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

OFFICE OF THE ADMINISTRATOR
SCIENCE ADVISORY

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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:

[First paragraph identifies client office and nature of advisory question].

[Next paragraph describes issues deserving the Administrator’s attention and SAB's advice as to actions, if any, that need to be taken by the Administrator]

[Middle paragraphs describe summary (“bottom line”) advice in lay terms].

[Final paragraph offers future help and identifies follow-up activities SAB would like to have with client office].

Sincerely,

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/signed/

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

/signed/

Dr. XXXX, Chair
XXX Committee

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NOTICE

This report has been written as part of the activities of the EPA **[Science Advisory Board/Clean Air Scientific Advisory Committee/ Advisory Council on Clean Air Compliance Analysis]**, a public advisory committee providing extramural scientific information and advice to the Administrator and other officials of the Environmental Protection Agency. The **[Board/CASAC/Council]** 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/Clean Air Scientific Advisory Committee/ Advisory Council on Clean Air Compliance Analysis]** are posted on the EPA Web site at: <http://www.epa.gov/sab>.

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**U.S. Environmental Protection Agency
Science Advisory Board
Arsenic Review Panel**

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

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

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

Dr. Claudia Marie Hopenhayn, Associate Professor, Department of Epidemiology, Markey Cancer Control Program, College of Public Health, University of Kentucky, Lexington, KY

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- 1
- 2 **Dr. James E. Klaunig**, Professor and Director, Department of Pharmacology and Toxicology,
- 3 School of Medicine , Indiana University , Indianapolis, IN
- 4
- 5 **Dr. X. Chris Le**, Professor, Department. of Public Health Sciences, Department of Chemistry &
- 6 Department of Laboratory Medicine & Pathology, University of Alberta, Edmonton, Alberta,
- 7 Canada
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- 9 **Dr. Michele Medinsky**, Toxicology Consultant, Toxcon, Durham, NC
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- 11 **Dr. Kenneth Portier**, Associate Professor. Institute of Food and Agricultural Sciences.
- 12 University of Florida. Gainesville, FL
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- 14 **Dr. Barry Rosen**, Professor and Chairman, Department of Biochemistry and Molecular Biology,
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- 17 **Dr. Toby Rossman**, Professor, Environmental Medicine, School of Medicine, New York
- 18 University, Tuxedo, NY
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- 20 **Dr. Miroslav Styblo**, Research Associate Professor, Department of Nutrition, University of
- 21 North Carolina , Chapel Hill, NC
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- 23 **Dr. Justin Teeguarden**, Senior Scientist, Pacific Northwest National Laboratory, Richland, WA
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- 25 **Dr. Michael Waalkes**, Chief, Inorganic Carcinogenesis Section, Laboratory of Comparative
- 26 Carcinogenesis, National Cancer Institute, National Institute of Environmental Health Science,
- 27 RTP, NC
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- 29 **Dr. Janice Yager**, Scientific Program Manager-Senior Research Manager, Electric Power
- 30 Research Institute, Palo Alto , CA
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- 35

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**U.S. Environmental Protection Agency
Science Advisory Board**

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TO BE ADDED

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

4
5 **1. EXECUTIVE SUMMARY [optional]**

6
7
8 **[Provide short introductory paragraph, followed by bullets, derived from the text**
9 **boxes in each chapter. Organize document by charge question, if appropriate./**

10
11 **Check that the substance and tone of the Executive Summary is consistent with the**
12 **Administrator Letter]**

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2. INTRODUCTION

2.1. Background

EPA's Office of Research and Development (ORD), in association with the EPA Office of Water and the EPA Office of Pesticide Programs (USEPA, 2005a), requested that the EPA Science Advisory Board (SAB) conduct a review of certain components of its draft assessment of potential human carcinogenicity associated with arsenic, and arsenic containing compounds. Generally, inorganic arsenic is found naturally in the environment and it is typically present in soil and water at some determinate level. Sources of human exposure to inorganic arsenic include drinking water, diet, air and anthropogenic sources such as wood preservatives and industrial wastes. Additionally, humans are exposed to organic arsenicals when they are used as pesticides.

Several laws require EPA to consider the 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 evaluate exposure to arsenic compounds at locations undergoing clean up or remediation. The Clean Air Act, requires EPA to set air emissions standards for 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. Under the mandate of the Food Quality Protection Agency (FQPA), EPA must reevaluate arsenical, and other, pesticide food tolerances (the legal limits of pesticides on/in food or animal feed) in the U.S. by August, 2006. Also, several organic arsenic herbicides are undergoing reregistration and/or tolerance reassessment including cacodylic acid (referred to as dimethylarsinic acid or DMA^V), monosodium, disodium, and calcium salts of methanearsonate acid (MSMA, DSMA, and CAMA, collectively as referred as MMA^V). In 2003, most residential uses of chromated copper arsenate (CCA) as a wood preservative were cancelled.

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

Since the 2001 NAS review, new information has been developed on the mode of carcinogenic action, metabolism and toxicokinetics for arsenic and its methylated species, and new epidemiology studies have been conducted on inorganic arsenic. EPA

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1 considered this new information in its hazard characterization for tolerance assessment of
2 DMA^V and MMA^V (USEPA OPP, 2005 and USEPA ORD, 2005). EPA also developed a
3
4 revised hazard and dose response assessment for inorganic Arsenic (USEPA OW, 2005)
5 which relies on the two NRC reviews and provides an updated human health effects and
6 dose-response assessment for inorganic arsenic.
7

8 In its Charge to the SAB (USEPA, 2005a), EPA asked for advice on the soundness of its
9 major science conclusions in the above cited documents developed by EPA during 2005. The
10 focus is on the carcinogenic assessments of DMA^V and inorganic arsenic.
11

12 **2.1.1. Metabolism and Toxic Responses of Arsenic Species**

13
14 **A1. Metabolism and pharmacokinetics:** *Please comment on how*
15 *pharmacokinetic processes are best considered regarding the use of data derived*
16 *from direct DMA^V exposure versus direct iAs exposure for cancer risk*
17 *assessment.*
18

19 **A2. Response to mixtures of metabolites:** *Given the toxicological response*
20 *profiles observed following direct exposures to iAs versus MMA^V and DMA^V, and*
21 *the differences in human and rodent toxicologic responses to arsenicals, please*
22 *comment on the use of data derived from rodent exposures to the organic*
23 *arsenicals versus use of data derived from direct iAs human exposure, in the*
24 *DMA^V assessment.*
25

26 **2.1.2. Modes of Carcinogenic Action for DMA^V and Inorganic Arsenic**

27
28 **B1. Mode of action of DMA^V:** *Please comment on the sufficiency of*
29 *evidence to establish the animal mode of carcinogenic action for DMA^V. Are the*
30 *scientific conclusions sound and consistent with the available evidence on DMA^V*
31 *and the current state of knowledge for chemical carcinogenesis.*
32

33 *Please comment on whether the key events in DMA's mode of action are*
34 *supported by the available data. Specifically comment on the role of: a) reactive*
35 *oxygen species in producing chromosomal damage and the strength of the*
36 *evidence supporting oxidative damage as a causal key event in DMA^V/DMA^{III}'s*
37 *mode of carcinogenic action versus an associative event or a secondary*
38 *consequence of cytotoxicity; b) cell proliferation and cytotoxicity and the*
39 *strength of the evidence as causal key events in DMA^V/DMA^{III}'s mode of*
40 *carcinogenic action versus associative or secondary events, and c) other*
41 *potential modes of action that have substantial scientific support that may be*
42 *contributing to the carcinogenicity of DMA.*
43

44 **B2. Human relevance of animal DMA^V MOA:** *Please comment on the*
45 *relevance of the postulated key events (see B1) to tumors in humans.*

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1
2 *Please comment on how, if at all, differences in the human population vs.*
3 *experimental animals should be accounted for in the risk assessment for DMA^V.*

4
5 *Please comment on the Agency's conclusion that the young are likely to respond*
6 *like the adult to the formation of bladder tumors following exposure to DMA.*

7
8 **B3. Modes of carcinogenic action from exposure to inorganic arsenic:**

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

12
13 **2.1.3. Selection of Data for Dose-Response Assessment**

14
15 **C1. Use of animal data for DMA^V :** *Please comment on the use of the*
16 *bladder tumor data from the DMA^V rat bioassay as the most suitable dataset for*
17 *quantifying potential human cancer risk to DMA^V, including the weight of*
18 *evidence to support this conclusion.*

19
20 *Please comment on whether the iAs epidemiology data can be used to inform the*
21 *DMA^V dose-response assessment derived from rat data with DMA^V. If so, please*
22 *discuss how such information might be used. (See Appendix).*

23
24 **C2. Use of human epidemiological data from direct iAs exposure:** *Does the*
25 *SAB agree that the Taiwanese dataset remains the most appropriate choice for*
26 *estimating cancer risk in humans? Please discuss the rationale for your*
27 *response.*

28
29 *Do these data provide adequate characterization of the impact of childhood*
30 *exposure to iAs? Please discuss the rationale for your response.*

31
32 **2.1.4. Approaches to Low-Dose Extrapolation for Inorganic Arsenic and**
33 **DMA^V**

34
35 **D1. Mode of carcinogenic action understanding for DMA^{V/III} and implications**
36 **for dose response extrapolation to estimate human cancer risk:** *Please comment*
37 *on the scientific evidence and biological rationale in support of nonlinear versus*
38 *linear low dose extrapolation approaches, which approach is more consistent with*
39 *the available data on DMA^V and current concepts of chemical carcinogenesis, and*
40 *how scientific uncertainty should most appropriately be incorporated into low-dose*
41 *extrapolation.*

42
43 **D2. Implementation of the recommendations of the NRC (2001):** *Does the panel*
44 *concur with the selection of a linear model following the recommendations of the*
45 *NRC (2001) to estimate cancer risk at this time? Please discuss your response in*
46 *light of the highly complex mode of action for iAs with its metabolites.*

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1
2 **D3. EPA re-implemented the model presented in the NRC (2001) in the**
3 **language R as well as in an Excel spreadsheet format. In addition, extensive**
4 **testing of the resulting code was conducted.** *Please comment upon precision and*
5 *accuracy of the re-implementation of the model.*
6

7 **D4. Available literature describing drinking water consumption rates for the**
8 **southwestern Taiwanese study population:** *What drinking water value does the*
9 *panel recommend for use in deriving the cancer slope factor for inorganic arsenic?*

10 **D5. Selection of an estimate of dietary intake of arsenic from food:** *What*
11 *background dietary intake (of arsenic) value does the panel recommend for both the*
12 *control population and study population of Southwestern Taiwan used in deriving the*
13 *cancer slope factor for inorganic arsenic?*
14

15

16 **2.2. Process for Developing this Report and the Structure of this Report**

17

18 This advisory was conducted by a Science Advisory Board *Ad Hoc* Panel
19 composed of members of the chartered SAB and its committees, members of the FIRRA
20 Scientific Advisory Panel, and invited outside experts. A *Federal Register* notice on
21 February 23, 2005 requested nominations of candidates for membership on the Arsenic
22 Review Panel (see GPO, 2005a). Panel Members were selected following procedures for
23 panel formation at the EPA Science Advisory Board (USEPA SAB 2005a). The Arsenic
24 Review Panel held a public telephone conference meeting to plan for the review on
25 August 11, 2005 (see GPO 2005b). The Panel' review meeting was held on September
26 12-13, 2005 and concluded with the articulation of a series of recommendations in
27 response to each of the EPA Charge questions. These recommendations became the core
28 of this report. The Arsenic Review Panel held its final discussions of the report during a
29 telephone conference meeting on January 24, 2006 (see GPO, 2005c, GPO, 2005d). The
30 chartered Science Advisory Board reviewed and approved the report in a meeting on
31 To Be Added.

