 5 villages) ranging from  
17 171-189 µg/day and for children of about 10 years ranging from 91-101 µg/day. These  
18 figures are ranges of means for two different geographic areas – standard deviations were  
19 not published. Although these data are not derived specifically from the area of Taiwan  
20 studied, they indicate along with ancillary information presented here and elsewhere that  
21 dietary exposure from food in this geographic area may be higher than previously  
22 thought. Raw rice, a staple of the area, has been shown in other studies to contain among  
23 the highest iAs values in food (Schoof, et al., 1999) while for vegetables approximately  
24 95% of total arsenic is organic arsenic (Chowdhury et al., 2001). Variation in arsenic  
25 concentration and speciation occurs relative to rice cultivar (Williams et al., 2005).  
26 Duxbury et al. (2003) estimates that 30-85% of arsenic in rice is inorganic arsenic.  
27

28 Diet is the largest source of total arsenic exposure in the U.S. relative to water and  
29 air exposures. Average intake is about 40 µg/day total arsenic (ATSDR, 2006) compared  
30 with the approximately five-fold higher total dietary arsenic intake observed in Asian  
31 studies cited in the foregoing paragraph. The estimated range of daily intake of total  
32 arsenic from food in the U.S. is reported at 2-92 µg/day (Tao and Bolger, 1999) while  
33 U.S. daily total intake of iAs at the 10th and 90th percentiles is estimated to be 1.8 to 11.4  
34 µg/day for males and 1.3 to 9.4 µg/day for females (Meacher, et al, 2002). The U.S.  
35 dietary intake of inorganic arsenic is estimated to range from 1 to 20 µg/day (ATSDR,  
36 2006). U.S. shellfish and other marine foods contain the highest total arsenic  
37 concentrations and are the largest dietary source (76% - 96%) of arsenic, however, most  
38 of the arsenic in seafood is present as the organic arsenic compound arsenobetaine, which

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1 is excreted rapidly and unchanged and does not appear to be harmful to humans  
2 (ATSDR, 2006). It is known, however, that fish may contain some portion of iAs further  
3 pointing to the need for the sensitivity analysis described below. Certain seafood may  
4 also contain DMA that may also contribute to background exposure from food relative to  
5 water sources (Huang, et al., 2003).

6  
7 It is clear that the adjustment for background iAs intake from food is extremely  
8 important given that the total exposure dose from all sources does likely matter in terms  
9 of toxicity and cancer induction and that the U.S. population likely has a considerably  
10 lower total arsenic intake from food than do populations in Asia.

11  
12 Sensitivity Analyses. The Panel recommends that a range of values from at least  
13 50 to 100  $\mu\text{g}/\text{day}$  and up to perhaps up to as high as 200  $\mu\text{g}/\text{day}$  be run in a sensitivity  
14 analysis to assess the impact of this range of dietary intakes on risk of lung and bladder  
15 cancer from exposure via drinking water in this population. The cancer risk model needs  
16 to be evaluated using a wider range of iAs food values above 50  $\mu\text{g}/\text{day}$  to determine if  
17 there is a change in the arsenic cancer exposure-response slope as a result. It also cannot  
18 be assumed that the control population has an intake of zero arsenic from food.

19  
20 Such a sensitivity analysis of the impact of dietary arsenic uptake using a range of  
21 data from high arsenic-exposed populations is unlikely to introduce larger uncertainty  
22 than the myriad dietary differences – protein deficiency, Se, Zn, folate deficiency etc. –  
23 between this Taiwanese population and the U.S. population

24  
25 Much greater rigor needs to be applied in discussing and presenting documented data  
26 sources and making clear the basis on which assumptions are being made and the relative  
27 strength of those assumptions. Comparisons of the impact of differing levels of iAs  
28 intake from food between the exposed and reference population need to be made on the  
29 basis of comparative relative risk. Clearer statements are needed on the data limitations  
30 of past daily dietary arsenic intake for the Blackfoot endemic area of Taiwan and for the  
31 reference population(s).

