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

OFFICE OF THE ADMINISTRATOR
SCIENCE ADVISORY

BOARD

READER NOTE:

The **Background Materials** for the Arsenic Review Panel’s meetings on the draft arsenic report consist of four documents. The documents are:

- 1. **December 27, 2005 Draft Report** – this is the “clean” draft report for ARP discussion and editing. It reflects edits made to the first draft that was circulated to members for comment on November 10, 2005.
- 2. **December 27, 2005 Draft Report with Comments** – this is the draft report (1 above) which embeds member questions and comments on that draft. This document was circulated to members for information and additional comment/edits on December 27, 2005.
- 3. **Embedded Comment Summary** – This is a summarization of the comments embedded in the December 27, 2005 Draft Report With Comments (2 above).
- 4. **Compilation of ARP Member Comments on the December 27, 2005 Draft Report With Comments** -- this is a compilation of member comments received on the Dec 27 2005 Draft report With Comments (2” above). These comments are not contained in 1, 2, or 3 above.

THIS DOCUMENT IS NUMBER 2 IN THE ABOVE LIST

[Date]

EPA-SAB-ADV-06-xxx

The Honorable Stephen L. Johnson
Administrator
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, N.W.

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1 Washington, D.C. 20460

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

7 Dear Administrator Johnson:

8
9

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

11
12 **[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]**

13
14
15 **[Middle paragraphs describe summary (“bottom line”) advice in lay terms].**

16
17 **[Final paragraph offers future help and identifies follow-up activities SAB would like to have with client office].**

18
19

20
21 Sincerely,

22
23

24 /signed/

/signed/

25
26

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

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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2
3 **U.S. Environmental Protection Agency**
4 **Science Advisory Board**
5 **Arsenic Review Panel**
6

7 **CHAIR**

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

10
11 **MEMBERS**

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

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

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

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

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

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

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

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

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

39
40 **Dr. Claudia Marie Hopenhayn**, Associate Professor, Department of Epidemiology,
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42 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
- 8
- 9 **Dr. Michele Medinsky**, Toxicology Consultant, Toxcon, Durham, NC
- 10
- 11 **Dr. Kenneth Portier**, Associate Professor. Institute of Food and Agricultural Sciences.
- 12 University of Florida. Gainesville, FL
- 13
- 14 **Dr. Barry Rosen**, Professor and Chairman, Department of Biochemistry and Molecular Biology,
- 15 School of Medicine, Wayne State University, Detroit, MI
- 16
- 17 **Dr. Toby Rossman**, Professor, Environmental Medicine, School of Medicine, New York
- 18 University, Tuxedo, NY
- 19
- 20 **Dr. Miroslav Styblo**, Research Associate Professor, Department of Nutrition, University of
- 21 North Carolina , Chapel Hill, NC
- 22
- 23 **Dr. Justin Teeguarden**, Senior Scientist, Pacific Northwest National Laboratory, Richland, WA
- 24
- 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
- 28
- 29 **Dr. Janice Yager**, Scientific Program Manager-Senior Research Manager, Electric Power
- 30 Research Institute, Palo Alto , CA
- 31
- 32 **SCIENCE ADVISORY BOARD STAFF**
- 33 **Mr. Thomas Miller**, Designated Federal Officer, EPA Science Advisory Board Staff Office
- 34 (1400F), 1200 Pennsylvania Avenue, NW, Washington, DC, 20460, Phone: 202-343-9982.
- 35

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

CHAIR

Dr. [To be Determined], Affiliation, City, State

MEMBERS

Dr. [To be Determined], Affiliation, City, State

SCIENCE ADVISORY BOARD STAFF

Mr. Thomas Miller, Designated Federal Officer, EPA Science Advisory Board Staff Office (1400F), 1200 Pennsylvania Avenue, NW, Washington, DC, 20460, Phone: 202-343-9982.

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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). EPA SAB **Panels** have considered inorganic arsenic issues (**USEPA SAB, 2000; USEPA 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 (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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3. 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.

{TR}(Dr. Rossman points out the need to scrub the document for “arsenic-compound” naming conventions and settle on a consistent name for the same compound throughout.)

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 ~~from~~ 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. ~~It should be noted that the p-~~ The Panel’s responses to questions A1 and A2 do not take

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1 into consideration potential byproducts of the microbial degradation of DMA^V in the
2 environment. This reflects statements from EPA representatives in the September, 2005
3 Panel meeting that the environmental conversion of DMA^V from organoarsenic
4 pesticides and the risk associated with exposures to these conversion products will be
5 addressed by EPA in an independent document.)

6 {SG}(Once we say that we have not taken into consideration potential byproducts
7 of microbial degradation, should we not state why? It seems to beg the question.)
8

9 {MS}(The change in the preceding sentence reflects the comment from SG).
10

11 {BR}(Dr. Rosen suggests several articles that are relevant to the introductory
12 statement from the “Bugs and Drugs” perspective. What should we do in that
13 regard? See item 4 in this subsection for more.)
14

15 {MS}(Responded on 11-04-2005...not aware of any paper addressing metabolism
16 of dimethylarsine by E coli or by gut microflora. Also, I do not think that arsenic
17 in any form causes intestinal cancer(?). The article you sent us may be referring
18 to two papers by Endo’s lab that found an unidentified cytotoxic metabolite in
19 urine and feces of rats exposed to DMA^V. The same compound was found to be a
20 product of the metabolism of DMA^V by E coli in presence of cysteine in an
21 vitro experiment. It is possible that this metabolite is DMA^{III} alone or in complex
22 with cysteine.)
23

24 {JT}(Since the charge question is directed towards administered As, it is OK to
25 ignore environmental metabolism, but if intestinal metabolism is to be ignored,
26 some explanation regarding why it is not an important consideration for oral
27 studies should be provided.)
28

29 The panel agrees with the Agency’s reasoning behind this question. In
30 mammalian (including human) tissues/cells, the metabolism of inorganic arsenic (iAs)
31 appears to be a one-way process in which iAs is converted to monomethyl-As (MMA),
32 dimethyl-As (DMA), and in some species to trimethyl-As (~~TMA~~ TMA^{III},
33 trimethylarsine){TR}metabolites containing As in +3 or +5 oxidation states (Vahter,
34 1999; Thomas, et al., 2001). There is no evidence for demethylation of methylated As
35 species in either animal or human tissues. While the step-wise addition of methyl groups
36 is likely a one-way process, a cycling between +3 and +5 As species may occur at each of
37 the methylation steps due to a spontaneous oxidation of +3 species (Gong, et al., 2001;
38 Aposhian, et al., 2003) and non-enzymatic (Delnomdedieu, et al., 1994; Scott et al.,
39 1993) or enzymatic (Zakharyn and Aposhian, 1999; Radabaugh and Aposhian, 2000;
40 Waters et al., 2004) reduction of +5 species. Given the one-way character of As
41 methylation, we do not expect to find significant amounts of MMA or iAs as products of
42 DMA^V metabolism in either rat or human tissues or urine.

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2 In contrast, exposure to iAs may result in the production, tissue retention, and
3 urinary excretion of all the above iAs and methylated As species. Both the uptake and
4 reduction of DMA^V to DMA^{III} are apparently critical steps in the activation of exogenous
5 DMA^V. It is not clear, where and to what extent (if at all) these processes occur in
6 humans exposed to DMA^V, although it appears that uptake may be the rate limiting for
7 further metabolism of DMA^V. {SHa} However, DMA^{III} is a major urinary metabolite in
8 individuals chronically exposed to iAs (Le et al., 2000; Valenzuela, et al., 2005),
9 {XCL}(Remove - Le et al 2000 showed the presence of DMAIII as a urinary
10 metabolite in individuals chronically exposed to iAs, but it did not show that it is
11 a “major” urinary metabolite. Therefore, I suggest removing this reference from
12 this context.)

13 indicating that the capacity to reduce DMA^V to DMA^{III} exists in human tissues.
14 However, ~~It should be pointed out that~~ even the conversion of a small amount/fraction of
15 exogenous DMA^V to DMA^{III} is of toxicological significance due to the significant
16 toxicity of DMA^{III}. Thus, strictly from the point of view of the metabolic pattern, data
17 derived from DMA^V exposure (in the rat), not from iAs exposure, {CH} is better suited
18 ~~should be used~~ for cancer risk assessment of DMA^V. However, ~~there is uncertainty~~
19 ~~associated with~~ this approach is uncertain because of specific ~~due to the following~~
20 metabolic differences, and other factors {CH}, ~~that are discussed in the following:~~

- 21
22 1. The uptake pathway or pathways for DMA^V is/are unidentified. The expression
23 or properties of DMA^V transporters may differ in rats and humans, leading to
24 differences in uptake of DMA^V in tissues and organs.
- 25 2. Results of laboratory and epidemiological studies suggest that the pattern for
26 DMA^V metabolism in rats is different from that in humans: Rat metabolize
27 DMA^V to DMA^{III}, trimethylarsine oxide (TMA^{VO}) (Yoshida et al., 1997; Yoshida
28 et al., 1998; Cohen et al., 2002), and possibly, trimethylarsine (TMA^{III}) (Waters et
29 al., 2004).

30 {BR}(Since TMAIII is volatile, it might be produced but expired through
31 the lungs rather than excreted in urine. I am not suggesting that this occurs
32 but just want to point out that absence of evidence is not evidence of
33 absence. The absence of TMAOV (and by association TMAIII) in human
34 urine is not evidence that they are not produced, and we should be
35 cautious about how we interpret negative data.)

36 DMA^V, DMA^{III}, and TMA^{VO} are major urinary metabolites of DMA^V in the rat.
37 In addition, TMA^{VO} was also detected in urine of rats chronically exposed to iAs
38 (Yoshida et al., 1998). In contrast, little or no TMA^{VO} was found in human urine
39 after a single dose of DMA^V (Marafante et al, 1987; Buchet et al., 1981) or after
40 acute (Mahieu, et al., 1981; Apostoli et al., 1997; Benramdane et al., 1999) or
41 chronic exposures to iAs (Vahter, 1999; Thomas et al., 2001). These data suggest
42 that the capacity to produce TMA^{VO} from iAs or DMA^V or to excrete TMA^{VO} in

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1 urine is limited in humans as compared to rats. Thus, while it is possible that the
2 urinary TMA^{V/III} metabolites significantly affect the overall toxic or cancerous
3 outcomes in the bladder of rats exposed to DMA^V, the relative lack of these
4 metabolites in human urine would suggest that the outcome in humans would not
5 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), {BR} 60 20-fold (Carbrey
30 et al., 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.

40 {JT} Some comment could be added in the paragraph above noting that the
41 experimental work conducted to support PBPK model development is an
42 investment in developing an understanding of the biochemical and other processes

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1 that influence As kinetics. That is, the work is not done for the sake of developing
2 a PBPK model, it is done to develop the understanding of the system we need to
3 understand the pharmacokinetic basis for risks across species whether or not we
4 use a PBPK model for dosimetry.)

5 At the present time the modeling work described by the agency is in the development
6 stages and is not considered sufficiently robust to conduct interspecies extrapolations.
7 However, the Panel ~~agency~~ is strongly encourages the Agency to proceed with PBPK
8 model development, including laboratory studies to obtain the kinetic constants needed to
9 describe rates of uptake, efflux, metabolism, and elimination of DMA^V in both rats and
10 humans. When sufficiently validated, this model could simulate concentrations of active
11 (toxic or carcinogenic) metabolites in urine and bladder tissue following exposure to
12 DMA^V. This approach could be used for dose response analysis in cancer risk
13 assessment. Such models must be validated for predicting tissue concentrations of active
14 species regardless of the source of arsenic exposure.