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RESPONSE TO THE CHARGE

3.1. Overview

The SAB Arsenic Review Panel is being asked to comment on several key science issues concerning the i) toxicity/metabolic profile/bioavailability for different arsenic species, ii) the Agency's understanding of the mode of action of arsenic carcinogenesis and implications of that on dose response extrapolation for DMA^V and inorganic arsenic, and iii) the implications of newer epidemiology and the 2001 National Research Council recommendations on modeling the human cancer slope factor for inorganic arsenic.

3.2. Metabolism and Toxic Responses of Arsenic Species

3.2.1. Metabolism and pharmacokinetics

“Evidence from *in vivo* and *in vitro* metabolism and pharmacokinetic studies with humans and laboratory animals suggests that the efficiency of the methylation reaction(s) and cellular uptake varies based on which arsenical compound is administered exogenously. Most available studies suggest that the metabolic process in most mammals is primarily a one-way process and that following direct exposure to DMA^V significant amounts of iAs^{III}, iAs^V, MMA^{III}, or MMA^V at the target tissue are not expected” (USEPA, 2005a).

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

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

The panel agrees with the Agency's reasoning behind this question. In mammalian (including human) tissues/cells, the metabolism of inorganic arsenic (iAs)

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1 appears to be a one-way process in which iAs is converted to monomethyl-As (MMA),
2 dimethyl-As (DMA), and in some species to trimethyl-As (TMA^{III}, trimethylarsine)
3 metabolites containing As in +3 or +5 oxidation states (Vahter, 1999; Thomas, et al.,
4 2001). There is no evidence for demethylation of methylated As species in either animal
5 or human tissues. While the step-wise addition of methyl groups is likely a one-way
6 process, a cycling between +3 and +5 As species may occur at each of the methylation
7 steps due to a spontaneous oxidation of +3 species (Gong, et al., 2001; Aposhian, et al.,
8 2003) and non-enzymatic (Delnomdedieu, et al., 1994; Scott et al., 1993) or enzymatic
9 (Zakharyn and Aposhian, 1999; Radabaugh and Aposhian, 2000; Waters et al., 2004)
10 reduction of +5 species. Given the one-way character of As methylation, we do not
11 expect to find significant amounts of MMA or iAs as products of DMA^V metabolism in
12 either rat or human tissues or urine.

13
14 In contrast, exposure to iAs may result in the production, tissue retention,
15 and urinary excretion of all the above iAs and methylated As species. Both the
16 uptake and reduction of DMA^V to DMA^{III} are apparently critical steps in the
17 activation of exogenous DMA^V. It is not clear, where and to what extent (if at all)
18 these processes occur in humans exposed to DMA^V, although it appears that
19 uptake may be the rate limiting for further metabolism of DMA^V. MA^{III} is a major
20 urinary metabolite in individuals chronically exposed to iAs (Valenzuela, et al.,
21 2005), indicating that the capacity to reduce DMA^V to DMA^{III} exists in human
22 tissues. However, even the conversion of a small amount/fraction of exogenous
23 DMA^V to DMA^{III} is of toxicological significance due to the significant toxicity of
24 DMA^{III}. Thus, strictly from the point of view of the metabolic pattern, data
25 derived from DMA^V exposure (in the rat), not from iAs exposure, is better suited
26 for cancer risk assessment of DMA^V. However, this approach is uncertain
27 because of specific metabolic differences, and other factors:

- 28
29 1. The uptake pathway or pathways for DMA^V is/are unidentified. The expression
30 or properties of DMA^V transporters may differ in rats and humans, leading to
31 differences in uptake of DMA^V in tissues and organs.
- 32 2. Results of laboratory and epidemiological studies suggest that the pattern for
33 DMA^V metabolism in rats is different from that in humans: Rat metabolize
34 DMA^V to DMA^{III}, trimethylarsine oxide (TMA^{VO}) (Yoshida et al., 1997; Yoshida
35 et al., 1998; Cohen et al., 2002), and possibly, trimethylarsine (TMA^{III}) (Waters et
36 al., 2004). DMA^V, DMA^{III}, and TMA^{VO} are major urinary metabolites of DMA^V
37 in the rat. In addition, TMA^{VO} was also detected in urine of rats chronically
38 exposed to iAs (Yoshida et al., 1998). In contrast, little or no TMA^{VO} was found
39 in human urine after a single dose of DMA^V (Marafante et al, 1987; Buchet et al.,
40 1981) or after acute (Mahieu, et al., 1981; Apostoli et al., 1997; Benramdane et
41 al., 1999) or chronic exposures to iAs (Vahter, 1999; Thomas et al., 2001). These
42 data suggest that the capacity to produce TMA^{VO} from iAs or DMA^V or to

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- 1 excrete TMA^VO in urine is limited in humans as compared to rats. Thus, while it
2 is possible that the urinary TMA^{V/III} metabolites significantly affect the overall
3 toxic or cancerous outcomes in the bladder of rats exposed to DMA^V, the relative
4 lack of these metabolites in human urine would suggest that the outcome in
5 humans would not be as severe as in rats.
- 6 3. Accumulation of DMA^{III} in rat erythrocytes (due to a high-affinity binding to
7 hemoglobin (Lu et al., 2004) contributes to a specific kinetic pattern for DMA^V in
8 rats. It is not clear how and to what extent this factor affects the yield and
9 concentration of the active As species (e.g., DMA^{III}, TMA^VO, or TMA^{sIII}) in
10 urine or in target tissues of rats and how lower accumulation in human
11 erythrocytes would alter the kinetic pattern for DMA^V and toxic/cancerous
12 outcomes of DMA^V exposure in humans.
- 13 4. Microorganisms, including intestinal bacteria, have a capacity to either methylate
14 or demethylate arsenicals (Hall et al., 1997; Cullen et al., 1984; Cullen et al, 1989;
15 Lehr et al., 2003; Bently and chasten, 2002; Tamaki and Frankenberger, 1992;
16 Mukhopadhyay et al, 2002; Ridley et al., 1977). Although the pattern and extent
17 of DMA^V metabolism by human intestinal microflora are not known, it is possible
18 that oral exposure to DMA^V results in the absorption of a wide spectrum of As
19 metabolites produced by bacteria in the gastrointestinal tract of exposed
20 individuals. In contrast, bacterial metabolism would not affect the absorption of
21 DMA^V after inhalation or dermal exposures. Thus, As species found in tissues
22 may differ with different routes of exposure. Interspecies differences in
23 endogenous intestinal bacteria may further complicate extrapolation from rats to
24 humans.
- 25 5. Additional factors may affect the metabolic profiles for DMA^V in humans,
26 including co-exposures to other environmental contaminants, deficiencies of
27 specific nutrients (e.g., selenium) or malnutrition (poor nutrition) has been shown
28 to induce expression of aquaglyceroporin-9 (AQP9), an iAs^{III}/MMA^{III} transporter
29 (Liu et al., 2002; Liu et al., 2004; Liu et al., submitted), 20-fold (Carbrey et al.,
30 2003).

31
32 All the above concerns should be considered in the risk assessment of DMA^V
33 exposure.

34
35 In their briefing documents the agency presented information on a physiologically
36 based pharmacokinetic (PBPK) model for As disposition and metabolism that is under
37 development. PBPK modeling might be a useful approach for integrating tissue and
38 excreta concentrations of As metabolites resulting from exposure to the various forms of
39 As, including DMA^V, in laboratory animals and humans. At the present time the
40 modeling work described by the agency is in the development stages and is not
41 considered sufficiently robust to conduct interspecies extrapolations. However, the Panel
42 strongly encourages the Agency to proceed with PBPK model development, including

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1 laboratory studies to obtain the kinetic constants needed to describe rates of uptake,
2 efflux, metabolism, and elimination of DMA^V in both rats and humans. When
3 sufficiently validated, this model could simulate concentrations of active (toxic or
4 carcinogenic) metabolites in urine and bladder tissue following exposure to DMA^V. This
5 approach could be used for dose response analysis in cancer risk assessment. Such
6 models must be validated for predicting tissue concentrations of active species regardless
7 of the source of arsenic exposure.
8

9 3.2.2. Response to mixtures of metabolites

10
11 “Tumorigenic profiles vary based on which arsenical compound is
12 administered exogenously. *In vivo* and *in vitro* studies indicate that each of the
13 arsenical compounds exhibit similarities and differences in their profiles of
14 biological activities. Direct exposure to iAs^{III} or iAs^V is expected to result in
15 more of a mixture of toxic metabolites than for direct exposure to DMA^V; the
16 mixture of metabolites is expected to vary based on which chemical is
17 administered exogenously. The potential mixture of metabolites following direct
18 exposure to DMA^V appears less complex as compared to iAs” (USEPA, 2005a).
19

20 *Given the toxicological response profiles observed following direct*
21 *exposures to iAs versus MMA^V and DMA^V, and the differences in human*
22 *and rodent toxicologic responses to arsenicals, please comment on the use*
23 *of data derived from rodent exposures to the organic arsenicals versus use*
24 *of data derived from direct iAs human exposure, in the DMA^V assessment.*
25

26 **A2. Response to mixtures of metabolites:** The answer to this charge question is
27 linked to the answer to the question in section 3.2.1 above. The metabolism of iAs yields
28 a wide spectrum of metabolites some of which (iAs^{III/V}, MMA^{III/V}) are apparently not
29 produced during the metabolism of exogenous DMA^V. The production of iAs and MMA
30 metabolites may be associated with specific toxic or cancerous endpoints that are absent
31 in DMA^V exposure in rats or humans unless there is a significant co-exposure to iAs from
32 drinking water, food or the environment. Therefore, data derived from human exposures
33 to iAs are not suitable for DMA^V risk assessment. It should be noted that there are no
34 published data on toxicological responses to DMA^V in humans. The toxic and
35 carcinogenic effects of DMA^V have been examined only in rodents, mainly in rats. Thus,
36 because there is no available alternative, this panel has no choice, but to recommend that
37 the data derived from rodent exposures to DMA^V be used for the risk assessment in
38 DMA^V exposure in humans.
39

40 However, a significant degree of uncertainty is associated with this approach due
41 to the metabolic differences between rats and humans and due to other factors, including
42 those listed in the response to the charge question in section 3.2.1 above. The differences

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1 in the production and urinary excretion of TMA^{III/V} species that could affect the toxic and
2 cancerous outcomes of DMA^V exposure are of a particular concern to this panel.
3 TMA^{VO} is a hepatocarcinogen in rats (Shen et al., 2003). TMA^{III} is apparently more
4 potent than DMA^{III} in damaging purified DNA in *in vitro* systems (Andrews, et al.,
5 2003). On the other hand, both TMA^{VO} and TMA^{III} are less acutely toxic or cytotoxic
6 than DMA^{III} (Yamauchi et al., 1990; Cullen, 2005; Sakurai et al., 1998; Oochi et al.,
7 1994). The contribution of these two metabolites to cytotoxicity and carcinogenesis in
8 the urinary bladder of rats exposed to DMA^V remains unclear. This uncertainty should be
9 properly addressed by the risk assessment analysis for DMA^V exposure in humans.