32

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

10  
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## APPENDIX A

### ABBREVIATIONS

Abbreviations	Meaning
ARP	US EPA SAB Arsenic Review Panel
As	Arsenic
BEIR	Biological Effects of Ionizing Radiation
CAMA	Calcium salt of MMA <sup>V</sup>
CCA	Chromated copper arsenate
DMA <sup>III</sup>	Dimethylarsinous acid
DMA <sup>V</sup>	Dimethylarsinic acid, Cacodylic Acid
DSMA	Disodium salt of MMA <sup>V</sup>
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 <sup>III</sup>	Arsenite, Trivalent inorganic arsenic
NFκB	Nuclear factor-kappa B
iAs <sup>V</sup>	Arsenate, Pentavalent inorganic arsenic
MLE	Maximum Likelihood Estimates
MMA <sup>III</sup>	Methylarsonous acid
MMA <sup>V</sup>	Methanearsonate acid, methylarsenic acid
MN	Micronuclei
MOA	Mode of Action
MSMA	Monosodium salt of MMA <sup>V</sup>
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

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PK	Pharmacokinetics
RCRA	Resource Conservation and Recovery Act
ROS	Reactive Oxygen Species
SAB	US EPA Science Advisory Board
TMA <sup>III</sup>	Trimethylarsine
TMA <sup>V</sup> O	Trimethylarsine oxide

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## APPENDIX B

### 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<sup>V</sup>), monosodium, disodium, and calcium salts of methanearsonate acid (MSMA, DSMA, and CAMA, collectively as referred as MMA<sup>V</sup>). 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<sup>V</sup> (and MMA<sup>V</sup>) 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

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1 on the two NRC reviews and provides an updated human health effects and dose-response  
2 assessment for inorganic arsenic. The Agency seeks comment and advice from the SAB on the  
3 scientific soundness of major science conclusions drawn in these two documents regarding the  
4 carcinogenic assessments of DMA<sup>V</sup> and inorganic arsenic and the appropriateness of the  
5 Agency's application of its own Guidelines for Carcinogen Risk Assessment for arsenicals.  
6

7 **Overview of Science and Assessment Issues:** Ingestion of inorganic arsenic has been  
8 demonstrated to cause cancer of the skin, lung, and urinary bladder in humans. Historically,  
9 standard chronic bioassays with exposure to inorganic arsenic in rodents have been negative for  
10 increased tumor formation. There are, however, more recent studies at high doses, in transgenic  
11 animals, and following transplacental exposures which have demonstrated cancer potential in  
12 rodent studies following exposure to inorganic arsenic. The NRC 1999 report advises that the  
13 bladder and lung cancer human mortality data, particularly from the southwestern Taiwanese  
14 studies provide the best dose-response data for evaluating the long-term effects of ingestion of  
15 inorganic arsenic. In the 2001 NRC report, a number of recommendations were made to EPA to  
16 revise the oral cancer slope for inorganic arsenic. Given the available database, and recognizing  
17 that the mode(s) of action by which inorganic arsenic causes cancer has not been fully  
18 established, the draft Toxicological Review of Arsenic, consistent with advice from the NRC uses  
19 linear low dose extrapolation to estimate cancer risks from ingestion of arsenic at low dose and  
20 has addressed many of the NRC recommendations.  
21

22 In approaching the cancer assessment on the pesticide cacodylic acid (DMA<sup>V</sup>), an organic  
23 arsenical, EPA has confronted a number of challenging issues. No human epidemiological  
24 information is available for DMA<sup>V</sup>. Rodent cancer bioassay data have shown that dietary  
25 administration of DMA<sup>V</sup> can result in bladder carcinogenesis in the rat. DMA, however, is a key  
26 urinary metabolite from exposure to inorganic arsenic. Thus, the question is raised regarding the  
27 extent the cancer epidemiology on inorganic arsenic may provide an appropriate dataset or may  
28 inform the low dose extrapolation for the cancer risk associated with direct exposure to DMA<sup>V</sup>.  
29 Available *in vivo* and *in vitro* pharmacokinetic, metabolism studies, and toxicology studies were  
30 reviewed to address this issue. The draft OPP Science Issue Paper states that the evidence  
31 indicates inorganic arsenic and DMA<sup>V</sup> have different pharmacokinetic and pharmacodynamic  
32 characteristics, EPA proposes to use the rat bioassay data on DMA<sup>V</sup> to estimate its cancer risk.  
33 The ORD Issue Paper (Appendix E of the OPP Science Issue Paper: Cancer Mode of Action of  
34 Cacodylic Acid (Dimethylarsinic Acid) and Recommendations for Dose Response Extrapolation)  
35 provides additional discussion on the MOA issues and perspective on the nexus between science  
36 issues for organic and inorganic arsenicals. The use of mode of action data in the assessment of  
37 potential carcinogens is a main focus of EPA's 2005 cancer guidelines. Mode of action data are  
38 available on DMA and were evaluated to guide the low dose extrapolation.  
39