15 {JT}(The last sentence should be modified to express the idea that the PBPK
16 model need not describe kinetics following exposure to As by any route, only
17 those routes necessary for interpreting animal studies (oral probably, dermal
18 maybe) and for human risk assessment (oral, maybe dermal).
19

20 3.2.2. Response to mixtures of metabolites

21
22 “Tumorigenic profiles vary based on which arsenical compound is
23 administered exogenously. *In vivo* and *in vitro* studies indicate that each of the
24 arsenical compounds exhibit similarities and differences in their profiles of
25 biological activities. Direct exposure to iAs^{III} or iAs^V is expected to result in
26 more of a mixture of toxic metabolites than for direct exposure to DMA^V; the
27 mixture of metabolites is expected to vary based on which chemical is
28 administered exogenously. The potential mixture of metabolites following direct
29 exposure to DMA^V appears less complex as compared to iAs” (USEPA, 2005a).
30

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

37 **A2. Response to mixtures of metabolites:** The answer to this charge question is
38 **essentially** linked to the answer to the **charge** question in section 3.2.1 above **A1**. The
39 metabolism of iAs yields a wide spectrum of metabolites some of which (iAs^{III/V},
40 MMA^{III/V}) are apparently not produced during the metabolism of exogenous DMA^V. The
41 production of iAs and MMA metabolites may be associated with specific toxic or
42 cancerous endpoints that are absent in DMA^V exposure in rats or humans unless there is a

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1 significant co-exposure to iAs from drinking water, food or the environment. Therefore,
2 data derived from human exposures to iAs are not suitable for DMA^V risk assessment. It
3 should be noted that there are no published data on toxicological responses to DMA^V in
4 humans. The toxic and carcinogenic effects of DMA^V have been examined only in
5 rodents, mainly in rats. Thus, because there is no available alternative, this panel has no
6 choice, but to recommend that the data derived from rodent exposures to DMA^V be used
7 for the risk assessment in DMA^V exposure in humans.

8 **{GMa}** (The wording in the bold type has undertones that are difficult to
9 interpret. Are you objecting to the policy or what? Better wording might be:
10 “Because there are no available data derived from human exposure to DMA^V, this
11 panel recommends that the data derived from rodent exposures be used for the
12 risk assessment of DMA^V exposures in humans.”)

13
14 **{SG}**(In the next draft, I would suggest we delete the phrase, “this panel has no
15 choice”. To me it seems an inappropriate phrase to use in a document of this
16 type.)

17
18 **{TR}**(This paragraph is difficult to understand because of the abbreviations used).

19
20 **{MS}** The phrase captures the deliberations which did not suggest in any way that
21 using rodent data is a good choice for evaluation of DMA^V metabolism and
22 carcinogenesis in humans. It is the only choice we had. Perhaps others can
23 suggest more appropriate wording without losing this perspective.)

24
25 **{JT}** I would urge the authors to add a paragraph assessing how sufficient the
26 data is. It is not very satisfying to end with a statement, as it now stands, that
27 there is no alternative to using the data. It will be important to convey how
28 good/bad we feel that approach is based on the sufficiency of the data.

29
30 However, a significant degree of uncertainty is associated with this approach due
31 to the metabolic differences between rats and humans and due to other factors, including
32 those listed in the response to the charge question in section 3.2.1 above ~~A1~~. The
33 differences in the production and urinary excretion of TMA^{III/V} species that could affect
34 the toxic and cancerous outcomes of DMA^V exposure are of a particular concern to this
35 panel. TMA^VO is a hepatocarcinogen in rats (Shen et al., 2003). TMA^{III} is apparently
36 more potent than DMA^{III} in damaging **{DB}** purified DNA in *in vitro* systems (Andrews,
37 et al., 2003). On the other hand, both TMA^VO and TMA^{sIII} are less acutely toxic or
38 cytotoxic than DMA^{III} (Yamauchi et al., 1990; Cullen, 2005; Sakurai et al., 1998; Oochi
39 et al., 1994). The contribution of these two metabolites to cytotoxicity and
40 carcinogenesis in the urinary bladder of rats exposed to DMA^V remains unclear. This
41 uncertainty should be properly addressed by the risk assessment analysis for DMA^V
42 exposure in humans.

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1
2 {JT}(The authors make it clear that there are some important metabolism/kinetics
3 issues that are poorly understood and advise that the uncertainty is addressed in
4 the risk assessment. This is not easy to do and so I would urge the authors to
5 advise that the research be conducted to understand the processes. Only this will
6 eventually reduce or allow uncertainty to be addressed. The bar certainly is lower
7 than the unequivocally implicated...)
8

9 **3.3. Modes of Carcinogenic Action for DMA^V and Inorganic Arsenic**

10 **3.3.1. Mode of Action of DMA^V:**

11
12
13 “When relying on laboratory animal data, two critical assumptions are made: (i)
14 data on animal tumors are predictive of human cancer, and (ii) animal tumor
15 effects found at high experimental doses predict human risk at lower exposures.
16 An understanding of a chemical mode of carcinogenic action can help inform the
17 above assumptions. In the case of DMA^V, mode of action (MOA) data are
18 available and were evaluated using the framework described in EPA’s cancer
19 guidelines” (USEPA, 2005a).
20

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

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

38 **B1. Mode of action of DMA^V:** The committee felt that there is adequate data to
39 support an MOA for bladder carcinogenesis induced by high doses of DMA^V in the rat
40 that involves cytotoxicity to the bladder epithelium and increased, sustained regenerative
41 proliferation as key events. The urine of DMA^V-treated rats contains DMA^{III} at levels
42 that cause necrotic cytotoxicity in vitro, so it is reasonable to postulate that DMA^{III} might

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1 mediate the necrotic cytotoxicity in the rat bladder. However, the rat (unlike the human)
2 metabolizes a significant fraction of exogenous DMA^V to trimethylarsine oxide
3 (TMA^{VO}) (Cohen et al., 2002; Yoshida et al., 1997, 1998) and possibly to trimethylarsine
4 (TMAs^{III}) (Waters et al., 2004). Thus, these compounds cannot be excluded as additional
5 mediators of the necrotic cytotoxicity in bladder of rats exposed to DMA^V.

6
7 The committee {SHa} ~~thought felt~~ that there is insufficient data to invoke reactive
8 oxygen species (ROS)-induced DNA damage as a key event in the carcinogenic process
9 associated with exposures to DMA^V or DMA^{III}, although contributions from that
10 mechanism cannot be ruled out. Cytotoxic concentrations of DMA^{III} have been shown to
11 induce DNA damage *in vitro* and in intact cells (Mass et al., 2001), possibly *via* an ROS-
12 mediated mechanism (Yamanaka et al., 2003; Kitchin and Ahmad, 2003). However, this
13 mechanism has not been unequivocally implicated as a causative factor in bladder
14 cancers induced in rats by DMA^V exposure. {JT}(This is a new topic and should start
15 another paragraph). Permanent genetic change is necessary for carcinogenesis, and it is
16 unlikely that increased proliferation alone in the absence of increased genomic instability
17 (increased mutation rate, aneuploidy, amplification, methylation changes, etc.) will result
18 in the 3 or more changes needed to transform a normal cell to a tumor cell. {CH} ~~In~~
19 addition, Chronic induction of cell proliferation, such as that seen with chloroform-
20 induced compensatory hyperplasia in the liver, is thought to induce genetic instability.

21 {JT}(The involvement of ROS in the mode of action is very important to how the
22 risk assessment is to be conducted. It will be heavily scrutinized. As such it is
23 important that the authors do more than(?) say that there is not enough evidence to
24 invoke ROS. We need to state what our criteria for enough evidence would be and
25 why the experimental data to date do not meet the criteria. Our analysis must be
26 more clear than it is now.)

27 {JT}(I encourage the authors to outline the MOA in more detail, as done in D1
28 either by cross referencing to D1 or taking it from D1 (we then would remove it).
29 We need to make sure that the MOA in D1 and B3 are in full agreement.)

30 {JT}(Understand that if the authors do not believe that increased cell proliferation
31 is not enough for carcinogenesis, they are arguing for the role of some other
32 processes, one involving gene tox, that will be low dose linear. The last line here,
33 that chloroform induced cytotoxicity and compensatory hyperplasia induces genetic
34 instability contradicts the first statement that more than cell proliferation is needed
35 for carcinogenesis.

36 Other sources

37 {JT}(Understand that the “other” sources of DNA damage you propose, at least as
38 written here, are as speculative as ROS is as a means of DNA damage. Again,

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1 dismissing ORS and eplacing it with speculations about what else could be involved
 2 is not satisfying. We seem to allow ourselves the freedom to rely on speculation,
 3 but don't accept speculation by the EPA regarding ROS. When we deal with mode
 4 of action, we are always faced with putting forth the best hypothesis we can based
 5 on available data.

6 of DNA damage exist (including spontaneous oxidative lesions) and arsenic may affect
 7 the ~~activity of enzymes that~~ repair of oxidative and other DNA damage (reviewed in
 8 Rossman, 2003). It is also possible that ~~live~~ cells exposed to the contents of necrotic cells
 9 may experience DNA damage (e.g. via “clastogenic factors” or via inflammatory cells).
 10 Although there is no direct evidence to support this mechanism, it is of interest that heat-
 11 killed *E. coli* instilled into the bladder was found to increase bladder carcinogenesis by
 12 MNU {BR: N-methyl-N-Nitrosourea} (Yamamoto et al., 1992), presumably by an
 13 inflammatory mechanism.

14 There are known direct effects of trivalent arsenicals, including DMA^{III}, on
 15 protein thiols that can affect cytoprotection and cell signaling. These effects may
 16 contribute not only to injury, but also to changes in gene expression and enhanced
 17 proliferation. Further, generation of low levels of oxidants from enzymatic sources
 18 (Smith et al., 2001) or possibly by uncoupling of mitochondrial oxidations

19 {JT} (“uncoupling mitochondrial oxidations” ... would this not lead to ROS?)

20 (if DMA^V can act in a manner similar to arsenate) may contribute to these effects on cell
 21 signaling and transcriptional activation. Finally, effects of inorganic and methylated
 22 arsenicals on thiols in tubulin and cytoskeletal proteins interfere with microfilament
 23 function and cytoskeletal changes that contribute to mitotic arrest and genomic instability
 24 (Li et al., 1992; Ling et al., 2002; Ochi et al., 1999). There is no evidence that hydroxyl
 25 or peroxy radicals play a significant role in these regulatory processes, especially at low
 26 concentrations of arsenicals. Thus, there are too many highly plausible alternative
 27 pathways through which arsenicals can affect the carcinogenic or tumorigenic processes
 28 to commit to oxyradical generation and oxidative damages as a primary key event in the
 29 toxicity of arsenicals. Other effects of trivalent arsenicals that may be applicable to
 30 DMA^{V/III} exposure include: alterations in DNA methylation, effects on DNA repair, and
 31 induction of aneuploidy (reviewed in Rossman, 2003).

32 {JT} (Plausible alternative pathways are not an argument against a stated MOA.
 33 Ruling out the influence from all plausible pathways will always be experimentally
 34 beyond our reach, and it is therefore not a good argument for dismissing ROS. It is
 35 fine to mention these, but much more attention is directed at pointing out the
 36 alternatives to ROS than is directed at clearly articulating why the existing data are
 37 not sufficient to support ROS. Is the existing data sufficient to rule it out? We need
 38 to give everyone a framework for experimentally determining if ROS plays a role,

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1 not just give them a list of alternatives.) (The authors of this section should
2 consider taking p29 L5-18 from the answer to D1 [DFO NOTE: The original
3 document pagination is cited here]. Around this text the authors can expand on why
4 the ROS data are not sufficient and lay the foundation for conducting the necessary
5 experimental work to refine the MOA.

6 The tumor response in the rat bladder system is non-linear, as is the key event (i.e.
7 necrotic cytotoxicity). ~~(Dr. Dragan: More on this please). (This statement really needs a
8 proper explanation + references) {TR}~~ Since the MOA involves cytotoxicity, doses
9 below those causing cytotoxicity would not be expected to cause tumors.