11 3.3. Modes of Carcinogenic Action for DMA^V and Inorganic Arsenic

13 3.3.1. Mode of Action of DMA^V:

15 “When relying on laboratory animal data, two critical assumptions are made: (i)
16 data on animal tumors are predictive of human cancer, and (ii) animal tumor
17 effects found at high experimental doses predict human risk at lower exposures.
18 An understanding of a chemical mode of carcinogenic action can help inform the
19 above assumptions. In the case of DMA^V, mode of action (MOA) data are
20 available and were evaluated using the framework described in EPA’s cancer
21 guidelines” (USEPA, 2005a).

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

28 *Please comment on whether the key events in DMA’s mode of action are
29 supported by the available data. Specifically comment on the role of: a)
30 reactive oxygen species in producing chromosomal damage and the
31 strength of the evidence supporting oxidative damage as a causal key
32 event in DMA^V/DMA^{III}’s mode of carcinogenic action versus an
33 associative event or a secondary consequence of cytotoxicity; b) cell
34 proliferation and cytotoxicity and the strength of the evidence as causal
35 key events in DMA^V/DMA^{III}’s mode of carcinogenic action versus
36 associative or secondary events, and c) other potential modes of action
37 that have substantial scientific support that may be contributing to the
38 carcinogenicity of DMA.*

40 **B1. Mode of action of DMA^V:** The committee felt that there is adequate data to
41 support an MOA for bladder carcinogenesis induced by high doses of DMA^V in the rat
42 that involves cytotoxicity to the bladder epithelium and increased, sustained regenerative

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1 proliferation as key events. The urine of DMA^V-treated rats contains DMA^{III} at levels
2 that cause necrotic cytotoxicity *in vitro*, so it is reasonable to postulate that DMA^{III} might
3 mediate the necrotic cytotoxicity in the rat bladder. However, the rat (unlike the human)
4 metabolizes a significant fraction of exogenous DMA^V to trimethylarsine oxide
5 (TMA^{VO}) (Cohen et al., 2002; Yoshida et al., 1997, 1998) and possibly to trimethylarsine
6 (TMA^{sIII}) (Waters et al., 2004). Thus, these compounds cannot be excluded as additional
7 mediators of the necrotic cytotoxicity in bladder of rats exposed to DMA^V.
8

9 The committee thought that there is insufficient data to invoke reactive oxygen
10 species (ROS)-induced DNA damage as a key event in the carcinogenic process
11 associated with exposures to DMA^V or DMA^{III}, although contributions from that
12 mechanism cannot be ruled out. Cytotoxic concentrations of DMA^{III} have been shown to
13 induce DNA damage *in vitro* and in intact cells (Mass et al., 2001), possibly *via* an ROS-
14 mediated mechanism (Yamanaka et al., 2003; Kitchin and Ahmad, 2003). However, this
15 mechanism has not been unequivocally implicated as a causative factor in bladder
16 cancers induced in rats by DMA^V exposure.

17 Permanent genetic change is necessary for carcinogenesis, and it is unlikely that
18 increased proliferation alone in the absence of increased genomic instability (increased
19 mutation rate, aneuploidy, amplification, methylation changes, etc.) will result in the 3 or
20 more changes needed to transform a normal cell to a tumor cell. Chronic induction of
21 cell proliferation, such as that seen with chloroform-induced compensatory hyperplasia in
22 the liver, is thought to induce genetic instability.

23 Other sources of DNA damage exist (including spontaneous oxidative lesions)
24 and arsenic may affect the repair of oxidative and other DNA damage (reviewed in
25 Rossman, 2003). It is also possible that cells exposed to the contents of necrotic cells
26 may experience DNA damage (e.g. *via* “clastogenic factors” or *via* inflammatory cells).
27 Although there is no direct evidence to support this mechanism, it is of interest that heat-
28 killed *E. coli* instilled into the bladder was found to increase bladder carcinogenesis by
29 MNU (N-methyl-N-Nitrosourea) (Yamamoto et al., 1992), presumably by an
30 inflammatory mechanism.

31 There are known direct effects of trivalent arsenicals, including DMA^{III}, on
32 protein thiols that can affect cytoprotection and cell signaling. These effects may
33 contribute not only to injury, but also to changes in gene expression and enhanced
34 proliferation. Further, generation of low levels of oxidants from enzymatic sources
35 (Smith et al., 2001) or possibly by uncoupling of mitochondrial oxidations (if DMA^V can
36 act in a manner similar to arsenate) may contribute to these effects on cell signaling and
37 transcriptional activation. Finally, effects of inorganic and methylated arsenicals on thiols
38 in tubulin and cytoskeletal proteins interfere with microfilament function and cytoskeletal
39 changes that contribute to mitotic arrest and genomic instability (Li et al., 1992; Ling et
40 al., 2002; Ochi et al., 1999). There is no evidence that hydroxyl or peroxy radicals play a

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1 significant role in these regulatory processes, especially at low concentrations of
2 arsenicals. Thus, there are too many highly plausible alternative pathways through which
3 arsenicals can affect the carcinogenic or tumorigenic processes to commit to oxyradical
4 generation and oxidative damages as a primary key event in the toxicity of arsenicals.
5 Other effects of trivalent arsenicals that may be applicable to DMA^{V/III} exposure include:
6 alterations in DNA methylation, effects on DNA repair, and induction of aneuploidy
7 (reviewed in Rossman, 2003).

8 The tumor response in the rat bladder system is non-linear, as is the key event (i.e.
9 necrotic cytotoxicity). Since the MOA involves cytotoxicity, doses below those causing
10 cytotoxicity would not be expected to cause tumors.

11 **3.3.2. Human relevance of animal DMA^V MOA:**

12
13
14 “There are little or no scientific data to suggest that if sufficient DMA^{III} were
15 present, key precursor events and ultimately tumor formation would not occur in
16 humans directly exposed to DMA^V” (USEPA, 2005a)

17
18 *Please comment on the relevance of the postulated key events (see B1) to*
19 *tumors in humans.*

20
21 *Please comment on how, if at all, differences in the human population vs.*
22 *experimental animals should be accounted for in the risk assessment for*
23 *DMA^V.*

24
25 **B2. Human relevance of animal DMA^V MOA:** If high enough (cytotoxic)
26 concentrations of DMA^V or DMA^{III} were present in the human urine or bladder after
27 exposure to DMA^V, it is plausible that a similar response (necrosis followed by
28 regenerative proliferation) would take place. However, no data are available to support or
29 reject this assumption. No studies have been carried out on DMA^V-induced bladder
30 cancer in humans, so it is not known at this time whether there have been any cases.
31 Concentrations high enough to cause necrosis in the bladder might be achievable in an
32 industrial accident or deliberate poisoning. It is not clear whether a repeated or chronic
33 exposure to DMA^V from the environment could produce cytotoxic concentrations of
34 critical metabolites in human urine. Even in the case of high exposure, the exposures
35 would probably have to be repeated often enough to produce persistent necrosis and
36 regeneration in order to cause cancer.

37
38 Already mentioned (in charge A1) is the fact that DMA^V is converted to TMA^{VO}
39 and possibly TMA^{III} more efficiently by rats than by humans. TMA^{VO} is a
40 hepatocarcinogen in rats (Shen et al., 2003). TMA^{III} is more potent than DMA^{III} in
41 damaging DNA in *in vitro* systems (Andrews et al, 2003). Thus, although acute toxicities
42 of TMA^{VO} and TMAs^{III} are lower than that of DMA^{III} (Ochi et al., 1994; Sakurai and

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1 Kaise, 1998; Yamauchi et al., 1990), these metabolites can contribute to the MOA for
2 DMA^V-induced bladder cancer in rats. The extent of this contribution is unknown.
3 However, it is possible that the rat data over-estimates the human risk for bladder cancers
4 from DMA^V.

5
6 There are no data to suggest that the young are at greater or lesser risk with regard
7 to DMA^V-induced carcinogenesis.

8
9 There are little to no chemical specific data regarding an increased susceptibility
10 of humans for bladder tumor development during different life stages.

11 **3.3.3. Modes of carcinogenic action from exposure to inorganic arsenic:**

12
13
14 “Inorganic arsenic (iAs) undergoes successive methylation steps in humans,
15 resulting in the intermediate production of iAs^{III}, MMA^V, MMA^{III}, DMA^V, and
16 DMA^{III}. Each arsenical metabolite exhibits its own toxicity” (USEPA, 2005a).

17
18 *Please comment on the conclusion that the available data support the*
19 *hypothesis that multiple modes of action may be operational following*
20 *exposure to inorganic arsenic.*

21
22 **B3. Modes of carcinogenic action from exposure to inorganic arsenic:** The
23 committee agrees that multiple modes of action may operate in carcinogenesis induced by
24 inorganic arsenic. This is because there is simultaneous exposure to multiple metabolic
25 products as well as multiple target organs. There are differences in metabolic capability
26 and probably transport into and out of different organs for different metabolic products,
27 so that the composition of the metabolites can differ in different organs as well. Each of
28 the metabolites has its own cytotoxic and genotoxic capability. In general, the
29 pentavalent compounds are less cytotoxic and genotoxic than are the trivalent
30 compounds. The primary genotoxic endpoint produced by both inorganic and organic
31 arsenic compounds *in vitro* is chromosome breakage, most likely mediated by DNA
32 strand breaks resulting from cytotoxicity (Kligerman et al., 2003). DNA strand breakage,
33 sister chromatid exchange induction and clastogenicity are limited almost exclusively to
34 trivalent species. There is no evidence of direct DNA binding of any arsenical to DNA.
35 Point mutations occur at low levels in arsenite-treated cells, and only at cytotoxic
36 concentrations (Rossman 2003), except as a secondary result of genomic instability
37 (Mure et al., 2003). Genotoxic activity *in vivo* is limited to a small number of studies in
38 rodents. IP injections of high doses of DMA(DMA^V) induced a slight but insignificant
39 increase in mutagenesis in the MutaTMMouse lung, but not in bladder or bone marrow.
40 Arsenite was also negative in this assay (Noda et al., 2002). Arsenite induced
41 micronuclei in mouse peripheral blood lymphocytes and in mouse bone marrow (Tinwell
42 *et al.*, 1991; Noda *et al.*, 2002). DMA did not induce micronuclei in mouse peripheral

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1 blood lymphocytes (Noda *et al.*, 2002), but did induce aneuploidy in mouse bone marrow
2 cells (Kashiwada *et al.*, 1998). Genotoxic activity found *in vivo* is limited to a small
3 number of studies in rodents indicating that highly toxic doses of arsenic compounds may
4 induce micronuclei and/or aneuploidy in non-target tissues.

5
6 Animal studies indicate that for some organs, transplacental carcinogenesis after
7 maternal exposure to inorganic arsenic occurs. This includes the formation in C3H mice
8 of tumors of the lung and liver, target sites of potential human relevance, after exposure
9 to arsenic *in utero*. In addition, *in utero* arsenic induces tumors of the ovary and adrenal,
10 sites not observed in humans to date. The C3H mouse was selected in these studies
11 because it is, in general, sensitive to chemical carcinogenesis, although this strain shows
12 spontaneous tumor formation in several tissues. Other studies indicate that in skin,
13 inorganic arsenic compounds are not complete carcinogens, but act as enhancers
14 (cocarcinogens, sometimes mistakenly called “promoters”) with other agents. Arsenite
15 acts as a cocarcinogen with solar UV light (Rossman *et al.* 2001; Burns *et al.*, 2004) and
16 arsenate is cocarcinogenic with 9,10 dimethyl-1-2-benzanthracene (Motiwale *et al.*,
17 2005). This leaves open the possibility that a cocarcinogenic MOA may also operate for
18 other organs, but this remains to be tested.