40 The Agency seeks comments and advice from the SAB on key science issues concerning  
41 (A) the metabolism and toxic responses of arsenic species, (B) the mode of action for

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1 carcinogenesis and implications for dose-response extrapolation for DMA<sup>V</sup> and inorganic arsenic,  
2 (C) the selection of data for dose-response, and (D) approaches to low-dose extrapolation. In  
3 addition, the Agency is requesting comment on the implications of newer epidemiology and the  
4 incorporation of the 2001 NRC recommendation on modeling the human cancer data for  
5 inorganic arsenic.

## 6 7 **Issues and Charge Questions**

### 8 9 **A. Metabolism and Toxic Responses of Arsenic Species**

10  
11 **A1. Metabolism and pharmacokinetics:** Evidence from *in vivo* and *in vitro*  
12 metabolism and pharmacokinetic studies with humans and laboratory animals suggests  
13 that the efficiency of the methylation reaction(s) and cellular uptake varies based on  
14 which arsenical compound is administered exogenously. Most available studies suggest  
15 that the metabolic process in most mammals is primarily a one-way process and that  
16 following direct exposure to DMA<sup>V</sup> significant amounts of iAs<sup>III</sup>, iAs<sup>V</sup>, MMA<sup>III</sup>, or  
17 MMA<sup>V</sup> at the target tissue are not expected.

18  
19 *Please comment on how pharmacokinetic processes are best considered*  
20 *regarding the use of data derived from direct DMA<sup>V</sup> exposure versus direct iAs*  
21 *exposure for cancer risk assessment.*

22  
23 **A2. Response to mixtures of metabolites:** Tumorigenic profiles vary based on  
24 which arsenical compound is administered exogenously. *In vivo* and *in vitro* studies  
25 indicate that each of the arsenical compounds exhibit similarities and differences in their  
26 profiles of biological activities. Direct exposure to iAs<sup>III</sup> or iAs<sup>V</sup> is expected to result in  
27 more of a mixture of toxic metabolites than for direct exposure to DMA<sup>V</sup>; the mixture of  
28 metabolites is expected to vary based on which chemical is administered exogenously.  
29 The potential mixture of metabolites following direct exposure to DMA<sup>V</sup> appears less  
30 complex as compared to iAs.

31  
32 *Given the toxicological response profiles observed following direct exposures to*  
33 *iAs versus MMA<sup>V</sup> and DMA<sup>V</sup>, and the differences in human and rodent*  
34 *toxicologic responses to arsenicals, please comment on the use of data derived*  
35 *from rodent exposures to the organic arsenicals versus use of data derived from*  
36 *direct iAs human exposure, in the DMA<sup>V</sup> assessment.*

### 37 38 **B. Modes of Carcinogenic Action for DMA<sup>V</sup> and Inorganic Arsenic**

39  
40 **B1. Mode of action of DMA<sup>V</sup>:** When relying on laboratory animal data, two critical  
41 assumptions are made: (i) data on animal tumors are predictive of human cancer, and (ii)

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1 animal tumor effects found at high experimental doses predict human risk at lower  
2 exposures. An understanding of a chemical mode of carcinogenic action can help inform  
3 the above assumptions. In the case of DMA<sup>V</sup>, mode of action (MOA) data are available  
4 and were evaluated using the framework described in EPA's cancer guidelines.

5  
6 *Please comment on the sufficiency of evidence to establish the animal mode of*  
7 *carcinogenic action for DMA<sup>V</sup>. Are the scientific conclusions sound and*  
8 *consistent with the available evidence on DMA<sup>V</sup> and the current state of*  
9 *knowledge for chemical carcinogenesis.*

10  
11 *Please comment on whether the key events in DMA's mode of action are*  
12 *supported by the available data. Specifically comment on the role of: a) reactive*  
13 *oxygen species in producing chromosomal damage and the strength of the*  
14 *evidence supporting oxidative damage as a causal key event in DMA<sup>V</sup>/DMA<sup>III</sup>'s*  
15 *mode of carcinogenic action versus an associative event or a secondary*  
16 *consequence of cytotoxicity; b) cell proliferation and cytotoxicity and the*  
17 *strength of the evidence as causal key events in DMA<sup>V</sup>/DMA<sup>III</sup>'s mode of*  
18 *carcinogenic action versus associative or secondary events, and c) other*  
19 *potential modes of action that have substantial scientific support that may be*  
20 *contributing to the carcinogenicity of DMA.*

21  
22 **B2. Human relevance of animal DMA<sup>V</sup> MOA:** There are little or no scientific data  
23 to suggest that if sufficient DMA<sup>III</sup> were present, key precursor events and ultimately  
24 tumor formation would not occur in humans directly exposed to DMA<sup>V</sup>.