10
11 {MS}(Forwarded on 11-28-2005 an article on Drosophila (Rizki et al., 2005) – “...that
12 may be of interest for elucidating MOA.)
13

14 3.3.2. Human relevance of animal DMA^V MOA:

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

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

22
23 *Please comment on how, if at all, differences in the human population vs.*
24 *experimental animals should be accounted for in the risk assessment for*
25 *DMA^V.*
26

27 **B2. Human relevance of animal DMA^V MOA:** If high enough (cytotoxic)
28 concentrations of DMA^V or DMA^{III} were present in the human urine or bladder after
29 exposure to DMA^V, it is plausible that a similar response (necrosis followed by
30 regenerative proliferation) would take place. However, no data are available to support or
31 reject this assumption. No studies have been carried out on DMA^V-induced bladder
32 cancer in humans, so it is not known at this time whether there have been any cases.
33 Concentrations high enough to cause necrosis in the bladder might be achievable in an
34 industrial accident or deliberate poisoning. It is not clear whether a repeated or chronic
35 exposure to DMA^V from the environment could produce cytotoxic concentrations of
36 critical metabolites in human urine. Even in the case of high exposure, the exposures
37 would probably have to be repeated often enough to produce {TR} persistent necrosis
38 and ~~/or cancer in the bladder~~ regeneration in order to cause cancer.
39

40 {TR}(Again, the confusion with TMAs exists in this paragraph and it appears that
41 there is an assumption that TMA^{III} is present in the rat bladder.) Already mentioned (in
42 charge A1) is the fact that DMA^V is converted to TMA^VO and possibly TMA^{III} more

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1 efficiently by rats than by humans. TMA^VO is a hepatocarcinogen in rats (Shen et al.,
2 2003). TMA^{III} is more potent than DMA^{III} in damaging DNA in *in vitro* systems
3 (Andrews et al, 2003). Thus, although acute toxicities of TMA^VO and TMA^{III} are lower
4 than that of DMA^{III} (Ochi et al., 1994; Sakurai and Kaise, 1998; Yamauchi et al., 1990),
5 these metabolites can contribute to the MOA for DMA^V-induced bladder cancer in rats.
6 The extent of this contribution is unknown. However, it is possible that the rat data over-
7 estimates the human risk for bladder cancers from DMA^V.
8

9 There are no data to suggest that the young are at greater or lesser risk with regard
10 to DMA^V-induced carcinogenesis. {GMA^{III}} {SHa} (The sentence is not clear about whether
11 there are no data because the effect on the young has not been tested or whether the tests
12 have not yielded clear results. Could we clarify?)
13

14 There are little to no chemical specific data regarding an increased susceptibility
15 of humans for bladder tumor development during different life stages. {CH} (The two
16 preceding short paragraphs could be integrated and or have more substance added.
17 Otherwise they could be deleted because they refer to a similar issue as discussed in the
18 last paragraph of 3.4.2, although the latter is not specific to bladder cancer. Also, what is
19 meant by “little to no chemical specific data. If there is little, then there is some, and
20 maybe it should be cited. If there are no data, then there is no data. And chemical
21 specific...does this refer to data specific to different chemical compounds of arsenic? It
22 isn't clear.”)
23

24 **3.3.3. Modes of carcinogenic action from exposure to inorganic arsenic:**

25
26 “Inorganic arsenic (iAs) undergoes successive methylation steps in humans,
27 resulting in the intermediate production of iAs^{III}, MMA^V, MMA^{III}, DMA^V, and
28 DMA^{III}. Each arsenical metabolite exhibits its own toxicity” (USEPA, 2005a).
29

30 *Please comment on the conclusion that the available data support the*
31 *hypothesis that multiple modes of action may be operational following*
32 *exposure to inorganic arsenic.*
33

34 **B3. Modes of carcinogenic action from exposure to inorganic arsenic:** The
35 committee agrees that multiple modes of action may operate in carcinogenesis induced by
36 inorganic arsenic. This is because there is simultaneous exposure to multiple metabolic
37 products as well as multiple target organs. There are differences in metabolic capability
38 and probably transport into and out of different organs for different metabolic products,
39 so that the composition of the metabolites can differ in different organs as well. Each of
40 the metabolites has its own cytotoxic and genotoxic capability. In general, the
41 pentavalent compounds are less cytotoxic and genotoxic than are the trivalent
42 compounds. The primary genotoxic endpoint produced by both inorganic and organic

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1 arsenic compounds *in vitro* is chromosome breakage, most likely mediated by DNA
2 strand breaks resulting from cytotoxicity (Kligerman et al., 2003). DNA strand breakage,
3 ~~sister chromatid exchange SCE~~ induction and clastogenicity are limited almost
4 exclusively to trivalent species. {TR} There is ~~little or~~ no evidence of direct DNA
5 binding of any arsenical to DNA. ~~or~~ Point mutations occur at low levels in arsenite-
6 treated cells, and only at cytotoxic concentrations (Rossman 2003), ~~from either trivalent~~
7 ~~or pentavalent arsenic compounds~~, except as a secondary result of genomic instability
8 (Mure et al., 2003). Genotoxic activity *in vivo* is limited to a small number of studies in
9 rodents. IP injections of high doses of DMA(DMA^V) induced a slight but insignificant
10 increase in mutagenesis in the MutaTM Mouse lung, but not in bladder or bone marrow.
11 Arsenite was also negative in this assay (Noda et al., 2002). Arsenite induced
12 micronuclei in mouse peripheral blood lymphocytes and in mouse bone marrow (Tinwell
13 *et al.*, 1991; Noda *et al.*, 2002). DMA did not induce micronuclei in mouse peripheral
14 blood lymphocytes (Noda *et al.*, 2002), but did induce aneuploidy in mouse bone marrow
15 cells (Kashiwada *et al.*, 1998). {CH}(Unless I am confused here, shouldn't the epi
16 studies that have found elevated micronuclei frequency in exfoliated bladder cells among
17 arsenic-exposed individuals be mentioned in the following sentences as evidence of
18 genotoxicity? E.g., Moore et al., papers). Genotoxic activity found *in vivo* is limited to a
19 small number of studies in rodents indicating that highly toxic doses of arsenic
20 compounds may induce micronuclei and/or aneuploidy in non-target tissues. {DBr} (the
21 sentence should be deleted – Andrews et al. 2003 shows TMA damages purified DNA in
22 vitro.) ~~There is no genotoxicity data available for other arsenic compounds found in~~
23 ~~rodents such as TMA.~~ {GMA}(What arsenic compounds are the genotoxic and what
24 compounds are you referring to as having no data? The way it is currently written, it
25 looks like the arsenic compounds are both genotoxic and non-genotoxic.)

26 {TR} I would like to respond to the suggestion that we include the Epi studies
27 showing elevated micronuclei (MN) in exfoliated bladder epithelial cells. While I
28 believe that the data is real (it has been found in many studies and in many
29 places), it does not really help with MOA. I will quote from something I wrote a
30 short while ago:

31
32 "MN are defined as small, round, DNA-containing cytoplasmic bodies formed
33 during cell division by loss of either acentric chromatin fragments or whole
34 chromosomes. The two basic phenomena leading to the formation of MN are
35 chromosome breakage (double strand breaks associated with clastogenesis) and
36 dysfunction of the mitotic apparatus (aneugeneis). Aneuploidy is defined as a
37 change in chromosome number from the normal diploid or haploid number other
38 than an exact multiple (polyploidy). MN as a result of clastogenesis contain
39 acentric chromosome or chromatid fragments and MN associated with aneuploidy
40 contain whole chromosomes that lag behind in anaphase and are left outside the
41 daughter nuclei in telophase. Lagging chromosomes cannot move to the poles,
42 because they are detached from the mitotic spindle.

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1 Because cell division is necessary for the generation of MN, the cytokinesis block
2 micronucleus (CBMN) assay is recommended for use with human lymphocytes
3 (Kirkland et al., 2003). In this assay, cultures are treated with cytochalasin B, an
4 inhibitor of actin polymerization. Cytochalasin B prevents cytokinesis but allows
5 nuclear division, resulting in cells with multiple nuclei. It is thus possible to
6 identify cells that have divided once, because they contain two nuclei. By
7 restricting scoring of micronuclei only to cells with two nuclei, problems caused
8 by variations in cell division due to exposure to toxicants are eliminated.
9

10 In the past, several attempts have been made to distinguish between the aneugenic
11 and clastogenic action of test compounds. Currently, the most widespread and
12 reliable assays identify whole chromosomes in MN by labeling their kinetochores
13 or centromeres. Kinetochore proteins can be identified by immunofluorescence
14 with anti-kinetochore antibody (labeled MN are termed K+) while centromeric
15 DNA sequences can be identified by FISH using repetitive DNA sequence probes
16 (labeled MN are termed C+). However, only a few laboratories routinely use these
17 techniques because they are very costly. When these techniques are used, the in
18 vitro MN assay is considered a suitable alternative to in vitro chromosome
19 aberrations tests for detection of clastogenic and aneugenic agents.
20 It is recommended that this assay should be performed under conditions of high
21 survival (an increase of >90% in number of viable cells). It is also recommended
22 that markers for apoptosis and necrosis be included (Kirsch-Volders et al., 2003).
23 At least 2000 cells should be scored per concentration (1000 per culture, in
24 duplicate)."
25

26 When MN are measured in Epi studies, there is not enough information to
27 determine whether the MN result from: 1) toxicity, 2) clastogenicity, or 3) non-
28 dysjunction (leading to aneuploidy). Thus, one cannot say that the MN result from
29 a genotoxic insult (i.e. clastogenesis). Aneuploidy is an event that has a threshold
30 (see papers by Kirsch-Volders), whereas many people assume that clastogenesis
31 does not (at least for ionizing radiation). Also, MN in cells is a trigger for
32 apoptosis, so many cells with MN will have no progeny.
33

34 Furthermore, Giri's studies on MN induction in India show that arsenic exposed
35 individuals have increased MN in bladder cells, lymphocytes and buccal cells,
36 with the greatest effect in lymphocytes. But arsenic exposure is not associated
37 with blood cancers or cancer of the mouth. Thus, MN does not help us to
38 understand bladder cancer.
39

40 Animal studies indicate that for some organs, transplacental carcinogenesis after
41 maternal exposure to inorganic arsenic occurs. This includes the formation in C3H mice
42 of tumors of the lung and liver, target sites of potential human relevance, after exposure

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1 to arsenic *in utero*. In addition, *in utero* arsenic induces tumors of the ovary and adrenal,
2 sites not observed in humans to date. The C3H mouse was selected in these studies
3 because it is, in general, sensitive to chemical carcinogenesis, although this strain shows
4 spontaneous tumor formation in several tissues. {TR} Other studies indicate that ~~in fox~~
5 skin, ~~inorganic arsenic compounds are not complete carcinogens, but act as enhancers~~
6 ~~(cocarcinogens, sometimes mistakenly called “promoters”)~~ with other agents. Arsenite
7 acts as a cocarcinogen with solar UV light (Rossman *et al.* 2001; Burns *et al.*, 2004) and
8 ~~arsenate is cocarcinogenic with 9,10 dimethyl-1-2-benzanthracene (Motiwale *et al.*,~~
9 ~~2005).~~ ~~and not a complete carcinogen.~~ This leaves open the possibility that a
10 cocarcinogenic MOA may also operate for other organs, but this remains to be tested
11 ~~(only money is needed)~~{StH, XCL, GMA}.

12
13 ~~At this time~~ One cannot dismiss the possibilities of hormesis effects in humans
14 exposed to low-dose arsenic or the essentiality of arsenic to humans {TR} (Snow *et al.*,
15 2005). Evidence for essentiality of arsenic has been reported for a number of mammalian
16 species as well as for chickens (reviewed in Uthus, 1992). {BR: Could this be
17 reworded? I’m uncomfortable with the suggestion that we are supporting the essentiality
18 of arsenic, which I personally don’t believe. For example, in chickens and other farm
19 animals, arsenicals may serve as antimicrobial agents that improve growth yields by
20 preventing infections, but this doesn’t mean that arsenic is an essential element. }

21 These may explain some of the apparent low-dose benefits seen in a variety of systems.
22 For example, inorganic arsenic has both positive and negative effects on the growth and
23 function of blood vessel (Soucy *et al.*, 2003, 2005; Kamat *et al.*, 2005). Low
24 concentrations fuel angiogenesis, while higher concentrations injure endothelial cells and
25 promote the vessels dysfunction seen in ischemic diseases and peripheral vascular
26 diseases. Thus at low levels arsenic may provide improved vascularization and growth
27 of normal tissues, which could reduce cardiovascular risks. However, this process poses
28 a high risk for arsenic increasing the vascularization and growth of both atherosclerotic
29 lesions (Simeonova and Luster, 2004) and tumors from a secondary source (Kamat *et al.*,
30 2005). The potential for arsenicals to enhance tumorigenesis through enhanced
31 vascularization has been demonstrated in mice drinking 10-250 ppb iAs^{III} (Kamat *et al.*,
32 2005). However, arsenic at high doses has been used to destroy the tumor vasculature
33 (Griffin *et al.*, 2003). {CH}(This entire section on essentiality is unclear and confusing. I
34 suggest deleting it here – the point about essentiality is in the preceding section.) If
35 arsenic is essential for humans and/or if epidemiological data could be strengthened at the
36 low-dose range to demonstrate either a low-dose benefit or no effect at low dose, then a
37 threshold is certain. However, at this time, the data are lacking or problematic with
38 regard to low-dose effects. This is an extremely important issue and should be
39 investigated.