19
20 One cannot dismiss the possibilities of hormesis effects in humans exposed to
21 low-dose arsenic or the essentiality of arsenic to humans (Snow *et al.*, 2005). Evidence
22 for essentiality of arsenic has been reported for a number of mammalian species as well
23 as for chickens (reviewed in Uthus, 1992). These may explain some of the apparent low-
24 dose benefits seen in a variety of systems. For example, inorganic arsenic has both
25 positive and negative effects on the growth and function of blood vessel (Soucy *et al.*,
26 2003, 2005; Kamat *et al.*, 2005). Low concentrations fuel angiogenesis, while higher
27 concentrations injure endothelial cells and promote the vessels dysfunction seen in
28 ischemic diseases and peripheral vascular diseases. Thus at low levels arsenic may
29 provide improved vascularization and growth of normal tissues, which could reduce
30 cardiovascular risks. However, this process poses a high risk for arsenic increasing the
31 vascularization and growth of both atherosclerotic lesions (Simeonova and Luster, 2004)
32 and tumors from a secondary source (Kamat *et al.*, 2005). The potential for arsenicals to
33 enhance tumorigenesis through enhanced vascularization has been demonstrated in mice
34 drinking 10-250 ppb iAs^{III} (Kamat *et al.*, 2005). However, arsenic at high doses has been
35 used to destroy the tumor vasculature (Griffin *et al.*, 2003). If arsenic is essential for
36 humans and/or if epidemiological data could be strengthened at the low-dose range to
37 demonstrate either a low-dose benefit or no effect at low dose, then a threshold is certain.
38 However, at this time, the data are lacking or problematic with regard to low-dose effects.
39 This is an extremely important issue and should be investigated.

40
41
42

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1 **3.4. Selection of Data for Dose-Response Assessment**

2
3 **3.4.1. Use of animal data for DMA^V**

4
5 “A number of different rodent bioassays (standard bioassay, transgenic animals,
6 susceptible rodent strains, initiation and promotion studies) are available on
7 DMA^V” (USEPA, 2005a).

8
9 *Please comment on the use of the bladder tumor data from the DMA^V rat*
10 *bioassay as the most suitable dataset for quantifying potential human*
11 *cancer risk to DMA^V, including the weight of evidence to support this*
12 *conclusion*

13
14 **C1: Use of animal data for DMA^V:** The consensus of the panel is that the
15 bladder tumor data from the DMA^V rat bioassay is the most suitable data set for
16 quantifying potential human cancer risk to DMA^V. Given the complex metabolic fates of
17 Arsenic and its various species, the use of human data from iAs exposure to predict risk
18 from DMA^V is not recommended. In this case, reliance on interspecies extrapolation
19 using the rat bioassay data is the best alternative.

20
21 This question indirectly raises the issue as to the largest source of uncertainty for
22 DMA^V risk assessment—conventional interspecies extrapolation or extrapolation across
23 various forms of arsenic. The available material suggests that extrapolation across
24 various forms of arsenic would lead to the greatest degree of uncertainty in a risk
25 assessment. Although the panel agreed that use of the rat bioassay data is the preferred
26 alternative, the panel also felt strongly that a discussion of the key uncertainties with
27 using data from testing in rats to conduct human risk assessment should be included in
28 EPA’s “*Science Issue Paper: Model of Carcinogenic Action for Cacodylic Acid*
29 (*Dimethylarsinic Acid, DMA^V) and Recommendations for Dose Response*
30 *Extrapolation.*”. Issues that panel members consider important to discuss in EPA’s
31 Science Issue Paper are discussed in more detail below. These issues relate to the
32 toxicokinetic and toxicodynamic differences between rats and humans in response to
33 arsenic exposure, the use of rodent bladder tumor models in general, and issues in the use
34 of rodent data for human risk assessment.

35
36 Data illustrating the mode of action for DMA^V as a bladder carcinogen in rats
37 seem quite convincing. However, rats are much more sensitive to DMA^V in
38 carcinogenicity testing than the mouse (Rossman, 2003; Arnold, et al., 2003). Several
39 toxicokinetic and toxicodynamic differences between rats and humans have also been
40 reported after arsenic exposure. For example, arsenic methylation in rat liver hepatocytes
41 proceeds at a faster rate than in human hepatocytes; and rats have a considerably slower
42 whole body clearance of DMA than humans. This slower whole body clearance in rats is

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1 because a significant portion of DMA is retained in the erythrocytes of rats (Vahter, et al.,
2 1984). There is a 15 to 20 fold higher binding of arsenic to rat hemoglobin than to human
3 hemoglobin (Lu, et al, 2004). Human bladder tumors are primarily transitional cell
4 carcinomas, and rat bladder tumors are reported to bear some similarity in pathology to
5 low-grade papillary tumors that occur in humans; however, they are not similar to
6 invasive human bladder tumors that display high grade malignancy (Cohen, 2002). The
7 foregoing, taken together, illustrate known substantial metabolic, pharmacokinetic and
8 pharmacodynamic differences between rats and humans and should be thoroughly
9 discussed in the final EPA documents as these data indicate that the rat is likely to be
10 considerably more sensitive to developing bladder cancer than humans after exposure to
11 DMA^V.

12
13 A second major uncertainty associated with using bladder tumor data from rats is
14 the lack of knowledge about levels of DMA^{III} produced in the human bladder upon
15 exposure to DMA^V and how that compares to levels of DMA^{III} produced in rats exposed
16 to DMA^V. The few human exposure studies that exist seem to indicate little if any
17 DMA^{III} production takes place. This is because DMA^V is not absorbed well --
18 approximately 80% of a dose of the parent compound is excreted in a short time after
19 exposure (Buchet, et al., 1981; Marafante, E., et al., 1987). Additionally rat urothelial
20 cells are 3.5 times more sensitive to DMA^{III} than are human urothelial cells in *in vitro*
21 studies (Cohen, et al., 2000).

22
23 These toxicokinetic and toxicodynamic factors should be taken into account in the
24 application of rat bladder tumor data to assess human bladder cancer risk. These factors
25 will impact the choice of uncertainty factors since the weight of evidence indicates that
26 the rat is considerably more sensitive to bladder tumor induction from direct exposure to
27 DMA^V than are humans. Although selection of a safety factor is the province of EPA's
28 policy choice, the Panel believes that in the case of the Food Quality Protection Act 10X
29 safety factor for this element of risk assessment, the science supporting a smaller factor
30 could lead EPA to choose to lower the factor for arsenic to some number less than 10.
31 The increased sensitivity of rats relative to humans could be taken into account. The
32 Arsenic Review Panel's analysis of the toxicokinetic data indicates that an uncertainty
33 factor for extrapolation from rat toxicokinetic data to human risk in this case is likely to
34 be less than one. The analysis of the toxicodynamic data indicates that the uncertainty
35 factor may also be lower than the default. The application of uncertainty factors has also
36 been addressed in the Panel's response to question D1. **{WE NEED TO POINT OUT
37 THIS IS A POLICY ISSUE THAT THE PANEL ADVISES UPON—CROSS WALK
38 TO D1 as WELL—SEE SECTION IN 3.5.1}**

39
40 The Agency should also discuss in its Science Issue Paper, differences between
41 rats and humans in the development of bladder tumors, and how these differences impact
42 interspecies extrapolation. For example, urinary bladder tumors in rats occur very late in

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1 life. Studies suggest that in rats it takes two or more years of continuous high dose
2 exposure to DMA^V to induce these tumors. This would equate to a human developing
3 cancer very late in life as well. The Science Issue Paper should specifically discuss the
4 similarities and differences in the time for induction of DMA^V related tumors in rats with
5 the pattern observed with humans and arsenic associated urinary bladder cancer.

6
7 EPA'S Science Issue Paper should also discuss general issues associated with rat
8 urinary bladder cancer. One such issue is the relationship between the non-specific
9 induction of tumors and high concentrations of arsenic in the urine. Also, there is a need
10 to address evidence that simple enhancement of proliferation is not associated with
11 carcinogenesis in many tissues. Studies by Gur et al. (listed on page 97 of the DMA
12 MOA Science Issue Paper) on the carcinogenicity of DMA^V were never published and
13 thus cannot be critically evaluated by the Panel. The Science Issue Paper notes that the
14 Gur studies in rats and mice are key bioassay studies. Reliance on these studies would be
15 stronger if the studies had the benefit of peer review.

16
17 EPA's Science Issue Paper is critical of the transplacental model for inorganic
18 arsenic carcinogenesis because the work was done in a sensitive strain of mouse (C3H)
19 that develops a significant background level of tumors in certain tissues. Implicit in this
20 criticism is the assumption that the presence of a high spontaneous tumor rate in the
21 organ of interest makes the interpretation of the animal data difficult. That difficulty
22 would extend to the ability to estimate the proportion of human tumors, if any, that could
23 be attributable to low exposure to a specific contaminant such as iAs. However, it is well
24 known that all cancers in rodent and human tissues can occur spontaneously. Thus, it
25 could be argued that no rodent carcinogenesis studies could be used to assess human
26 carcinogenicity. Clearly, this is not the case as rodent studies are used routinely for
27 human risk assessment. The EPA's position on the issue of using a sensitive strain to
28 extrapolate to humans should be expanded and clarified in the Science Issue Paper
29 especially as it relates to arsenic. As part of this clarification, requirements for target site
30 concordance between human and rodents in order to validate a rodent bioassay and the
31 relative weight placed on fatal versus not fatal cancers should be discussed as they apply
32 to arsenic. EPA's Science Issue Paper does not even address the question of
33 cocarcinogenesis of inorganic arsenic. It confuses tumors with paps and promotion with
34 cocarcinogenesis (p. 39) and Section 3.B (p. 40) contains numerous errors as well.)