25  
26 *Please comment on the relevance of the postulated key events (see B1) to tumors*  
27 *in humans.*

28  
29 *Please comment on how, if at all, differences in the human population vs.*  
30 *experimental animals should be accounted for in the risk assessment for DMA<sup>V</sup>.*

31  
32 There are little to no chemical specific data regarding an increased susceptibility of  
33 humans for bladder tumor development during different life stages.

34  
35 *Please comment on the Agency's conclusion that the young are likely to respond*  
36 *like the adult to the formation of bladder tumors following exposure to DMA.*

37  
38 **B3. Modes of carcinogenic action from exposure to inorganic arsenic:** Inorganic  
39 arsenic (iAs) undergoes successive methylation steps in humans, resulting in the  
40 intermediate production of iAs<sup>III</sup>, MMA<sup>V</sup>, MMA<sup>III</sup>, DMA<sup>V</sup>, and DMA<sup>III</sup>. Each arsenical  
41 metabolite exhibits its own toxicity.

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*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<sup>V</sup>:** A number of different rodent bioassays (standard bioassay, transgenic animals, susceptible rodent strains, initiation and promotion studies) are available on DMA<sup>V</sup>.

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

*Please comment on whether the iAs epidemiology data can be used to inform the DMA<sup>V</sup> dose-response assessment derived from rat data with DMA<sup>V</sup>. 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<sup>V</sup>

**D1. Mode of carcinogenic action understanding for DMA<sup>V/III</sup> 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<sup>V</sup> to support development of such a model.

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1 *Please comment on the scientific evidence and biological rationale in support of*  
2 *nonlinear versus linear low dose extrapolation approaches, which approach is*  
3 *more consistent with the available data on DMA<sup>V</sup> and current concepts of*  
4 *chemical carcinogenesis, and how scientific uncertainty should most*  
5 *appropriately be incorporated into low-dose extrapolation.*  
6

7 **D2. Implementation of the recommendations of the NRC (2001):** EPA has  
8 determined that the most prudent approach for modeling cancer risk from exposure to iAs  
9 is to use a linear model because there are significant remaining uncertainties regarding  
10 which of the metabolite(s) may be the ultimate carcinogenic moiety and whether or not  
11 mixtures of toxic metabolites interact at the site(s) of action.  
12

13 *Does the panel concur with the selection of a linear model following the*  
14 *recommendations of the NRC (2001) to estimate cancer risk at this time? Please*  
15 *discuss your response in light of the highly complex mode of action for iAs with*  
16 *its metabolites.*  
17

18 **D3. EPA re-implemented the model presented in the NRC (2001) in the language R**  
19 **as well as in an Excel spreadsheet format. In addition, extensive testing of the**  
20 **resulting code was conducted.**  
21

22 *Please comment upon precision and accuracy of the re-implementation of the*  
23 *model.*  
24

25 **D4. Available literature describing drinking water consumption rates for the**  
26 **southwestern Taiwanese study population:** NRC (2001) stated that the drinking water  
27 consumption rate, as well as variability of that rate in both US and Taiwanese  
28 populations, are important factors to consider. In calculating risk estimates for U.S.  
29 populations exposed to arsenic through drinking water, NRC used a drinking water  
30 consumption rate of 1 L/day for the US population and two possible consumption rates  
31 for the Taiwanese population: 1 L/day (identical to the US population) and 2.2 L/day  
32 with little or no supporting rationale. Since publication of NRC 2001, a number of new  
33 studies have become available and are summarized in the Cancer Slope Factor  
34 Workgroup Issue Paper. Agency reviews of the relevant literature suggests that the mean  
35 drinking water (for the Taiwanese study population) consumption rate is between 1 to 4.6  
36 L/day. EPA's current cancer modeling includes water intake adjustments for 2.0 and 3.5  
37 L/day.  
38

39 *What drinking water value does the panel recommend for use in deriving the*  
40 *cancer slope factor for inorganic arsenic?*  
41

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

10  
11  
12  
13

*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?*