40
41 {DB – The document references that there are insufficient experimental data
42 showing that cytotoxic effects from DMAIII/V exposure produce damage at animal dose

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1 levels associated with tumorigenic responses in the bladder. I believe that EPA should be
2 encouraged to investigate this area more thoroughly in order to fill gaps in the MOA as
3 proposed in the second paragraph of page 16. The EPA should want to resolve the issue
4 of the precise role of DNA in the MOA.}

6 3.4. Selection of Data for Dose-Response Assessment

8 3.4.1. Use of animal data for DMA^V

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

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

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

26 This question indirectly raises the issue as to the largest source of uncertainty for
27 DMA^V risk assessment—conventional interspecies extrapolation or extrapolation across
28 various forms of arsenic. The available material suggests that extrapolation across
29 various forms of arsenic would lead to the greatest degree of uncertainty in a risk
30 assessment. Although the panel agreed that use of the rat bioassay data is the preferred
31 alternative, the panel also felt strongly that a discussion of the key uncertainties with
32 using data from testing in rats to conduct human risk assessment should be included in
33 EPA’s “*Science Issue Paper: Model of Carcinogenic Action for Cacodylic Acid
34 (Dimethylarsinic Acid, DMA^V) and Recommendations for Dose Response
35 Extrapolation.*”. Issues that panel members consider important to discuss in EPA’s
36 Science Issue Paper are discussed in more detail below. {XCL: Referring to Section
37 3.2.1 would be useful.} These issues relate to the toxicokinetic and toxicodynamic
38 differences between rats and humans in response to arsenic exposure, the use of rodent
39 bladder tumor models in general, and issues in the use of rodent data for human risk
40 assessment.

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1 Data illustrating the mode of action for DMA^V as a bladder carcinogen in rats
2 seem quite convincing. However, rats are much more sensitive to DMA^V in
3 carcinogenicity testing than the mouse (Rossman, 2003; Arnold, et al., 2003). Several
4 toxicokinetic and toxicodynamic differences between rats and humans have also been
5 reported after arsenic exposure. For example, arsenic methylation in rat liver hepatocytes
6 proceeds at a faster rate than in human hepatocytes; and rats have a considerably slower
7 whole body clearance of DMA than humans. This slower whole body clearance in rats is
8 because a significant portion of DMA is retained in the erythrocytes of rats (Vahter, et al.,
9 1984). There is a 15 to 20 fold higher binding of arsenic to rat hemoglobin than to human
10 hemoglobin (Lu, et al, 2004). Human bladder tumors are primarily transitional cell
11 carcinomas, and rat bladder tumors are reported to bear some similarity in pathology to
12 low-grade papillary tumors that occur in humans; however, they are not similar to
13 invasive human bladder tumors that display high grade malignancy (Cohen, 2002). The
14 foregoing, taken together, illustrate known substantial metabolic, pharmacokinetic and
15 pharmacodynamic differences between rats and humans and should be thoroughly
16 discussed in the final EPA documents as these data indicate that the rat is likely to be
17 considerably more sensitive to developing bladder cancer than humans after exposure to
18 DMA^V. {MW: I do have an issue with the text on page 20 line 32-35—I think that the
19 distinction made between the rat urinary bladder tumors (“Low grade” transitional cell
20 papillomas) and human UB tumors associated with arsenic (“high grade” invasive
21 transitional cell carcinomas) in this text is not one of qualitative substance. First of all,
22 the majority of the rat tumors induced by DMA were diagnosed as transitional cell
23 carcinoma not low grade papilloma. So the rat and human UB tumors are the same cello
24 type and are both carcinoma (which by pathological definition has the quality of
25 invasiveness). The human bladder tumors in these third world countries are likely only
26 recognized when they cause overt symptoms and therefore at a late stage. The rat UB
27 tumors in the Fukushima study were discovered at the 2 year necropsy (mostly) in animals
28 intentionally killed at this time. Had the authors let the rats go until death these lesions
29 may well have progressed to “more” invasive carcinoma. ---- So this is largely an esoteric
30 argument concerning comparative pathology and tumor progression, not a major
31 qualitative difference. I think this text should be deleted or at least corrected to be in line
32 with the facts of this study. To say that these rat tumors “bear some similarity in
33 pathology to low grade papillary tumors” is a clear distortion. There may be biokinetic
34 and biodynamic issues but there are not real issues in UB tumor pathology. }

35
36 A second major uncertainty associated with using bladder tumor data from rats is
37 the lack of knowledge about levels of DMA^{III} produced in the human bladder upon
38 exposure to DMA^V and how that compares to levels of DMA^{III} produced in rats exposed
39 to DMA^V. The few human exposure studies that exist seem to indicate little if any
40 DMA^{III} production takes place. {MS} (Preceding sentence needs to be reworded. There
41 are no studies on DMA^{III} formation in humans exposed to DMA^V. In humans exposed
42 to iAs, DMA^{III} is the major urinary metabolite when fresh urines are analyzed (see

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1 **responses to A1).)** This is because DMA^V is not absorbed well -- approximately 80% of
2 a dose of the parent compound is excreted in a short time after exposure (Buchet, et al.,
3 1981; Marafante, E., et al., 1987). Additionally rat urothelial cells are 3.5 times more
4 sensitive to DMA^{III} than are human urothelial cells in *in vitro* studies (Cohen, et al.,
5 2000).
6

7 These toxicokinetic and toxicodynamic factors should be taken into account in the
8 application of rat bladder tumor data to assess human bladder cancer risk. These factors
9 will impact the choice of uncertainty factors since the weight of evidence indicates that
10 the rat is considerably more sensitive to bladder tumor induction from direct exposure to
11 DMA^V than are humans. **{MS}(The preceding sentence needs to be reworded. There is
12 no evidence, per se, that rats are more sensitive to DMA carcinogenesis than humans,
13 although the urinary excretion of trimethyl-As metabolites that are not found in humans
14 may suggest so.)** Although selection of a safety factor is the province of EPA's policy
15 choice, the Panel believes that in the case of the Food Quality Protection Act 10X safety
16 factor for this element of risk assessment, the science supporting a smaller factor could
17 lead EPA to choose to lower the factor for arsenic to some number less than 10. The
18 increased sensitivity of rats relative to humans could be taken into account. The Arsenic
19 Review Panel's analysis of the toxicokinetic data indicates that an uncertainty factor for
20 extrapolation from rat toxicokinetic data to human risk in this case is likely to be less than
21 one. The analysis of the toxicodynamic data indicates that the uncertainty factor may
22 also be lower than the default. The application of uncertainty factors has also been
23 addressed in the Panel's response to question D1. **{JT and MM: There is a question of
24 how the issue of the Safety Factor of 10 should be handled. This issue is in common with
25 the discussions here in 3.4.1 (i.e., C1) and 3.5.1 (i.e., D1). The issue has been dealt with
26 in the two sections as having PD and PK components and there is a suggestion that the
27 Safety Factor can be reduced. There is an issue of whether to suggest some factor that
28 the components could be reduced to or just to suggest to EPA that they should consider
29 reducing the factor. The issue needs to be discussed at the Panel meeting. WE NEED TO
30 POINT OUT THIS IS A POLICY ISSUE THAT THE PANEL ADVISES UPON—
31 CROSS WALK TO D1 as WELL—SEE SECTION IN 3.5.1}**
32

33 The Agency should also discuss in its Science Issue Paper, differences between
34 rats and humans in the development of bladder tumors, and how these differences impact
35 interspecies extrapolation. For example, urinary bladder tumors in rats occur very late in
36 life. **{GMa} (I am not sure why the comment is frequently made that bladder cancers in
37 rodents occur very late. That is true of human cancers as well. Bladder cancer is usually
38 one of the latest occurring cancers in humans. However, the age of occurrence also may
39 relate to the age of first exposure. Therefore, this is a complicated issue in humans and it
40 hardly seems an appropriate reason to try to refute the use of animal data for human
41 extrapolation because of the age of onset of disease in rats.)** Studies suggest that in rats
42 it takes two or more years of continuous high dose exposure to DMA^V to induce these

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1 tumors. This would equate to a human ~~being~~—developing cancer very late in life as well.
2 The Science Issue Paper should specifically discuss the similarities and differences in the
3 time for induction of DMA^V related tumors in rats with the pattern observed with humans
4 and arsenic associated urinary bladder cancer.
5

6 EPA'S Science Issue Paper should also discuss general issues associated with rat
7 urinary bladder cancer. One such issue is the relationship between the non-specific
8 {TR}(What is meant by the non-specific induction of tumors and high concentrations...?)
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. {TR}(The issue is not that tumors can occur spontaneously. It is that the
33 strain of mouse used in the transplacental studies has a very high incidence of
34 spontaneous tumors. If these results cannot be reproduced in a more normal strain of
35 mouse, one must view the results as a kind of cocarcinogenesis (arsenic enhancing an
36 endogenous carcinogenic process). EPA's Science Issue Paper does not even address the
37 question of cocarcinogenesis of inorganic arsenic. It confuses tumors with paps and
38 promotion with cocarcinogenesis (p. 39) and Section 3.B (p. 40) contains numerous
39 errors as well.)
40

41 *Please comment on whether the iAs epidemiology data can be used to*
42 *inform the DMA^V dose-response assessment derived from rat data with*

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1 *DMA^V. If so, please discuss how such information might be used. (See*
2 *Appendix).*

3
4 **C. 1 (B).** :The panel consensus was that without more detailed information on
5 target tissue dosimetry of arsenic species the iAs epidemiology data would be of limited
6 use to inform the DMA^V dose-response assessment derived from rat data with DMA^V.
7 Direct exposure to iAs elicits a different cascade of metabolite concentrations with
8 related differential kinetics compared to direct exposure to DMA^V, therefore the iAs
9 epidemiology data cannot reasonably be used to inform the DMA^V dose-response
10 assessment derived from rat data with DMA^V. In the absence of specific information on
11 target tissue levels, assumptions would have to be made regarding the proportion of the
12 iAs for human and DMA^V for rodents that reaches the bladder tissue as the toxic DMA
13 species.

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

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

26
27 “Since the NRC (2001) report on iAs, an additional body of literature has
28 developed describing epidemiology data from populations in the US exposed to
29 iAs in drinking water” (USEPA, 2005a).

30
31 *Does the SAB agree that the Taiwanese dataset remains the most*
32 *appropriate choice for estimating cancer risk in humans? Please discuss*
33 *the rationale for your response.*

34
35 **C2. Use of human epidemiological data from direct iAs exposure:** The
36 Taiwanese dataset consists of population and mortality data from 42 villages in southwest
37 Taiwan for the years 1973-1986. Arsenic levels in wells from these villages were
38 measured in 1964-1966. The database is one of the largest that has been evaluated for
39 cancer risk relative to arsenic exposures. A total of almost 900,000 person years of
40 follow-up were included, with 1,152 cancer deaths (637 males, 515 females). Among the
41 cancer deaths were 181 due to bladder cancer (85 males, 96 females), 268 lung cancer
42 (147 males, 121 females), and several hundred due to other types of cancer. These data

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1 have been subject to several ecologic analyses, starting with the original publications by
2 Chen et al. (1988) and Wu et al. (1989), followed by further analyses by Morales et al.
3 (2000) and by the National Research Council (1999 and 2001).
4

5 Among the 42 villages, the arsenic concentration ranged from 10 to 934 ppb ($\mu\text{g/L}$).
6 Twenty of these 42 villages used one well. Among many of the 21 {GM}(Where is the 1
7 missing village in these counts? The next page has 22.) villages with multiple wells,
8 many had wide variability in the measured arsenic level in their wells. Analyses using
9 the full dataset give results comparable to results from a reduced dataset including only
10 the villages with single wells, providing some confidence in the stability of the overall
11 results (National Research Council, 1999). The Panel recognizes the limitations of the
12 southwest Taiwan database, including its ecologic character, lack of smoking
13 information, limited precision of exposure estimates, especially among villages with
14 multiple wells, and the possible issue of compromised nutrition among segments of the
15 exposed population. {GM}(Isn't the fact that the data sets from Taiwan have been
16 subjected to many years of peer review, as part of published studies, an important plus for
17 these data as well?) However, in view of the size and statistical stability of the database
18 relative to other studies, the reliability of the population and mortality counts, the stability
19 of residential patterns, and the reliability of the exposure assessment {GM}(You talk
20 about the "reliability of exposure" here but just above say it lacks precision. That needs
21 clarification), it is the Panel's view that this database remains, at this time, the most
22 appropriate choice for estimating cancer risk among humans.