35
36 *Please comment on whether the iAs epidemiology data can be used to*
37 *inform the DMA^V dose-response assessment derived from rat data with*
38 *DMA^V. If so, please discuss how such information might be used. (See*
39 *Appendix).*

40
41 **C. 1 (B).** :The panel consensus was that without more detailed information on
42 target tissue dosimetry of arsenic species the iAs epidemiology data would be of limited

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1 use to inform the DMA^V dose-response assessment derived from rat data with DMA^V.
2 Direct exposure to iAs elicits a different cascade of metabolite concentrations with
3 related differential kinetics compared to direct exposure to DMA^V, therefore the iAs
4 epidemiology data cannot reasonably be used to inform the DMA^V dose-response
5 assessment derived from rat data with DMA^V. In the absence of specific information on
6 target tissue levels, assumptions would have to be made regarding the proportion of the
7 iAs for human and DMA^V for rodents that reaches the bladder tissue as the toxic DMA
8 species.
9

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

20 **3.4.2. Use of human epidemiological data from direct iAs exposure:**

21
22 “Since the NRC (2001) report on iAs, an additional body of literature has
23 developed describing epidemiology data from populations in the US exposed to
24 iAs in drinking water” (USEPA, 2005a).
25

26 *Does the SAB agree that the Taiwanese dataset remains the most*
27 *appropriate choice for estimating cancer risk in humans? Please discuss*
28 *the rationale for your response.*
29

30 **C2. Use of human epidemiological data from direct iAs exposure:** The
31 Taiwanese dataset consists of population and mortality data from 42 villages in southwest
32 Taiwan for the years 1973-1986. Arsenic levels in wells from these villages were
33 measured in 1964-1966. The database is one of the largest that has been evaluated for
34 cancer risk relative to arsenic exposures. A total of almost 900,000 person years of
35 follow-up were included, with 1,152 cancer deaths (637 males, 515 females). Among the
36 cancer deaths were 181 due to bladder cancer (85 males, 96 females), 268 lung cancer
37 (147 males, 121 females), and several hundred due to other types of cancer. These data
38 have been subject to several ecologic analyses, starting with the original publications by
39 Chen et al. (1988) and Wu et al. (1989), followed by further analyses by Morales et al.
40 (2000) and by the National Research Council (1999 and 2001).
41

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1 Among the 42 villages, the arsenic concentration ranged from 10 to 934 ppb ($\mu\text{g/L}$).
2 Twenty of these 42 villages used one well. Among many of the 21 villages with multiple
3 wells, many had wide variability in the measured arsenic level in their wells. Analyses
4 using the full dataset give results comparable to results from a reduced dataset including
5 only the villages with single wells, providing some confidence in the stability of the
6 overall results (National Research Council, 1999). The Panel recognizes the limitations
7 of the southwest Taiwan database, including its ecologic character, lack of smoking
8 information, limited precision of exposure estimates, especially among villages with
9 multiple wells, and the possible issue of compromised nutrition among segments of the
10 exposed population. However, in view of the size and statistical stability of the database
11 relative to other studies, the reliability of the population and mortality counts, the stability
12 of residential patterns, and the reliability of the exposure assessment it is the Panel's view
13 that this database remains, at this time, the most appropriate choice for estimating cancer
14 risk among humans.

15
16 The Panel recommends that other epidemiologic databases from studies of
17 arsenic-exposed populations be used to scale the unit risks at high exposure levels that
18 emerge from the Taiwan data. Several of these studies had the advantage of data with
19 excellent exposure assessment. In addition, some populations likely differed from the
20 Taiwanese population with regard to their nutritional status. The accuracy and precision
21 of exposure assessment is a major issue in all environmental epidemiologic studies, and
22 in particular, in studies of arsenic in drinking water. Misclassification of exposure in
23 such studies (when non-differential) can have a profound effect in depressing the
24 magnitude of the observed risk. The excellence of exposure assessment is an especially
25 strong aspect of several studies from northern Chile, and the Panel recommends that the
26 findings of Smith et al. (1998) and of Ferreccio et al. (2000) be considered by EPA. In
27 addition, arsenic exposures appear to be well characterized in cohort studies of Chiou et
28 al. (2001) of transitional cell carcinoma (mostly bladder cancers) and Chen et al. (2004)
29 of lung cancer, from arsenic-exposed cohorts in southwest and northeast Taiwan. The
30 latter study also provides data on the joint effects of arsenic and cigarette smoking in the
31 Taiwanese population. It should be possible to go through a complete risk assessment
32 using at least one other of these databases.

33
34 The accuracy of estimated long-term exposures to arsenic is of concern for some
35 recent studies under 100 ppb. This may compromise their overall utility in assessing
36 concordance with risk estimates obtained from the Taiwan study. The Panel suggests that
37 results on bladder cancer risk from published epidemiology studies of US and other
38 populations chronically exposed from 0.5 to 160 $\mu\text{g/L}$ inorganic arsenic in drinking water
39 be critically evaluated. EPA should determine their potential utility in exploring overall
40 concordance of the cancer risk estimates derived from their data with risk estimates
41 obtained from extrapolation of the Taiwan data [Bates (1995), Lewis (1999), Steinmaus
42 (2003), Michaud (2004), Bates (2004)].

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1
2 When reviewing these “low-level” studies, as well as the “high level” studies, at
3 least the following should be considered: The effect of exposure misclassification on
4 estimates of risk; temporal variability in assigning past arsenic levels from recent
5 measurements; the extent of reliance on imputed exposure levels; the number of persons
6 exposed at various estimated levels of waterborne arsenic; study response/participation
7 rates; estimates of exposure variability; and the resulting influence of these factors on the
8 magnitude and statistical stability of risk estimates. US and other populations differ from
9 the Taiwanese population of interest in genetic background, dietary intake, and
10 background exposure concentrations to inorganic arsenic, and if one or more of these
11 studies are shown to be of potential utility, comparative analyses of the US and Taiwan
12 data may lead to further insights into the possible influence of these differences on
13 population responses to arsenic in drinking water. For compounds such as arsenic for
14 which there are human data beyond the Taiwanese study on which human cancer risk has
15 been based, data from the other, investigations at high exposure levels (>150 ug/l) can be
16 used to gauge the Taiwanese findings.

17
18 All of these studies including those from Taiwan, Chile, Argentina and the U.S. as
19 described above should be judged by the same set of criteria, with the comparative
20 assessment of those criteria across studies clearly laid out in a tabular format. Some of
21 the criteria have been listed in the previous paragraph. The relative strengths and
22 weaknesses of each study need to be described in relation to each criterion. The caveats
23 and assumptions used should be presented so that they are apparent to anyone who uses
24 the data. Included in the risk assessment background document should be a complete
25 and transparent treatment of variability within and among studies and how it affects risk
26 estimates. The present lack of transparency in the application of the criteria in the
27 process of study selection was pointed out by several panel members.

28
29 As recommended in the preceding sections, aggregate results, particularly on
30 bladder cancer risk, from multiple published epidemiology studies of low level arsenic-
31 exposed populations need to be taken into consideration in a more formal secondary
32 integrative analysis and compared with the main analysis for concordance. Data from the
33 epidemiologic studies of relatively low exposure can be informative and need to be
34 formally evaluated beginning with a comparative analysis of strengths and weaknesses as
35 described above.

36
37 A sensitivity analysis to formally evaluate the potential impact of sources of bias
38 (non-random error) in the low level case control and cohort studies is recommended since
39 non-differential misclassification cannot be routinely assumed. These several recent
40 arsenic epidemiology studies have the advantage of data with exposure assessment at a
41 range of exposure levels relevant to those experienced by the US population—exposure
42 levels in these studies range from 0.5 to 160 µg/L inorganic arsenic in drinking water

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1 (Bates et al., 1995; Karagas et al., 2004; Lewis et al., 1999; Kurttio et al., 1999;
2 Steinmaus et al., 2003; Bates et al., 2004). Most of these populations have a nutritional
3 and genetic background similar to that of U.S. or were conducted in a U.S. population.
4

5 Precedents for formally integrating health outcome information from a number of
6 epidemiology studies are readily available. Although, ideally, one would prefer
7 individual measures of exposure to be available in all studies, it is recognized that the
8 Taiwan study of 42 villages herein recommended as the basis for arsenic cancer risk
9 estimation is an ecological study with uncertainty as to individual exposure levels.
10 Recommendations for assessing the range of uncertainty have been put forth in this report
11 in the section immediately following.
12

13 Arsenic epidemiological literature is an instance in which a number of quality (but
14 not ideal) epidemiology studies are available. Quantitative exposure-response modeling
15 for other compounds for which integrative risk analyses were carried out utilizing
16 multiple epidemiology studies have been conducted and health risks for defined
17 outcomes estimated. For example, NRC/NAS (2000) conducted an integrative analysis
18 of three studies of *in utero* exposure to methylmercury (MeHg) and a number of
19 neurodevelopmental outcomes in children. Statistical power among studies was
20 examined and was found not to be principally accountable for observed study-to-study
21 differences in outcomes at similar exposure levels; likewise *p* values for outcomes were
22 not found to be particularly useful in comparing studies, but rather comparative dose-
23 response estimates (i.e., regression slopes) were chosen as the most optimum comparative
24 basis for integration. Likewise, four recent studies (Konig et al., 2005; Bouzan et al.,
25 2005; Cohen et al., 2005a; Cohen et al., 2005b) amply illustrate the conduct of integrative
26 exposure analyses and health outcome. In an integrative analysis of fish consumption
27 and coronary heart disease mortality, eight studies (29 exposure groups) were identified
28 that met pre-established study quality criteria, had quantified exposure (e.g., fish intake)
29 and had reported the precision of relative risk estimates (Konig et al., 2005). Averaged
30 relative risk results were weighted proportionately by precision. In another integrative
31 analysis, a quantitative exposure-response function for prenatal MeHg exposure and IQ
32 was developed using data from three different epidemiology studies (Cohen et al.,
33 2005a). Weights were assigned to measures of cognitive performance for each of seven
34 test domains; an integrated sensitivity analysis was conducted to assess the impact of
35 alternative assumptions on the final integrative study results.
36

37 Studies for inclusion in each integrative analysis were selected on the basis of *a*
38 *priori* established criteria. As previously stated, inclusive evaluation of all arsenic
39 epidemiology studies (both “low” and “high” exposure studies) by pre-set standard
40 criteria and presentation of results in tabular format has been recommended by this Panel.
41 This is the initial step in conducting an integrative analysis.
42

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1 For most compounds of human health concern, epidemiologic data are generally not
2 available (see A2); but occasionally, as in the case of arsenic, one or perhaps a few
3 epidemiology studies will be available. To improve validity, it is important to support
4 human cancer risk estimates using the maximum available scientific information and
5 contemporary risk assessment methodology. The current cancer risk assessment
6 methodology for iAs relies on choosing a single epidemiological study to derive a cancer
7 slope factor that is then used to extrapolate health effects considerably below the
8 exposure levels observed in that study. There are a number of arsenic epidemiology
9 studies now available; there are published methods for quantitatively integrating results
10 from multiple studies (Coull et al., 2003; Ryan, 2005).

11
12 Integrative analyses result in improved statistical power and precision of the
13 estimates that represent an additional advantage of utilizing a larger dataset, as has been
14 pointed out for the Taiwan dataset. Although the “low” arsenic exposure epidemiology
15 studies cannot by themselves provide a basis for dose-response modeling because of lack
16 of data at the higher exposure levels (see D2), they do provide data on the relative risks of
17 bladder cancer for humans exposed at low levels. The Panel suggests, as described in
18 detail in this section that an effort be made to conduct a secondary integrative analysis
19 applying similar approaches to those described above to assess concordance with
20 exposure-response models derived from the outcome of the primary analysis.

21
22 Given the concerns regarding the use of the median well water concentrations in
23 some of the 42 villages in Southwest Taiwan that have more than one measurement, the
24 Panel recommends that EPA conduct a sensitivity analysis. This should include the range
25 of exposures in said villages to provide a range of risk estimates. One alternative
26 (suggested in response to D-3) is a full Monte Carlo analysis in which the individual well
27 concentrations for 22 villages with multiple wells are taken into account. The Panel
28 recognizes the difficulties with this approach including the issue of how to allocate cases
29 to wells within villages. A simpler, but useful first approach would be to test the
30 sensitivity of the model fitting when arsenic concentrations for multiple-well villages are
31 set to: 1) a low level concentration from the range for the village (10th percentile, 20th
32 percentile); 2) the median (current procedure); and 3) a high level concentration from the
33 village range (90th percentile, 80th percentile).