23 {JT}(I urge the panel to revise the ending of the paragraph to reflect the text that
24 follows describing the limitations of this data set. For example:

25 "...this database remains, at this time, the most appropriate choice for estimating
26 cancer risks in humans, but given its limitations, alone it is not sufficient for
27 estimating risks to humans. Additional, work to test, validate and compare risk
28 estimates made using this data set must be completed, as suggested in the
29 following sections."
30

31 The Panel recommends that other epidemiologic databases from studies of
32 arsenic-exposed populations be used to scale the unit risks at high exposure levels that
33 emerge from the Taiwan data. Several of these studies had the advantage of data with
34 excellent exposure assessment. In addition, some populations likely differed from the
35 Taiwanese population with regard to their nutritional status. The accuracy and precision
36 of exposure assessment is a major issue in all environmental epidemiologic studies, and
37 in particular, in studies of arsenic in drinking water. Misclassification of exposure in
38 such studies (when non-differential) can have a profound effect in depressing the
39 magnitude of the observed risk. The excellence of exposure assessment is an especially
40 strong aspect of several studies from northern Chile, and the Panel recommends that the
41 findings of Smith et al. (1998) and of Ferreccio et al. (2000) be considered by EPA
42 {JT}(What does it mean "be considered by EPA." Be more specific, how should it be

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1 ~~used?)~~ In addition, arsenic exposures appear to be well characterized in cohort studies of
 2 Chiou et al.(2001) of transitional cell carcinoma (mostly bladder cancers) and Chen et al.
 3 (2004) of lung cancer, from arsenic-exposed cohorts in southwest and northeast Taiwan.
 4 The latter study also provides data on the joint effects of arsenic and cigarette smoking in
 5 the Taiwanese population. It should be possible to go through a complete risk assessment
 6 using at least one other of these databases. {JT} (“it should be possible” ~~should be revised~~
 7 ~~to stronger language and expand the reason this is important: model and data set~~
 8 ~~validation, uncertainty assessment, sensitivity to data sets, etc.)~~
 9

10 ~~{JY} In contrast, problems in t~~ The accuracy of estimated long-term exposures to
 11 arsenic ~~in some recent studies with individual data conducted in the United States and~~
 12 ~~elsewhere among populations exposed to levels~~ is of concern for some recent studies
 13 under 100 ppb. ~~compromises~~ This may compromise their overall utility ~~of these data for~~
 14 ~~long-term estimates of exposure (>20 years)~~ in assessing concordance with risk estimates
 15 ~~obtained from the Taiwan study.~~ The Panel suggests that results on bladder cancer risk
 16 from published epidemiology studies of US and other populations chronically exposed
 17 from 0.5 to 160 µg/L inorganic arsenic in drinking water be critically evaluated. EPA
 18 ~~should determine regarding~~ their potential utility in exploring overall concordance of the
 19 cancer risk estimates derived from their data with risk estimates obtained from
 20 extrapolation of the Taiwan data [Bates (1995), Lewis (1999), Steinmaus (2003),
 21 Michaud (2004), Bates (2004)]. {SHa} ~~(corss-reference this line with section 3.5.1.)~~
 22

23 When reviewing these “low-level” studies, {JY} ~~as well as the “high level”~~
 24 ~~studies,~~ at least the following should be considered: The effect of exposure
 25 misclassification on estimates of risk; temporal variability in assigning past arsenic levels
 26 from recent measurements; the extent of reliance on imputed exposure levels; the number
 27 of persons exposed at various estimated levels of waterborne arsenic; study
 28 response/participation rates; estimates of exposure variability; and the resulting influence
 29 of these factors on the magnitude and statistical stability of risk estimates. US and other
 30 populations differ from the Taiwanese population of interest in genetic background,
 31 dietary intake, and background exposure concentrations to inorganic arsenic, and if one
 32 or more of these studies are shown to be of potential utility, comparative analyses of the
 33 US and Taiwan data may lead to further insights into the possible influence of these
 34 differences on population responses to arsenic in drinking water. For compounds such as
 35 arsenic for which there are human data beyond the Taiwanese study on which human
 36 cancer risk has been based, data from the other, {JY} ~~less robust,~~ investigations at high
 37 exposure levels (>150 ug/l) can be used to gauge the Taiwanese findings (REFERENCE).
 38

39 All of these studies including those from Taiwan, Chile, Argentina and the U.S. as
 40 described above should be judged by the same set of criteria, with the comparative
 41 assessment of those criteria across studies clearly laid out in a tabular format. {JY} ~~We~~
 42 ~~recommend that~~ At least some the criteria ~~be listed and described,~~ have been listed in the

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1 ~~previous paragraph.~~ ~~with~~ The relative strengths and weaknesses of each study ~~spelled~~
2 ~~out~~ ~~need to be described~~ in relation to each criterion. The caveats and assumptions used
3 should be presented so that they are apparent to anyone who uses the data. Included in
4 the risk assessment background document should be a complete and transparent treatment
5 of variability within and among studies and how it affects risk estimates. The present
6 lack of transparency in the application of the criteria in the process of study selection was
7 pointed out by several panel members.

8
9 {JY Insertion}

10
11 As recommended in the preceding sections, aggregate results, particularly on bladder
12 cancer risk, from multiple published epidemiology studies of low level arsenic-exposed
13 populations need to be taken into consideration in a more formal secondary integrative
14 analysis and compared with the main analysis for concordance. Data from the
15 epidemiologic studies of relatively low exposure can be informative and need to be
16 formally evaluated beginning with a comparative analysis of strengths and weaknesses as
17 described above.

18
19 A sensitivity analysis to formally evaluate the potential impact of sources of bias (non-
20 random error) in the low level case control and cohort studies is recommended since non-
21 differential misclassification cannot be routinely assumed. These several recent arsenic
22 epidemiology studies have the advantage of data with exposure assessment at a range of
23 exposure levels relevant to those experienced by the US population—exposure levels in
24 these studies range from 0.5 to 160 µg/L inorganic arsenic in drinking water (Bates et al.,
25 1995; Karagas et al., 2004; Lewis et al., 1999; Kurtzio et al., 1999; Steinmaus et al., 2003;
26 Bates et al., 2004). Most of these populations have a nutritional and genetic background
27 similar to that of U.S. or were conducted in a U.S. population.

28
29 Precedents for formally integrating health outcome information from a number of
30 epidemiology studies are readily available. Although, ideally, one would prefer
31 individual measures of exposure to be available in all studies, it is recognized that the
32 Taiwan study of 42 villages herein recommended as the basis for arsenic cancer risk
33 estimation is an ecological study with uncertainty as to individual exposure levels.
34 Recommendations for assessing the range of uncertainty have been put forth in this report
35 in the section immediately following.

36
37 Arsenic epidemiological literature is an instance in which a number of quality (but not
38 ideal) epidemiology studies are available. Quantitative exposure-response modeling for
39 other compounds for which integrative risk analyses were carried out utilizing multiple
40 epidemiology studies have been conducted and health risks for defined outcomes
41 estimated. For example, NRC/NAS (2000) conducted an integrative analysis of three
42 studies of *in utero* exposure to methylmercury (MeHg) and a number of

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1 neurodevelopmental outcomes in children. Statistical power among studies was
2 examined and was found not to be principally accountable for observed study-to-study
3 differences in outcomes at similar exposure levels; likewise p values for outcomes were
4 not found to be particularly useful in comparing studies, but rather comparative dose-
5 response estimates (i.e., regression slopes) were chosen as the most optimum comparative
6 basis for integration. Likewise, four recent studies (Konig et al., 2005; Bouzan et al.,
7 2005; Cohen et al., 2005a; Cohen et al., 2005b) amply illustrate the conduct of integrative
8 exposure analyses and health outcome. In an integrative analysis of fish consumption
9 and coronary heart disease mortality, eight studies (29 exposure groups) were identified
10 that met pre-established study quality criteria, had quantified exposure (e.g., fish intake)
11 and had reported the precision of relative risk estimates (Konig et al., 2005). Averaged
12 relative risk results were weighted proportionately by precision. In another integrative
13 analysis, a quantitative exposure-response function for prenatal MeHg exposure and IQ
14 was developed using data from three different epidemiology studies (Cohen et al.,
15 2005a). Weights were assigned to measures of cognitive performance for each of seven
16 test domains; an integrated sensitivity analysis was conducted to assess the impact of
17 alternative assumptions on the final integrative study results.

18
19 Studies for inclusion in each integrative analysis were selected on the basis of *a priori*
20 established criteria. As previously stated, inclusive evaluation of all arsenic
21 epidemiology studies (both “low” and “high” exposure studies) by pre-set standard
22 criteria and presentation of results in tabular format has been recommended by this Panel.
23 This is the initial step in conducting an integrative analysis.

24
25 For most compounds of human health concern, epidemiologic data are generally not
26 available (see A2); but occasionally, as in the case of arsenic, one or perhaps a few
27 epidemiology studies will be available. To improve validity, it is important to support
28 human cancer risk estimates using the maximum available scientific information and
29 contemporary risk assessment methodology. The current cancer risk assessment
30 methodology for iAs relies on choosing a single epidemiological study to derive a cancer
31 slope factor that is then used to extrapolate health effects considerably below the
32 exposure levels observed in that study. There are a number of arsenic epidemiology
33 studies now available; there are published methods for quantitatively integrating results
34 from multiple studies (Coull et al., 2003; Ryan, 2005).

35
36 Integrative analyses result in improved statistical power and precision of the estimates
37 that represent an additional advantage of utilizing a larger dataset, as has been pointed out
38 for the Taiwan dataset. Although the “low” arsenic exposure epidemiology studies cannot
39 by themselves provide a basis for dose-response modeling because of lack of data at the
40 higher exposure levels (see D2), they do provide data on the relative risks of bladder
41 cancer for humans exposed at low levels. The Panel suggests, as described in detail in
42 this section that an effort be made to conduct a secondary integrative analysis applying

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1 similar approaches to those described above to assess concordance with exposure-
2 response models derived from the outcome of the primary analysis.
3

4

5 {JY} In response to Dr. Harlow’s questions in her comments, Dr. Yager notes:
6 The charge to the committee states that we must address all questions AND
7 THEN WE ARE FREE TO COMMENT ON ANY OTHER ASPECT WE WISH.
8 These paragraphs were submitted in the latter vein as stated.
9

10 The suggestion being made in these paragraphs is that, in general, EPA needs to
11 consider at some point changing the paradigm of using just one epidemiology
12 study to conduct a risk analysis and consider methods to integrate results when
13 several epidemiology studies evaluated on the same set of criteria are available. It
14 appears that this suggestion is not particularly controversial. Of course, this Panel
15 cannot recommend EXACTLY how EPA should conduct an integrative analysis
16 for arsenic. As correctly pointed out by Sioban, that is outside the scope of this
17 Panel.
18

19 These paragraphs make very clear, however, that an integrative approach is being
20 suggested for future consideration by US EPA and is giving EXAMPLES (note
21 the term exemplify used in these paragraphs) of how integrative analyses have
22 been conducted for ANOTHER compound—methylmercury. NO WHERE is it
23 suggested in these paragraphs that EPA conduct an analysis for arsenic
24 EXACTLY as was carried out in the examples provided. The fact that these
25 integrative analyses and methods are published (and therefore referenced) verifies
26 the fact that the integrative approach is being applied; that this is not simply some
27 random idea that cannot be carried out in the real world. Again, referencing these
28 studies does NOT in any way specifically suggest that EPA follow these exact
29 examples.
30

31 It may very well be that the quality of current arsenic epidemiology studies in
32 general simply cannot meet the requirements for a reasonably rigorous integrative
33 analytical approach at this time. So be it. The point is to make the suggestion
34 that US EPA be considering such an approach in general, and at the very least in
35 this instance, compare ALL of the arsenic epidemiology studies currently in
36 existence by the same set of criteria.
37
38

39

40

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1 ~~Regarding the southwest Taiwan data from 42 villages, the Panel suggests that EPA~~
 2 ~~conduct sensitivity analyses, using the range of exposures in the villages with more than~~
 3 ~~one well to provide a range of the risk.~~

4
 5 ~~(Note: The following is an adaptation of the response originally to D2):~~

6
 7 {GMA} (This paragraph seems out of place where it is – do we need a lead in or
 8 something?) {CH} (This should be merged with the preceding paragraph. See following
 9 changes) Given the concerns regarding the use of the median well water concentrations
 10 in some of the 42 villages in Southwest Taiwan that have with more than one well
 11 measurement, the Panel recommends that EPA the Agency conduct a sensitivity
 12 analysis. This should include the range of exposures in said villages to provide a range
 13 of risk estimates. ~~of the estimated model for arsenic exposure hazard to the assumption~~
 14 ~~that all village residents were exposed to the median well water concentration in~~
 15 ~~communities served by multiple wells.~~ One alternative (suggested in response to D-3)
 16 is a full Monte Carlo analysis in which the individual well concentrations for 22
 17 villages with multiple wells are taken into account. The Panel recognizes the difficulties
 18 with this approach including the issue of how to allocate cases to wells within villages.
 19 A simpler, but useful first approach would be to test the sensitivity of the model fitting
 20 when arsenic concentrations for multiple-well villages are set to: 1) a low level
 21 concentration from the range for the village ({SHA} ~~minimum~~, 10th percentile, 20th
 22 percentile); 2) the median (current procedure); and 3) a high level concentration from
 23 the village range ({SHA} ~~maximum~~, 90th percentile, 80th percentile).

24
 25 *Do these data provide adequate characterization of the impact of*
 26 *childhood exposure to iAs? Please discuss the rationale for your*
 27 *response.*

28
 29 The Taiwanese data are inadequate to characterize the impact of childhood
 30 exposure to inorganic arsenic with respect to carcinogenesis. That is, it is not clear
 31 whether children differ from adults with regard to their sensitivity to the carcinogenic
 32 effects of arsenic in drinking water. More data are needed to fully characterize the impact
 33 {CH rewords as follows} of transplacental exposures. However, data from the studies in
 34 Southwestern Taiwan which include and childhood exposures in the calculation of
 35 lifetime dose, show that in the population under 30 years of age there were no bladder
 36 cancer cases, and only 5 lung cancer cases. ~~however the Southwestern Taiwanese data~~
 37 ~~are as good as other studies in this regard and do have more information than most others.~~
 38 ~~In these data, no bladder and 5 lung cases were observed in population age <30 years.~~
 39 Childhood exposures are included in the lifetime dose estimates. Smith et al (1998) report
 40 the highest excessive risk for male lung cancer in the 30-39 year old age group,
 41 suggesting the importance of childhood exposure and risk {JY} and perhaps smoking
 42 behavior as young adults. For 533 women exposed to arsenic in drinking water from tube

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1 wells at greater than 50 µg/L compared with those exposed at 50 µg/L, or less, findings
2 suggest that there are significantly increased odds ratios for spontaneous abortion,
3 stillbirth and neonatal death (Milton et al., 2005). Another reproductive study in Chile,
4 which followed over 800 pregnancies, found that pregnant women drinking water
5 containing 40 ug/L gave birth to infants of lower birth weight than a comparable group
6 drinking water containing very low arsenic concentrations (<1 ug/L) (Hopenhayn et al,
7 2003). Thus maternal exposure at {CH}moderately high levels may have untoward
8 toxicity effects; the issue of childhood carcinogenic susceptibility has not been {JY} well
9 extensively addressed.

10 3.5. Approaches to Low-Dose Extrapolation for Inorganic Arsenic and DMA^V

11 3.5.1. Mode of carcinogenic action understanding for DMA^{V/III} and 12 implications for dose response extrapolation to estimate human cancer risk:

13 “The use of mode of action data in the assessment of potential carcinogens is a
14 main focus of EPA’s 2005 cancer guidelines. As stated in these guidelines “The
15 approach to dose-response assessment for a particular agent is based on the
16 conclusion reached as to its potential mode(s) of action”. Although a biological-
17 based model is the preferred approach to estimating cancer risk, there are
18 insufficient data on DMA^V to support development of such a model” (USEPA,
19 2005a).
20
21
22

23 *Please comment on the scientific evidence and biological rationale in*
24 *support of nonlinear versus linear low dose extrapolation approaches,*
25 *which approach is more consistent with the available data on DMA^V and*
26 *current concepts of chemical carcinogenesis, and how scientific*
27 *uncertainty should most appropriately be incorporated into low-dose*
28 *extrapolation.*
29

30 **D1: Mode of carcinogenic action understanding for DMA^{V/III} and**
31 **implications for dose response:** (1) *Please comment on the scientific evidence*
32 *and biological rationale in support of the nonlinear versus linear low dose*
33 *extrapolation approaches,*
34

35 The committee felt that there are adequate data to support a MOA for bladder
36 carcinogenesis induced by high doses of DMA^V in the rat {JT}(see B3). The MOA that
37 involves cytotoxicity of the bladder epithelium and increased, sustained regenerative
38 proliferation as a key events. {GMA}(Initially you mentioned that carcinogenesis needed
39 3 steps but here you only talk about the cytotoxicity and regeneration as a route to
40 cancer. Don’t we need to clarify this relative to the previous statements?) The urine of
41 DMA^V-treated rats contains DMA^{III} at levels that cause necrotic cytotoxicity in these

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1 **cells *in vitro***, so it is reasonable to postulate that DMA^{III} might mediate the necrotic
2 cytotoxicity in the rat bladder. A role for other rat DMA^V metabolites, trimethylarsine
3 oxide (TMA^Vne (TMAs^{III})) (Waters, et al., 2004) cannot be excluded as contributors of
4 the necrotic cytotoxicity in rats exposed to DMA^V.

5
6 The committee felt that there are insufficient data to invoke ROS-induced DNA
7 damage as a key event in the carcinogenic process, associated with exposures to DMA^V
8 or DMA^{III}, **although contributions from that mechanism cannot be ruled out. {BR: Can**
9 **we remove “although contributions from.....ruled out?” Almost nothing can be ruled out,**
10 **so this statement places undue emphasis on ROS.} {SG}(Here, and 6 paragraphs forward**
11 **-- are we being inconsistent in our comments in these instances? It seems on the one hand**
12 **we don't think much of the ROS mechanism in relation to MOA, but on the other hand,**
13 **there is ample evidence that ROS can be involved in the MOA. Am I misinterpreting**
14 **something?)**

15
16 The **postulated revised** MOA {JT} for DMA^V is:

- 17
- 18 1. Reductive metabolism of DMA^V to DMA^{III}.
- 19 2. High concentrations of DMA^{III} in urine cause urothelial cytotoxicity.
- 20 3. DNA damage by an unknown mechanism unrelated to direct genotoxicity. The
21 clastogenic action of DMA^{III/V} is likely involved. {TR}{**The fact that the bladder**
22 **contains increased 8-oxo-dG does not mean that DMA caused it. See Section B.)**
- 23 4. Regenerative cell proliferation drives conversion of DNA damage into heritable
24 mutations and clonal expansion of altered cells.
- 25 5. Continuous exposure and persistent regenerative proliferation leads to production
26 of additional mutations, including those necessary for multistep carcinogenesis.
- 27

28 Neither the revised MOA nor those postulated by ORD or OPP (USEPA OPP,
29 2005; USEPA ORD, 2005b) contain key events expected to be a linear function of dose.
30 Reductive metabolism of DMA^V is likely to be saturable and therefore non-linear. In
31 vitro, cytotoxicity of uroepithelial cells occurs {TR} ***in vitro*** only at concentrations
32 greater than 0.4 µM DMA^{III} (Inferred from Dr Cohen's paper, but should be confirmed
33 with the author. The range of doses tested was not described **PLEASE ADD THE**
34 **CITATION HERE){GMA}{Is the Cohen paper published?}). In rats, cytotoxicity of the**
35 **uroepithelium occurred at the lowest tested dose (2 ppm in the diet), but the incidence**
36 **and severity increased, and the latency decreased significantly as a function of dose.**
37 **Statistically significant increases in regenerative cell proliferation only occur in rats at**
38 **DMA^V doses greater than 40 ppm ~~in rats~~, again, a non linear or apparent threshold**
39 **response. Even the production of ROS and its interaction with DNA, a key event in the**
40 **MOA postulated by OPP and ORD would be nonlinear functions of DMA^V dose.**
41 **{TR}{This makes no sense to me. It completely leaves out DNA Repair!}** Production of
42 ROS would likely be linear low dose, but nonlinear across a larger dose range if saturable

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1 metabolic processes are involved. Formation of heritable alterations in DNA by ROS is
2 believed to be nonlinear (sublinear) effect best represented by a quadratic function
3 (USEPA OPP, 2005). The formation rate is a function of the rate of DNA damage and
4 the rate of DNA misreplication (USEPA OPP, 2005). The latter being a function of cell
5 proliferation, which in the case of DMA^V, is a highly nonlinear function of dose (USEPA
6 ORD, 2005). {TR}(This also leaves out DNA repair!)

7
8 It was therefore the consensus opinion that the available data support the
9 nonlinear approach for the low dose extrapolation.

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

19
20 Based on results from genotoxicity studies conducted DMA^V and DMA^{III} appear
21 to lack significant reactivity directly with DNA. These studies are discussed in the
22 Science Issue Paper (pages 52 to 59) and summarized in Table B4 (with references). The
23 panel agrees with the conclusion in the Science Issue Paper that DMA^V is only genotoxic
24 at concentrations producing cytotoxicity or cytolethality. For example, DMA^V was not
25 mutagenic in the Ames assay (Kligerman, et al., 2003) or the transgenic “Muta” mouse
26 assay (Noda, et al., 2002); DMA^V exposure did not result in micronuclei formation
27 (Noda, et al, 2002). In the mouse lymphoma assay a low frequency of mutations were
28 seen only at concentrations that were cytolethal (Moore, et al, 1997). Chromosome
29 aberrations in human lymphocytes were only seen at cytotoxic levels (Moore et al, 1997).
30 In contrast, there is some evidence that DMA^{III} is clastogenic *in vitro* at concentrations
31 below those that are cytotoxic. For example, in Chinese hamster ovary cells low
32 concentrations of DMA^{III} (1 to 5 micromolar) resulted in micronuclei, well below
33 cytolethal concentrations (Dopp, et al., 2004). {TR}(1-5 micromolar DMAIII is NOT A
34 LOW CONCENTRATION, BUT IS LETHAL. The Dopp et al. paper makes an error in
35 using Trypan blue exclusion to assay cytotoxicity. This is an extremely poor choice, as is
36 shown in Komissarova et al., 2005.) However, the induction of chromosomal damage in
37 *in vitro* and in non target cell types is not necessarily related to cytotoxicity in bladder cells
38 or genotoxicity in bladder cells.

39
40 Overall, there is a critical mass of data from *in vitro* studies with DMA^{V/III} in
41 animal tissue that supports the types of mechanisms typically associated with indirect
42 (i.e., threshold) types of carcinogens. {GMA}(The section that follows does not seem to

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1 **be a sentence**). For example, production of reactive oxygen species and DNA disruption
 2 (nicks and breaks) formed in association with toxic levels of DMA species, inhibition of
 3 some DNA repair processes, DNA-protein cross-links, and altering the expression of
 4 pathways associated with the production of tumors (e.g., p53 and telomerase proteins).
 5 Data that might argue for a linear, non-threshold mode of action such as DNA binding
 6 and point mutation induction have not been produced. **{SG}(Here, and 6 paragraphs**
 7 **back -- are we being inconsistent in our comments in these instances? It seems on the one**
 8 **hand we don't think much of the ROS mechanism in relation to MOA, but on the other**
 9 **hand, there is ample evidence that ROS can be involved in the MOA. Am I**
 10 **misinterpreting something?)** Other studies *in vivo* that show induction of DNA strand
 11 breaks and the formation of oxidative DNA species also support secondary effects on the
 12 DNA. While there are studies which show *in vivo* clastogenicity with inorganic arsenic
 13 compounds, no solid evidence of *in vivo* chromosome damage exists for DMA^{V/III}.
 14 Thus, data produced with animal cells and tissues points strongly to a secondary mode of
 15 action for DMA^{V/III}.