34
35 *Do these data provide adequate characterization of the impact of*
36 *childhood exposure to iAs? Please discuss the rationale for your*
37 *response.*
38

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

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1 of transplacental exposures. However, data from the studies in Southwestern Taiwan
2 which include childhood exposures in the calculation of lifetime dose, show that in the
3 population under 30 years of age there were no bladder cancer cases, and only 5 lung
4 cancer cases. Childhood exposures are included in the lifetime dose estimates. Smith et
5 al (1998) report the highest excessive risk for male lung cancer in the 30-39 year old age
6 group, suggesting the importance of childhood exposure and risk and perhaps smoking
7 behavior as young adults. For 533 women exposed to arsenic in drinking water from tube
8 wells at greater than 50 µg/L compared with those exposed at 50 µg/L, or less, findings
9 suggest that there are significantly increased odds ratios for spontaneous abortion,
10 stillbirth and neonatal death (Milton et al., 2005). Another reproductive study in Chile,
11 which followed over 800 pregnancies, found that pregnant women drinking water
12 containing 40 ug/L gave birth to infants of lower birth weight than a comparable group
13 drinking water containing very low arsenic concentrations (<1 ug/L) (Hopenhayn et al,
14 2003). Thus maternal exposure at moderately high levels may have untoward toxicity
15 effects; the issue of childhood carcinogenic susceptibility has not been extensively
16 addressed.

17 **3.5. Approaches to Low-Dose Extrapolation for Inorganic Arsenic and DMA^V**

18 **3.5.1. Mode of carcinogenic action understanding for DMA^{V/III} and** 19 **implications for dose response extrapolation to estimate human cancer risk:**

20 “The use of mode of action data in the assessment of potential carcinogens is a
21 main focus of EPA’s 2005 cancer guidelines. As stated in these guidelines “The
22 approach to dose-response assessment for a particular agent is based on the
23 conclusion reached as to its potential mode(s) of action”. Although a biological-
24 based model is the preferred approach to estimating cancer risk, there are
25 insufficient data on DMA^V to support development of such a model” (USEPA,
26 2005a).
27
28
29
30

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

37 **D1: Mode of carcinogenic action understanding for DMA^{V/III} and**
38 **implications for dose response:** (1) *Please comment on the scientific evidence*
39 *and biological rationale in support of the nonlinear versus linear low dose*
40 *extrapolation approaches,*
41

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1 The committee felt that there are adequate data to support a MOA for bladder
2 carcinogenesis induced by high doses of DMA^V in the rat (see B3). The MOA involves
3 cytotoxicity of the bladder epithelium and increased, sustained regenerative proliferation
4 as a key events. The urine of DMA^V-treated rats contains DMA^{III} at levels that cause
5 necrotic cytotoxicity in these cells *in vitro*, so it is reasonable to postulate that DMA^{III}
6 might mediate the necrotic cytotoxicity in the rat bladder. A role for other rat DMA^V
7 metabolites, trimethylarsine oxide (TMA^{Ve} (TMA^{sIII})) (Waters, et al., 2004) cannot be
8 excluded as contributors of the necrotic cytotoxicity in rats exposed to DMA^V.

9
10 The committee felt that there are insufficient data to invoke ROS-induced DNA
11 damage as a key event in the carcinogenic process, associated with exposures to DMA^V
12 or DMA^{III}, although contributions from that mechanism cannot be ruled out.

13
14 The postulated MOA for DMA^V is:

- 15
- 16 1. Reductive metabolism of DMA^V to DMA^{III}.
- 17 2. High concentrations of DMA^{III} in urine cause urothelial cytotoxicity.
- 18 3. DNA damage by an unknown mechanism unrelated to direct genotoxicity. The
19 clastogenic action of DMA^{III/V} is likely involved.
- 20 4. Regenerative cell proliferation drives conversion of DNA damage into heritable
21 mutations and clonal expansion of altered cells.
- 22 5. Continuous exposure and persistent regenerative proliferation leads to production
23 of additional mutations, including those necessary for multistep carcinogenesis.
- 24

25 Neither the revised MOA nor those postulated by ORD or OPP (USEPA OPP,
26 2005; USEPA ORD, 2005b) contain key events expected to be a linear function of dose.
27 Reductive metabolism of DMA^V is likely to be saturable and therefore non-linear. In
28 vitro, cytotoxicity of uroepithelial cells occurs *in vitro* only at concentrations greater than
29 0.4 μM DMA^{III} (Inferred from Dr Cohen's paper, but should be confirmed with the
30 author. The range of doses tested was not described (PLEASE ADD THE CITATION
31 HERE). In rats, cytotoxicity of the uroepithelium occurred at the lowest tested dose (2
32 ppm in the diet), but the incidence and severity increased, and the latency decreased
33 significantly as a function of dose. Statistically significant increases in regenerative cell
34 proliferation only occur in rats at DMA^V doses greater than 40 ppm, again, a non linear or
35 apparent threshold response. Even the production of ROS and its interaction with DNA,
36 a key event in the MOA postulated by OPP and ORD would be nonlinear functions of
37 DMA^V dose. Production of ROS would likely be linear low dose, but nonlinear across a
38 larger dose range if saturable metabolic processes are involved. Formation of heritable
39 alterations in DNA by ROS is believed to be nonlinear (sublinear) effect best represented
40 by a quadratic function (USEPA OPP, 2005). The formation rate is a function of the rate
41 of DNA damage and the rate of DNA misreplication (USEPA OPP, 2005). The latter

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1 being a function of cell proliferation, which in the case of DMA^V, is a highly nonlinear
2 function of dose (USEPA ORD, 2005).

3
4 It was therefore the consensus opinion that the available data support the
5 nonlinear approach for the low dose extrapolation.

6
7 The linear approach would be consistent with evidence for direct genotoxicity of
8 DMA^{III/V}. There is no compelling data that DMA^{III/V} are directly genotoxic. It is generally
9 accepted that DMA^V is not directly genotoxic (not DNA reactive). This conclusion is
10 well supported by the data presented in the “Science Issue Paper: Model of Carcinogenic
11 Action for Cacodylic Acid (Dimethylarsinic Acid, DMA^V) and Recommendations for
12 Dose Response Extrapolation.” While DMA^{III} may be indirectly genotoxic under some
13 circumstances, genotoxicity does not appear to be the driving factor in the mode of action
14 of DMA^{III}. We summarize these data below.

15
16 Based on results from genotoxicity studies conducted DMA^V and DMA^{III} appear
17 to lack significant reactivity directly with DNA. These studies are discussed in the
18 Science Issue Paper (pages 52 to 59) and summarized in Table B4 (with references). The
19 panel agrees with the conclusion in the Science Issue Paper that DMA^V is only genotoxic
20 at concentrations producing cytotoxicity or cytolethality. For example, DMA^V was not
21 mutagenic in the Ames assay (Kligerman, et al., 2003) or the transgenic “Muta” mouse
22 assay (Noda, et al., 2002); DMA^V exposure did not result in micronuclei formation
23 (Noda, et al, 2002). In the mouse lymphoma assay a low frequency of mutations were
24 seen only at concentrations that were cytolethal (Moore, et al, 1997). Chromosome
25 aberrations in human lymphocytes were only seen at cytotoxic levels (Moore et al, 1997).
26 In contrast, there is some evidence that DMA^{III} is clastogenic *in vitro* at concentrations
27 below those that are cytotoxic. For example, in Chinese hamster ovary cells low
28 concentrations of DMA^{III} (1 to 5 micromolar) resulted in micronuclei, well below
29 cytolethal concentrations (Dopp, et al., 2004). However, the induction of chromosomal
30 damage *in vitro* and in non target cell types is not necessarily related to cytotoxicity in
31 bladder cells or genotoxicity in bladder cells.

32
33 Overall, there is a critical mass of data from *in vitro* studies with DMA^{V/III}
34 in animal tissue that supports the types of mechanisms typically associated with
35 indirect (i.e., threshold) types of carcinogens. For example, production of reactive
36 oxygen species and DNA disruption (nicks and breaks) formed in association with
37 toxic levels of DMA species, inhibition of some DNA repair processes, DNA-
38 protein cross-links, and altering the expression of pathways associated with the
39 production of tumors (e.g., p53 and telomerase proteins). Data that might argue
40 for a linear, non-threshold mode of action such as DNA binding and point
41 mutation induction have not been produced. Other studies *in vivo* that show
42 induction of DNA strand breaks and the formation of oxidative DNA species also

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1 support secondary effects on the DNA. While there are studies which show *in*
2 *vivo* clastogenicity with inorganic arsenic compounds, no solid evidence of *in vivo*
3 chromosome damage exists for DMA^{V/III}. Thus, data produced with animal cells
4 and tissues points strongly to a secondary mode of action for DMA^{V/III}.
5

6 The Science Issue Paper states that the limited ability of DMA^{III} to induce sister
7 chromatid exchanges coupled with its clastogenicity and cytotoxicity are features of a
8 genotoxin whose mode of action is likely via the production of reactive oxygen species
9 (ROS). However, the Panel was not in agreement that ROS play a role in the mechanism
10 of action of DMA. Although *in vitro* studies with isolated DNA have shown oxidative
11 DNA adducts and damage, these results do not necessarily mean that resulting
12 chromosomal or DNA mutational events will occur *in vivo*. Oxidative DNA adduct
13 formation is readily repaired in mammalian cells and unless there is direct evidence for
14 the formation of oxidative DNA adducts resulting in the induction of mutational events in
15 the bladder, the relationship between these two events associative at best and probably
16 not related to each other in the context of bladder cancer in the rat following DMA
17 treatment. In contrast, the induction of oxidative damage and oxidative stress following
18 cytotoxicity is well documented. This frequently is the result of necrotic events in the
19 target tissue resulting in the sequelae of inflammatory events.
20

21 *(2)...which approach is more consistent with the available data on DMA^V and*
22 *current concepts of chemical carcinogenesis,*
23

24 The non-linear approach is more consistent with the available data and current
25 concepts of chemical carcinogenesis (See (1), above).
26

27 *(3)...how [should] scientific uncertainty should most appropriately be*
28 *incorporated into low-dose extrapolation*
29

30 After some discussion, we viewed this question from the perspective of the EPA's
31 RfC guidelines (EPA 1994). Similar guidelines for the derivation of chemical specific
32 uncertainty factors have been developed by the International Program for Chemical
33 Safety (IPCS 2001). These guidelines provide an approach for incorporating uncertainty
34 into risk assessments in the form of uncertainty factors. Uncertainties in the dose-
35 response assessment can be broadly grouped into a) those related to interspecies
36 differences in pharmacokinetics, b) those related to interspecies differences in
37 pharmacodynamics, to which we add, c) those related to misspecification of the MOA. In
38 the case of the latter, the dose response would change significantly only if evidence
39 became available that DMA^{III/V} caused DNA damage through direct reactivity with DNA.
40 The low dose extrapolation would then become linear. This appears unlikely at this time
41 and the panel concludes that conducting the low-dose extrapolation using the linear
42 assumption to allow evaluation of uncertainty in the MOA by comparison to the non

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1 linear approach is not an appropriate way to address this uncertainty. The preferred
2 approach is to conduct additional research (an outline is found in B3).
3

4 Although selection of uncertainty factors is the province of EPA’s policy choice,
5 the Panel believes that in the case of the Food Quality and Protection Act 10X safety
6 factor for this element of risk assessment, the science supporting a smaller factor could
7 lead EPA to choose to lower the factor for arsenic to some number less than 10. As a
8 result of the Arsenic Review Panel’s analysis of the data for the key toxicodynamic
9 response, uroepithelial cell cytotoxicity, the consensus was the EPA could assemble a
10 case for toxicodynamic equivalency between the test species, rats, and humans from
11 existing experimental data. In the context of EPA and IPCS guidelines, this finding could
12 be incorporated in the assessment as a reduction of the toxicodynamic component of the
13 interspecies uncertainty factor, which is 3, to a value of one. The application of
14 uncertainty factors has also been addressed in the Panel’s response to question C1. [THIS
15 IS A POLICY ISSUE THAT THE PANEL ADVISES UPON—CROSS WALK TO C1 -
16 -SEE SECTION IN 3.4.1].