16 **{GMa}(Didn't we also recommend looking at the dose consistency using other**
 17 **studies?)**

18
 19 **{JT}(Suggests striking this paragraph. It seems redundant.)**

20
 21 The Science Issue Paper states that the limited ability of DMA^{III} to induce sister
 22 chromatid exchanges coupled with its clastogenicity and cytotoxicity are features of a
 23 genotoxin whose mode of action is likely via the production of reactive oxygen species
 24 (ROS). However, the Panel was not in agreement that ROS play a role in the mechanism
 25 of action of DMA. Although *in vitro* studies with isolated DNA have shown oxidative
 26 DNA adducts and damage, these results do not necessarily mean that resulting
 27 chromosomal or DNA mutational events will occur *in vivo*. Oxidative DNA adduct
 28 formation is readily repaired in mammalian cells and unless there is direct evidence for
 29 the formation of oxidative DNA adducts resulting in the induction of mutational events in
 30 the bladder, the relationship between these two events **are** associative **ed** at best and
 31 probably not related to each other in the context of bladder cancer in the rat following
 32 DMA treatment. In contrast, the induction of oxidative damage and oxidative stress
 33 following cytotoxicity, ~~however,~~ is well documented. This frequently is the result of
 34 necrotic events in the target tissue resulting in the sequelae of inflammatory events.

35 **{JT}(This text...the whole paragraph, should be a part of the answer to charge**
 36 **question B3. Around this text the authors can expand on why the ROS data are**
 37 **not sufficient and lay the foundation for conducting the necessary experimental**
 38 **work to refine the MOA.)**

39
 40 (2)...which approach is more consistent with the available data on DMA^V and
 41 current concepts of chemical carcinogenesis,
 42

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

3

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

After some discussion, we viewed this question from the perspective of the EPA's RfC guidelines (EPA 1994). Similar guidelines for the derivation of chemical specific uncertainty factors have been developed by the International Program for Chemical Safety (IPCS 2001). These guidelines provide an approach for incorporating uncertainty into risk assessments in the form of uncertainty factors. Uncertainties in the dose-response assessment can be broadly grouped into a) those related to interspecies differences in pharmacokinetics, b) those related to interspecies differences in **{JT} pharmacodynamics**, to which we add, c) those related to misspecification of the MOA. In the case of the latter, the dose response would change significantly only if evidence became available that DMA^{III/V} caused DNA damage through direct reactivity with DNA. The low dose extrapolation would then become linear. This appears unlikely at this time and the panel concludes that conducting the low-dose extrapolation using the linear assumption to allow evaluation of uncertainty in the MOA by comparison to the non linear approach is not an appropriate way to address this uncertainty. **{JT} The preferred approach is to conduct additional research (an outline is found in B3).**

{MM—Asks whether for EPA we should use pharmacokinetics or toxicokinetics throughout the document?}

{MM—the following is MM's revision to reflect both the policy issue and the crosswalk. Also, we need to decide whether to call these "uncertainty" factors or "safety" factors". Section C1 calls them safety factors, although the rest of the para refers to uncertainty factors. It is probably a policy convention, so let's do whatever the agency usually does.}

Although selection of uncertainty factors is the province of EPA's policy choice, the Panel believes that in the case of the Food Quality and Protection Act 10X safety factor for this element of risk assessment, the science supporting a smaller factor could lead EPA to choose to lower the factor for arsenic to some number less than 10. As a result of the Arsenic Review Panel's analysis of the data for the key toxicodynamic response, uroepithelial cell cytotoxicity, the consensus was the EPA could assemble a case for toxicodynamic equivalency between the test species, rats, and humans from existing experimental data. In the context of EPA and IPCS guidelines, this finding could be incorporated in the assessment as a reduction of the toxicodynamic component of the interspecies uncertainty factor, which is 3, to a value of one. The application of uncertainty factors has also been addressed in the Panel's response to question C1. ~~For the key pharmacodynamic response, uroepithelial cell cytotoxicity, the consensus was the~~

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~~EPA could assemble a case for pharmacodynamic equivalency between the test species, rats, and humans from existing experimental data. In the context of EPA and IPCS guidelines, this finding could be incorporated in the assessment as a reduction of the pharmacodynamic component of the interspecies uncertainty factor, which is 3, to a value of one.~~

[{JT and MM: There is a question of how the issue of the Safety Factor of 10 should be handled. This issue is in common with the discussions here in 3.5.1 (i.e., D1) and 3.4.1 (i.e., C1). The issue has been dealt with in the two sections as having PD and PK components and there is a suggestion that the Safety Factor can be reduced. There is an issue of whether to suggest some factor that the components could be reduced to or just to suggest to EPA that they should consider reducing the factor. The issue needs to be discussed at the Panel meeting. AGAIN, WE NEED TO POINT OUT THIS IS A POLICY ISSUE THAT THE PANEL ADVISES UPON—CROSS WALK TO C1 --SEE SECTION IN 3.4.1 FOR HOW THAT WAS DONE].

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

{MM}(The conclusion drawn in this paragraph seems to be somewhat at odds with the panel conclusions in A1, A2 and C1 as I read it. It is also somewhat at odds with the discussion of safety factors in C1. Basically, it boils down to whether or not the panel believes that the scientific data points to humans' metabolism of As leading to less toxic species, and as such putting them at less risk than rats. If that is the case the safety factor could be reduced. I think this either needs to be resolved, and the two sections brought into harmony, or if there is not consensus then this needs to be stated. What I think would be a mistake is to have one section (C1) saying to reduce the safety factor for toxicokinetics and another section (D1) saying that there is not sufficient data to do this.

3.5.2. Implementation of the recommendations of the NRC (2001)

“EPA has determined that the most prudent approach for modeling cancer risk from exposure to iAs is to use a linear model because there are significant remaining uncertainties regarding which of the metabolite(s) may be the ultimate

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1 carcinogenic moiety and whether or not mixtures of toxic metabolites interact at
2 the site(s) of action” (USEPA, 2005A).
3

4 *Does the panel concur with the selection of a linear model following the*
5 *recommendations of the NRC (2001) to estimate cancer risk at this time?*
6 *Please discuss your response in light of the highly complex mode of action for*
7 *iAs with its metabolites.*
8

9 **D2: Implementation of the recommendations of the NRC (2001):** There is a
10 lack of adequate human data at the lower range of iAs due to limitations in
11 epidemiologic studies conducted to date. These studies have been discussed in
12 response to charge question C-2. In summary, there have been a number of studies in
13 different populations across different countries that seem to support a possible linear
14 dose-response between exposure from drinking water and internal cancer risks
15 (particularly in Taiwan, Chile and Argentina). However, the dose-response
16 relationships are observed at higher exposure levels (>100 ppb). Although some
17 recent studies have included populations with exposures in the lower range (<100
18 ppb), they are not appropriate for using in dose-response analysis for lower exposure
19 levels since they have problems related to study design, exposure assessment and
20 statistical power. Estimations of low dose risk based on studies in populations with
21 only low dose exposure are unstable with high uncertainty and studies are
22 underpowered (Lamm et al, 2004; Bates et al, 2003; Steinmaus et al, 2003). For
23 example, in the Lamm et al. (2004) [PLEASE PROVIDE A FULL CITATION FOR
24 THIS LAMM 2004] ecological study, exposure assessment is not only highly
25 problematic given that a single median county-level exposure value is assigned to all
26 the person-years contributed by each county in the analysis, but 82% of the 133
27 counties are assigned exposure levels of 3-5 ug/L with only 6 counties assigned
28 values between 15 and 60 ug/L. A recent follow-up of the Taiwanese cohort reports a
29 monotonic trend in lung cancer risk for exposure to arsenic levels ranging from <10
30 to 700 ug/L, however this study also has limited power to examine the form of the
31 dose-response relationship within the 10-100 ug/L range (Chen et al 2004). There is
32 no human data available that is adequate to characterize the shape of the dose
33 response curve below a given point of departure.

34 At present the experimental evidence on mode of action of inorganic arsenic
35 supports a possible non-linear dose-response at low exposure levels yet there is no
36 clear indication of what shape a non-linear dose-response would take for application
37 to human cancer risks at low exposures (<50 or 100 ppb). In examining the dose-
38 response relationships of arsenicals in inducing mutagenic responses (including
39 effects thought to be clastogenic in nature), it is clear that effects are only seen at
40 doses that induce cytotoxicity. This implies a threshold (Rossman, T.G. 2003).
41 Until more is learned about the complex properties and MOAs of iAs and its
42 metabolites there is insufficient justification for the choice of a specific non-linear

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1 form of the dose-response relationship. Under these circumstances, the EPA’s 2005
2 Guidelines for Cancer Risk Assessment are clear that linear extrapolation below the
3 point of departure is the method to be used.
4

5 Although the EPA has chosen a linear model for the arsenic dose component of
6 the hazard model for lung and bladder cancer, the Panel encourages the Agency to
7 test the sensitivity of the assumption of linearity by comparing its corresponding
8 estimate of excess life risk to an alternative hazard model that has a dose contribution
9 that is multiplicative and quadratic in form. The following equation is the form of the
10 model that NRC (2001) found to have best fit to the data based on the Akaike
11 Information Criterion (AIC):
12

13 **[Corrected equation follows:]**
14

$$15 \lambda_{i,C} = \exp(a_1 + a_2 \cdot \text{age}_i + a_3 \cdot \text{age}_i^2) \cdot \exp(\beta_0 + \beta_1 \cdot \text{dose} + \beta_2 \cdot \text{dose}^2)$$

16
17 **Summary:** ~~F~~ In summary, the Panel recognizes the potential for a highly complex
18 mode of action of iAs and its metabolites, but until more is learned about the complex
19 PKPD properties of iAs and its metabolites there is insufficient justification for the
20 choice of a specific nonlinear form of the dose-response relationship. Based on this
21 and the EPA’s 2005 Guidelines for Cancer Risk Assessment, the final
22 recommendation of NRC (2001) to base current risk assessments on a linear dose
23 response model that includes the SW Taiwan population as a comparison group
24 seems the most appropriate approach. However, the Panel also recommends a)
25 performing a sensitivity analysis with different exposure metrics with the subgroup of
26 villages with more than one well measurement; b) using a multiplicative model that
27 includes a quadratic term for dose, as performed by NRC (2001).
28

29 **3.5.3. EPA Model Re-implementation**

30
31 “EPA re-implemented the model presented in the NRC (2001) in the language R
32 as well as in an Excel spreadsheet format. In addition, extensive testing of the
33 resulting code was conducted” (USEPA, 2005a).
34

35 *Please comment upon precision and accuracy of the re-implementation of*
36 *the model.*
37

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

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1 Pre-meeting Comments/Clarifications on the Question

2
3 Question D-3 suggests that the estimation of the dose-response model and the
4 hazard assessment were originally programmed in the R language. Page 63 of the issue
5 paper indicates that the Poisson hazard model was originally estimated in the R language
6 (optim routine) but neither the main text of the paper nor its appendices provided any
7 additional information. A clarifying question from the panel through **the Designated**
8 **Federal Officer:**

9
10 *“The reference to the implementation in R in question D.3 is outdated, and should*
11 *have been removed. This was an oversight on EPA's part. The model*
12 *implementation in Excel is our implementation of record, and was used to*
13 *prepare the results in the draft toxicological review. We would ask the Panel to*
14 *please review and comment only on the implementation in Excel. (Background:*
15 *EPA did originally implement its model in R. However we found that version to*
16 *be not very transparent, and hard to debug. We then re-implemented the model in*
17 *Excel, found and corrected some errors, and used that corrected version to*
18 *prepare the tox review. While Excel may not be the best choice from the*
19 *standpoint of numerical accuracy, it is greatly superior in the transparency of the*
20 *implementation, and is powerful enough to perform the entire model calculation*
21 *from start to finish, even including the nonlinear optimization. Once the Panel is*
22 *satisfied that the implementation in Excel is correct and appropriate, then the*
23 *model can be re-implemented in R or some other numerically superior*
24 *language.)”*

25
26 The Agency staff is to be commended for deciding to test its original R-language
27 version of the model program through a separate implementation in EXCEL. The
28 EXCEL version serves as a check of programming performed in alternative systems (e.g.
29 R, SPlus) and provides transparency for review by non-specialists. For the calculations
30 of hazard and excess risk implemented in this model, the EXCEL computations will
31 provide sufficient numerical accuracy. If the EPA returns to another programming
32 environment, it should begin with the original model formulas and not simply transcribe
33 the EXCEL model program. As a debugging and error-checking tool, comparisons of
34 intermediate results from the two model implementations should be performed to verify
35 the equivalence of the models.