17 While it was the opinion that rats *might* deliver a higher dose of the proximate
18 toxicant, DMA^{III}, to the bladder for a given dose of DMA^V than humans, the committee
19 recognized that there was insufficient data on the comparative dosimetry for these species
20 to make any conclusive statements about species differences in pharmacokinetics. There
21 appears to be emerging data on DMA^V kinetics which might be brought to bear on the
22 question and the agency is encouraged to consider these data with respect to
23 pharmacokinetic differences between the species and the characterization of this
24 component of uncertainty in the dose response assessment.

25 26 **3.5.2. Implementation of the recommendations of the NRC (2001)**

27
28 “EPA has determined that the most prudent approach for modeling cancer risk
29 from exposure to iAs is to use a linear model because there are significant
30 remaining uncertainties regarding which of the metabolite(s) may be the ultimate
31 carcinogenic moiety and whether or not mixtures of toxic metabolites interact at
32 the site(s) of action” (USEPA, 2005A).
33

34 *Does the panel concur with the selection of a linear model following the*
35 *recommendations of the NRC (2001) to estimate cancer risk at this time?*
36 *Please discuss your response in light of the highly complex mode of action for*
37 *iAs with its metabolites.*
38

39 **D2: Implementation of the recommendations of the NRC (2001):** There is a
40 lack of adequate human data at the lower range of iAs due to limitations in
41 epidemiologic studies conducted to date. These studies have been discussed in

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1 response to charge question C-2. In summary, there have been a number of studies in
2 different populations across different countries that seem to support a possible linear
3 dose-response between exposure from drinking water and internal cancer risks
4 (particularly in Taiwan, Chile and Argentina). However, the dose-response
5 relationships are observed at higher exposure levels (>100 ppb). Although some
6 recent studies have included populations with exposures in the lower range (<100
7 ppb), they are not appropriate for using in dose-response analysis for lower exposure
8 levels since they have problems related to study design, exposure assessment and
9 statistical power. Estimations of low dose risk based on studies in populations with
10 only low dose exposure are unstable with high uncertainty and studies are
11 underpowered (Lamm et al, 2004; Bates et al, 2003; Steinmaus et al, 2003). For
12 example, in the Lamm et al. (2004) ecological study, exposure assessment is not only
13 highly problematic given that a single median county-level exposure value is assigned
14 to all the person-years contributed by each county in the analysis, but 82% of the 133
15 counties are assigned exposure levels of 3-5 ug/L with only 6 counties assigned
16 values between 15 and 60 ug/L. A recent follow-up of the Taiwanese cohort reports a
17 monotonic trend in lung cancer risk for exposure to arsenic levels ranging from <10
18 to 700 ug/L, however this study also has limited power to examine the form of the
19 dose-response relationship within the 10-100 ug/L range (Chen et al 2004). There is
20 no human data available that is adequate to characterize the shape of the dose
21 response curve below a given point of departure.

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

34
35 Although the EPA has chosen a linear model for the arsenic dose component of
36 the hazard model for lung and bladder cancer, the Panel encourages the Agency to
37 test the sensitivity of the assumption of linearity by comparing its corresponding
38 estimate of excess life risk to an alternative hazard model that has a dose contribution
39 that is multiplicative and quadratic in form. The following equation is the form of the
40 model that NRC (2001) found to have best fit to the data based on the Akaike
41 Information Criterion (AIC):
42

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[Corrected Equation follows]

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

In summary, the Panel recognizes the potential for a highly complex mode of action of iAs and its metabolites, but until more is learned about the complex PKPD properties of iAs and its metabolites there is insufficient justification for the choice of a specific nonlinear form of the dose-response relationship. Based on this and the EPA's 2005 Guidelines for Cancer Risk Assessment, the final recommendation of NRC (2001) to base current risk assessments on a linear dose response model that includes the SW Taiwan population as a comparison group seems the most appropriate approach. However, the Panel also recommends a) performing a sensitivity analysis with different exposure metrics with the subgroup of villages with more than one well measurement; b) using a multiplicative model that includes a quadratic term for dose, as performed by NRC (2001).

3.5.3. EPA Model Re-implementation

"EPA re-implemented the model presented in the NRC (2001) in the language R as well as in an Excel spreadsheet format. In addition, extensive testing of the resulting code was conducted" (USEPA, 2005a).

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

Question D.3 *EPA re-implemented the model presented in the NRC(2001) in the language R as well as in EXCEL spreadsheet format. In addition, extensive testing of the resulting code was conducted. "Please comment on the precision and accuracy of the re-implementation of the model."*

Pre-meeting Comments/Clarifications on the Question

Question D-3 suggests that the estimation of the dose-response model and the hazard assessment were originally programmed in the R language. Page 63 of the issue paper indicates that the Poisson hazard model was originally estimated in the R language (optim routine) but neither the main text of the paper nor its appendices provided any additional information. A clarifying question from the panel through the Designated Federal Officer:

"The reference to the implementation in R in question D.3 is outdated, and should have been removed. This was an oversight on EPA's part. The model implementation in Excel is our implementation of record, and was used to

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1 *prepare the results in the draft toxicological review. We would ask the Panel to*
2 *please review and comment only on the implementation in Excel. (Background:*
3 *EPA did originally implement its model in R. However we found that version to*
4 *be not very transparent, and hard to debug. We then re-implemented the model in*
5 *Excel, found and corrected some errors, and used that corrected version to*
6 *prepare the tox review. While Excel may not be the best choice from the*
7 *standpoint of numerical accuracy, it is greatly superior in the transparency of the*
8 *implementation, and is powerful enough to perform the entire model calculation*
9 *from start to finish, even including the nonlinear optimization. Once the Panel is*
10 *satisfied that the implementation in Excel is correct and appropriate, then the*
11 *model can be re-implemented in R or some other numerically superior*
12 *language.)”*
13

14 The Agency staff is to be commended for deciding to test its original R-language
15 version of the model program through a separate implementation in EXCEL. The
16 EXCEL version serves as a check of programming performed in alternative systems (e.g.
17 R, SPlus) and provides transparency for review by non-specialists. For the calculations
18 of hazard and excess risk implemented in this model, the EXCEL computations will
19 provide sufficient numerical accuracy. If the EPA returns to another programming
20 environment, it should begin with the original model formulas and not simply transcribe
21 the EXCEL model program. As a debugging and error-checking tool, comparisons of
22 intermediate results from the two model implementations should be performed to verify
23 the equivalence of the models.
24

25 Overview of the EXCEL spreadsheet implementation of the model: The EXCEL
26 model implementation is described in Appendix B (pages 105-106) of the Issue Paper.
27 The Issue Paper (page 65) referenced a URL, www.epa.gov/waterscience.sab that proved
28 to be not available. EPA staff notified the panel of the correct address,
29 <http://epa.gov/waterscience/sab/>. The Issue Paper suggests that a listing is provided of
30 the variable and parameter input fields in Table B-3 but the current draft of the Issue
31 Paper did not include this table. The fields in the spreadsheet model were interpreted by
32 the Panel based on the description provided in the text of the Issue Paper and general
33 understanding of the model fitting procedure employed.
34

35 The spreadsheet model requires two Excel files and associated macros. The first
36 of these is MCCancerfit.XLS. This workbook consists of eight worksheets in four pairs
37 (e.g. fblad and MC fblad for female bladder cancer) that cover the two cancers of interest
38 (lung and bladder) and gender (male, female). The initial worksheet (e.g. fblad) in each
39 of the four cancer/gender pairs contains the input data for fitting the hazard model. The
40 first step in the model fitting algorithm is to employ the EXCEL Solver to find initial
41 values of a_1, a_2, a_3 and β (Cells G2:G5) that maximize the Poisson likelihood under the
42 following model:

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2

[Corrected equation follows]

3

4

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

5

6

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).

8

9

10

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, β) about the maximum likelihood estimates found using Solver. The perturbation involves independent, random, and uniformly distributed deviations of the coefficient estimates in a relative range of +/- 10% about the point estimates. Parameter draws outside this range were not performed since the posterior likelihood takes on a near zero value outside the +/- 10% of MLE boundaries. The corresponding macro (e.g. mcfblad) is then invoked and uses the observed data and the set of perturbed coefficient values to predict values 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 using the following equations.

21

22

23

24

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

25

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

with,

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

26

27

The estimated UCL(b) is then copied to the Bier.xls spreadsheet which implements the BIER.IV computations of excess lifetime risk.

28

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1
2 Based on its review, the Panel noted that for the given data inputs, the empirical
3 Bayes estimation algorithm programmed in the MCCancerFit.xls spreadsheet does match
4 the model form and general description of the parameter fitting algorithm outlined in the
5 Issue Paper.

6
7 The Panel recommends that the EPA verify the data on “person years of
8 exposure” for the male and female controls (Southwestern Taiwan). There is no
9 particular evidence that these values are in error but they exhibit a demographic
10 relationship that suggests a check on the accuracy of the data inputs is prudent. As
11 presently input, female person years of exposure for five year age groups are generally
12 less than that for males up to about age 60, a fact that is not consistent with general adult
13 population structures and dynamics. These EPA data inputs agree with Morales, et al.
14 (2000) for the reference population but the question of the gender balance in these data
15 should be investigated to be confident that these inputs correspond to the correct
16 population values. In general, the panel recommends that all tables of model data inputs
17 be published in appendices to the Issue Paper so that reviewers can independently
18 reference and verify the critical inputs to the hazard and excess risk analysis.

19
20 The MCCancerft.xls spreadsheet includes an adjustment of 50 µg/day of arsenic
21 from food intake. Based on the formula provided on page 103 of the Issue Paper, the
22 current model assumes a combined daily intake of 2 liters/day of cooking and drinking
23 water. The Issue Paper suggests that the current analysis uses 30 µg/day of arsenic from
24 this source. Although the Issue Paper notes the NRC (2001) finding that dietary intake
25 had no significant effect on the estimated cancer slope factor, the apparent discrepancy
26 between the value of 30 µg/day cited in the Issue Paper and the 50 µg/day value used in
27 the spreadsheet model should be resolved. The model does not allocate an arsenic food
28 input for the control population. This decision presumes food-based intake of arsenic
29 originates from cooking water only and is an assumption that should be subjected to a
30 sensitivity analysis.

31
32 The second EXCEL workbook in the risk assessment model employs estimates of
33 the dose response model parameter, β , and its upper bound to evaluate excess lifetime
34 risk under the Bier-IV formula. The Bier.xls workbook includes four worksheets, one for
35 each cancer type by gender combination (flung, mlung, fblad, mblad). The estimates of
36 the linear dose response parameter and its estimated 95% UCL (see above) are manually
37 input using the value obtained from the corresponding worksheet in MCCancerFit.xls.
38 The excess risk is computed in cell T15. Solver can be applied to the dose value in Cell
39 T11 (not U10 as indicated on Page 105 in the Issue Paper) to establish the dose level
40 requirements for user-specified values of excess risk (i.e., ED₀₁).

41

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1 The notation on Page 102 in the Issue Paper does not distinguish between total
2 survivorship (S_i) and survivorship adjusted for the added risk of cancer. However, the
3 spreadsheet implementation of the model decomposes survival into the product of
4 baseline survival and a survival factor that reflects excess cancer deaths due to arsenic
5 exposure in prior years. The version of the spreadsheet downloaded from the Office of
6 Water website has calculation of cancer-specific survival (Row 13) appearing to
7 incorporate mortality through time I, not time I-1 as indicated in the Issue Paper. This
8 should be checked. The calculation of baseline survival appears to be correct with
9 survival at time I including only mortality through the end of time period I-1. Other than
10 this exception, calculation of Excess Risk follows the Bier IV formula.

11
12 The Bier.xls spreadsheet implementation of the Bier.IV excess risk calculation
13 includes a 3-fold divisor which is assumed included to allow transforming of the risk to a
14 U.S. population base (based on the assumption that exposure per kg is 3-fold higher in
15 the SW Taiwanese population compared to the US population). This scaling occurs in
16 the calculation of the age-specific cancer hazard (Row 11). This multiplier should be
17 documented and included as a factor in future sensitivity studies. Since this is truly a
18 model parameter it should be identified as a distinct input on the spreadsheet interface
19 and not simply embedded in the calculations.

20
21 Following the series of checks and minor corrections to the model listed above,
22 the Panel encourages the Agency to extend its testing of the model's sensitivity to
23 alternative models forms and model assumptions. Specific areas where the Panel felt
24 additional sensitivity testing is warranted include:

- 25
26 • A Monte Carlo analysis in which the individual well concentrations for 22
27 villages with multiple wells are taken into account. The Panel recognizes the
28 difficulties with this approach including the issue of how best to allocate cases to
29 wells for those villages having multiple wells. A practical approach to this
30 sensitivity analysis has been described in the Panel's response to Question 3.4.2
31 (above).
- 32 • MCCancerFit.xls :
- 33 ○ Examine the sensitivity of the model to the choice of the reference
 - 34 population (SW Taiwan).
 - 35 ○ Examine the sensitivity of model results to the assumption that the
 - 36 reference population has 0 intake of arsenic via food.
 - 37 ○ A contrast of results for the linear dose model employed in this program to
 - 38 an alternative hazard model that has a dose contribution that is
 - 39 multiplicative and quadratic in form. This is the form of the model that
 - 40 NRC(2001) found to have best fit to the data based on the Akaike
 - 41 Information Criterion (AIC):
 - 42

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1 [Corrected equation follows]
2

$$3 \lambda_{i,C} = \exp(a_1 + a_2 \cdot age_i + a_3 \cdot age_i^2) \cdot \exp(\beta_0 + \beta_1 \cdot dose + \beta_2 \cdot dose^2)$$

- 4
5 • Bier.xls
6 ○ The Panel recommends a sensitivity analysis in which the age groupings
7 used to estimate the baseline hazard and excess lifetime risk are altered. A
8 logical choice is to test the sensitivity of the model results to using 10-year
9 groupings (e.g. 20-29, 30-39) in both spreadsheets.
10 The exposure/kg parameter used to transfer the dose/response model from
11 the original SW Taiwanese population to a U.S. general population should
12 be a major driver in the computation of excess lifetime risk. In preparing
13 its final risk assessment, the EPA should conduct a sensitivity analysis to
14 determine how the choice of 3 for the conversion factor impacts the final
15 estimates of excess lifetime risk.
16

17 **3.5.4. Available literature describing drinking water consumption rates for**
18 **the southwestern Taiwanese study population:**
19

20 “NRC (2001) stated that the drinking water consumption rate, as well as
21 variability of that rate in both US and Taiwanese populations, are important
22 factors to consider. In calculating risk estimates for U.S. populations exposed to
23 arsenic through drinking water, NRC used a drinking water consumption rate of 1
24 L/day for the US population and two possible consumption rates for the
25 Taiwanese population: 1 L/day (identical to the US population) and 2.2 L/day
26 with little or no supporting rationale. Since publication of NRC 2001, a number
27 of new studies have become available and are summarized in the Cancer Slope
28 Factor Workgroup Issue Paper. Agency reviews of the relevant literature suggests
29 that the mean drinking water (for the Taiwanese study population) consumption
30 rate is between 1 to 4.6 L/day. EPA’s current cancer modeling includes water
31 intake adjustments for 2.0 and 3.5 L/day” (USEPA, 2005a).
32

33 *What drinking water value does the panel recommend for use in deriving*
34 *the cancer slope factor for inorganic arsenic?*
35

36 **D4. Available literature describing drinking water consumption rates for**
37 **the southwestern Taiwanese study population:** Assumptions about water
38 consumption levels in the US and in Taiwan have a substantial impact on the risk
39 assessment. Relative to US consumption, overestimating water consumption in Taiwan
40 decreases risk estimates and underestimating consumption increases risk estimates.
41 Evidence for sex differences in consumption is limited, but considerable within-
42 population variability in consumption occurs (NRC, 2001).

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1
2 US water consumption data are obtained from comprehensive US surveys
3 including surveys by USDA and as part of NHANES (as cited in EPA 2005), among
4 others. These studies provide information on tap water consumption as well as water
5 consumption attributable to other beverage consumption and food preparation. Estimates
6 of mean daily drinking water consumption and total water consumption (including water
7 used in food preparation) range from 1.0 to 2.8 and from 1.2 to 3.2 respectively.
8

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

21 We recommend that:

- 22
- 23 a) the EPA incorporate variability parameters for individual water consumption in
24 their analysis for the Taiwanese population as they have done for the US population
25 as per NRC recommendation;
26
 - 27 b) given that assumptions about water consumption are an important source of
28 variability in the risk estimates, that the EPA conduct sensitivity analyses of the
29 impact of using a range of consumption values for the Taiwanese population.
30
 - 31 c) Data on sex differences in consumption in Taiwan are limited, and a better
32 justification for assuming different consumption levels by sex is needed, particularly
33 given lack of sex difference in consumption in US and observed in studies from other
34 countries (Watanabe et al., 2004). In the absence of such a justification, the panel
35 recommends an additional sensitivity analysis to examine the impact of equalizing the
36 sex-specific consumption level.
37
 - 38 d) The source of data for intake from other beverages and cooking water needs to be
39 more fully discussed and documented. Specifically, the document should more clearly
40 articulate how different sources of water intake are incorporated into the risk model
41 including beverages other than water (e.g. green tea) and water used in food

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1 preparation. Clarification of both the assumed consumption level and how water
2 consumption and consumption variability is introduced within the model is needed.
3

4 **3.5.5. Selection of an estimate of dietary intake of arsenic from food:**

5
6 “The issue of intake of arsenic from food (e.g., dry rice, sweet potatoes) has been
7 distinguished from the issue of intake of arsenic from drinking water. The NRC
8 addressed the issue of arsenic in food by determining how sensitive the
9 calculation of ED₀₁ was to the consumption rate. NRC found that changing the
10 consumption rate from 50 µg/day to 30 µg/day did not change the calculated ED₀₁
11 significantly (about 1% difference). Since the publication of NRC 2001, a
12 number of new studies have become available, summarized in the Cancer Slope
13 Factor Workgroup Issue Paper. EPA’s current cancer modeling includes dietary
14 intake adjustments for 0, 10, 30, and 50 µg/day” (USEPA, 2005a).
15

16 *What background dietary intake (of arsenic) value does the panel*
17 *recommend for both the control population and study population of*
18 *Southwestern Taiwan used in deriving the cancer slope factor for*
19 *inorganic arsenic?*
20

21 **Question D5.** Three studies summarizing arsenic consumption per day derived from
22 food in areas of high arsenic intake are listed in Table 4 (1). Based on NRC
23 recommendations, US EPA used a range of 30-50 µg per day arsenic intake from dry rice
24 (uncooked) and dried yams in the diet of Southeastern Taiwan that also was based on the
25 work of Schoof et al., 1998 (2) as listed in this table. In materials presented and
26 submitted to the committee (3), Dr. Schoof, however, affirmed that these 1998 data were
27 obtained during the dry season in Taiwan when arsenical pesticides were not in use.
28 Findings in the soil (5 ppm) indicated that arsenical pesticides had not been applied at
29 this time even though it is known that arsenic was applied to soil (and taken up in food
30 crops) during the wetter season. Thus these data may underestimate the dietary arsenic
31 intake from food in this population. Daily intake of arsenic from food obtained by
32 Chowdhury et al (2001) and Watanabe et al., (2004) suggest arsenic intakes of from 120
33 to 285 µg/day from food in Bangladesh and Indian populations exposed to high levels of
34 naturally occurring arsenic. Although these data are not derived specifically from the
35 area of Taiwan studied, they indicate along with ancillary information presented here and
36 elsewhere that dietary exposure from food may be somewhat higher than previously
37 thought. Raw rice, a staple of the area, has been shown in other studies to contain among
38 the highest iAs values in food (4). In comparison, daily total intake of iAs at the 10th and
39 90th percentiles in the US are estimated to be 1.8 to 11.4 µg/day for males and 1.3 to 9.4
40 µg/day for females (5). It is clear that the adjustment for background Asi intake from
41 food, given that the total exposure dose *does* matter in terms of toxicity and cancer
42 induction, is extremely important.

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Sensitivity Analyses. It is recommended that a range of values from at least 50 µg/day up to perhaps as high as 100 µg/day be run in a sensitivity analysis to assess the impact of this range of dietary intakes on risk of lung and bladder cancer from exposure via drinking water in this population. The cancer risk model needs to be weighted with a wider range of iAs food values above 50 µg/day to determine if there is a change in slope as a result.

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

It is known that fish contain some portion of iAs further pointing to the need for the sensitivity analysis described above. Seafood may also contain DMA that may also contribute to background exposure from food relative to water sources (Huang, et al., 2003).

Much greater rigor needs to be applied in discussing and presenting documented data sources and making clear the basis on which assumptions are being made and the relative strength of those assumptions. Comparisons of the impact of differing levels of iAs intake from food between the exposed and reference population (if one is used in the analysis) need to be made on the basis of absolute risk as well as relative risk.

An awareness and discussion of methodological issues related to reported arsenic concentrations in food. These are likely somewhat dependent upon differential extraction processes and different analytical procedures used in different laboratories on different food stuffs. Further, laboratory extraction procedures are not usually designed to equate with that portion of arsenic in food that may be bioavailable. The bioavailability issue is an important area for research. Additionally, a clearer statement of the limited data on daily dietary intake is needed.

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Crosswalk of Charge Questions with Report Sections

Charge Question	Report Section
A1	3.2.1
A2	3.2.2
B1	3.3.1
B2	3.3.2
B3	3.3.3
C1	3.4.1
C2	3.4.2
D1	3.5.1
D2	3.5.2
D3	3.5.3
D4	3.5.4
D5	3.5.5

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