36
37 Overview of the EXCEL spreadsheet implementation of the model: The EXCEL
38 model implementation is described in Appendix B (pages 105-106) of the Issue Paper.
39 The Issue Paper (page 65) referenced a URL, www.epa.gov/waterscience.sab that proved
40 to be not available. EPA staff notified the panel of the correct address,
41 <http://epa.gov/waterscience/sab/>. The Issue Paper suggests that a listing is provided of
42 the variable and parameter input fields in Table B-3 but the current draft of the Issue

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1 Paper did not include this table. The fields in the spreadsheet model were interpreted by
2 the Panel based on the description provided in the text of the Issue Paper and general
3 understanding of the model fitting procedure employed.
4

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

13
14 [Corrected equation follows]

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

15
16
17
18
19 This is the model described by the EPA in the Issue Paper and is one of two models that
20 appeared to provide best fit to the data based on the Akaike Information Criterion (NRC,
21 2001).
22

23 The second worksheet in each the four disease/gender pairs (e.g. MC fblad) is
24 used in conjunction with the initial starting values, generated by Solver and stored in Cell
25 N2, to simulate the empirical Bayes posterior distribution of the model parameters based
26 on a set of 1000 random perturbations of the coefficient vector (a_1, a_2, a_3, β) about the
27 maximum likelihood estimates found using Solver. The perturbation involves
28 independent, random, and uniformly distributed deviations of the coefficient estimates in
29 a relative range of +/- 10% about the point estimates. Parameter draws outside this range
30 were not performed since the posterior likelihood takes on a near zero value outside the
31 +/- 10% of MLE boundaries. The corresponding macro (e.g. mcfblad) is then invoked
32 and uses the observed data and the set of perturbed coefficient values to predict values of
33 the posterior log-likelihood for each of the 1000 draws. The empirical Bayes estimate of
34 the slope parameter and its lower confidence limit are then estimated based on the mean
35 and standard deviation of the simulated posterior distribution using the following
36 equations.
37

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

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

with,

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

2
3 The estimated UCL(b) is then copied to the Bier.xls spreadsheet which implements
4 the BIER.IV computations of excess lifetime risk.

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

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

23
24 {GMA}(Maybe we should not emphasize the problem of male/female
25 imbalance compared to other countries. I've looked at World Bank data several
26 years ago and found more male children than female. I'm not sure that occurred
27 because of biology or other reasons. However, if true in Taiwan as in mainland
28 China then it would set the stage for more males than females. Certainly a check
29 is worthwhile.)

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{SHa}(This gender differential may be a result of high female mortality associated with high maternal death rates – without information to the contrary for this subpopulation I would temper the statements. I can't get data on line for relevant time periods but what's there suggests very high maternal mortality was likely. Perhaps add "...population structures and dynamics in the absence of high maternal mortality.")

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

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

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

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1 The Bier.xls spreadsheet implementation of the Bier.IV excess risk calculation
 2 includes a 3-fold divisor which is assumed included to allow transforming of the risk to a
 3 U.S. population base (based on the assumption that exposure per kg is 3-fold higher in
 4 the SW Taiwanese population compared to the US population). This scaling occurs in
 5 the calculation of the age-specific cancer hazard (Row 11). This multiplier should be
 6 documented and included as a factor in future sensitivity studies. Since this is truly a
 7 model parameter it should be identified as a distinct input on the spreadsheet interface
 8 and not simply embedded in the calculations.
 9

10 Following the series of checks and minor corrections to the model listed above,
 11 the Panel encourages the Agency to extend its testing of the model's sensitivity to
 12 alternative models forms and model assumptions. Specific areas where the Panel felt
 13 additional sensitivity testing is warranted include:
 14

- 15 • A Monte Carlo analysis in which the individual well concentrations for 22
 16 villages with multiple wells are taken into account. The Panel recognizes the
 17 difficulties with this approach including the issue of how best to allocate cases to
 18 wells for those villages having multiple wells. {StH} A practical approach to this
 19 sensitivity analysis has been described in the Panel's response to Question 3.4.2
 20 (above).
- 21 • MCCancerFit.xls :
 - 22 ○ Examine the sensitivity of the model to the choice of the reference
 23 population (SW Taiwan).
 - 24 ○ Examine the sensitivity of model results to the assumption that the
 25 reference population has 0 intake of arsenic via food.
 26 {JY} This recommendation needs some expansion.
 - 27 ○ A contrast of results for the linear dose model employed in this program to
 28 an alternative hazard model that has a dose contribution that is
 29 multiplicative and quadratic in form. This is the form of the model that
 30 NRC(2001) found to have best fit to the data based on the Akaike
 31 Information Criterion (AIC):
 32

33 [Corrected equation follows]
 34

$$35 \lambda_{i,C} = \exp(a_1 + a_2 \cdot age_i + a_3 \cdot age_i^2) \cdot \exp(\beta_0 + \beta_1 \cdot dose + \beta_2 \cdot dose^2)$$

- 36
- 37 • Bier.xls
 - 38 ○ The Panel recommends a sensitivity analysis in which the age groupings
 39 used to estimate the baseline hazard and excess lifetime risk are altered. A
 40 logical choice is to test the sensitivity of the model results to using 10-year
 41 groupings (e.g. 20-29, 30-39) in both spreadsheets.

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1 The exposure/kg parameter used to transfer the dose/response model from
2 the original SW Taiwanese population to a U.S. general population should
3 be a major driver in the computation of excess lifetime risk. In preparing
4 its final risk assessment, the EPA should conduct a sensitivity analysis to
5 determine how the choice of 3 for the conversion factor impacts the final
6 estimates of excess lifetime risk.

7
8 **3.5.4. Available literature describing drinking water consumption rates for**
9 **the southwestern Taiwanese study population:**

10
11 “NRC (2001) stated that the drinking water consumption rate, as well as
12 variability of that rate in both US and Taiwanese populations, are important
13 factors to consider. In calculating risk estimates for U.S. populations exposed to
14 arsenic through drinking water, NRC used a drinking water consumption rate of 1
15 L/day for the US population and two possible consumption rates for the
16 Taiwanese population: 1 L/day (identical to the US population) and 2.2 L/day
17 with little or no supporting rationale. Since publication of NRC 2001, a number
18 of new studies have become available and are summarized in the Cancer Slope
19 Factor Workgroup Issue Paper. Agency reviews of the relevant literature suggests
20 that the mean drinking water (for the Taiwanese study population) consumption
21 rate is between 1 to 4.6 L/day. EPA’s current cancer modeling includes water
22 intake adjustments for 2.0 and 3.5 L/day” (USEPA, 2005a).

23
24 *What drinking water value does the panel recommend for use in deriving*
25 *the cancer slope factor for inorganic arsenic?*

26
27 **GENERAL COMMENTS:**
28 {SHA}{...I do think that we need to consider whether we really want to
29 recommend analyses based on extremes measured in populations (whether of
30 water consumption, food consumption or arsenic levels in water). Scientifically,
31 we probably do not, and consistency in recommending high and low values that
32 are reflective of a measure of standard deviations would be encouraged.)

33
34 **D4. Available literature describing drinking water consumption rates for**
35 **the southwestern Taiwanese study population:** Assumptions about water
36 consumption levels in the US and in Taiwan have a substantial impact on the risk
37 assessment. Relative to US consumption, overestimating water consumption in Taiwan
38 decreases risk estimates and underestimating consumption increases risk estimates.
39 Evidence for sex differences in consumption is limited, but considerable within-
40 population variability in consumption occurs (NRC, 2001).

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

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

20 We recommend that:

- 21
- 22 a) the EPA incorporate variability parameters for individual water consumption in
23 their analysis for the Taiwanese population as they have done for the US population
24 as per NRC recommendation;
25
- 26 b) given that assumptions about water consumption are an important source of
27 variability in the risk estimates, that the EPA conduct sensitivity analyses of the
28 impact of using a range of consumption values for the Taiwanese population.
29
- 30 c) Data on sex differences in consumption in Taiwan are limited, and a better
31 justification for assuming different consumption levels by sex is needed, particularly
32 given lack of sex difference in consumption in US and observed in studies from other
33 countries (Watanabe et al., 2004). In the absence of such a justification, the panel
34 recommends an additional sensitivity analysis to examine the impact of equalizing the
35 sex-specific consumption level.
36
- 37 d) The source of data for intake from other beverages and cooking water needs to be
38 more fully discussed and documented. Specifically, the document should more clearly
39 articulate how different sources of water intake are incorporated into the risk model
40 including beverages other than water (e.g. green tea) and water used in food
41 preparation. Clarification of both the assumed consumption level and how water
42 consumption and consumption variability is introduced within the model is needed.

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

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

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

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

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1 *Sensitivity Analyses.* It is recommended that a range of values from at least 50
2 µg/day up to perhaps as high as 100 200 µg/day ~~for males and perhaps somewhat lower~~
3 {SHa}--(The data do not support 200 for women or child/young adult exposures,
4 and baed on the data represents a most extreme value)
5 ~~for females~~, be run in a sensitivity analysis to assess the impact of this range of dietary
6 intakes on risk of lung and bladder cancer from exposure via drinking water in this
7 population.

8 {SHa}(Re: the following sentences in the paragraph – “It is important not to insert
9 too many presumptions where we have no data. Although, it is reasonable to
10 assume that men had the “best” diet – it is much more difficult to understand how
11 that translated into arsenic consumption. Women may well have had less fish but
12 also considerably less total food. Thus it is not possible to assess quantitatively
13 the implications of “relatively more rice” in terms of its impact on women’s
14 arsenic consumption.—She suggests the following deletion:)

15 ~~With regard to selection of the highest value to run in the sensitivity analysis, it is of note~~
16 ~~that according to information presented from Yang and Blackwell, Taiwanese males in~~
17 ~~the study population were afforded the “best” diet (presumably higher in protein) thus~~
18 ~~women and perhaps children may have ingested relatively more rice and less protein~~
19 ~~thereby perhaps also exposing them to relatively high levels of total arsenic levels from~~
20 ~~diet.~~ The cancer risk model {SHa}(Clarify this sentence with edits shown) thus needs to
21 be weighted with a wider range of iAs food values above 50 µg/day to determine if there
22 is a change in slope as a result

23 {SHa}(Clarify what “in slope” is expected in the model here).

25 {SHa}(Given the lack of data on consumption in Taiwan and absolutely no
26 information on variability in consumption by village, I think this line should be
27 struck—It would be excellent to do if there were any relevant data – but none
28 have been presented.)

29 ~~In addition, if possible, an analysis needs to be considered to determine the impact of~~
30 ~~differences in iAs background (from dietary sources) for each village in the Taiwan~~
31 ~~study.~~

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

38 It is known that fish contain some portion of iAs further pointing to the need for
39 the sensitivity analysis described above. Seafood may also contain DMA that may also
40 contribute to background exposure from food relative to water sources (Huang, et al.,
41 2003).

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

6 {CH}(I suggest either deleting the last sentence, or expanding to explain what is
7 meant by “on the basis of absolute risk as well as relative risk.”)
8

9 An awareness and discussion of methodological issues {JY} ~~around~~ related to
10 reported arsenic concentrations in food. ~~that~~ These are likely somewhat dependent upon
11 differential extraction processes and different analytical procedures used in different
12 laboratories on different food stuffs. ~~needs to be included.~~ Further, laboratory extraction
13 procedures are not usually designed, ~~however,~~ to equate with that portion of arsenic in
14 food that may be bioavailable. The bioavailability issue is an important area for
15 research. Additionally, a clearer statement of the limited data on daily dietary intake is
16 needed.
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4. ACRONYM TABLE

[Explain all acronyms used in a table format]

ACRONYM

EXPLANATION

iAS

Inorganic Arsenic

6
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11

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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