PUBLIC STAKEHOLDER WORKSHOP
TO INFORM EPA'S UPCOMING IRIS

TOXICOLOGICAL REVIEW OF INORGANIC ARSENIC

SESSION 4:

ROUNDTABLE DISCUSSION ON PLANNING AND SCOPING

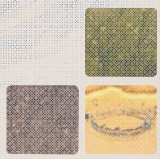
Tuesday, January 8 &
Wednesday, January 9
RTP, North Carolina

HOSTED BY EPA'S NATIONAL CENTER
FOR ENVIRONMENTAL ASSESSMENT

Identifying Client Needs: Draft planning and scoping summary discussion

What are client needs for the Toxicological Review of iAs?

- Discuss key themes from the workshop to be considered during the development of the draft Toxicological Review of iAs.



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Key Themes from Sessions I-3 of the iAs Public Stakeholder Workshop



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Office of Research and Development
National Center for Environmental Assessment

Disclaimer: the views expressed in this presentation are those of the speaker and do not necessarily represent the views or policies of the U.S. EPA.

Key Themes from Introduction

- Partnership and outreach
- Engagement of stakeholders, including at-risk populations
- Consideration of at-risk populations in scope

Key Themes – Session 1
Applying Systematic Review

- Process that is transparent and reproducible
- Iterative process with combination of search strategies
- Relevancy - use previous iAs work to guide, engage stakeholders grappling with iAs issues
- Set up key questions – e.g., focus on low-dose effects?
- Consider bias - e.g., negative data bias
- Feasibility (time and resources)

Key Themes – Session 2
Hazard ID

- Susceptibility factors – in utero, smoking/carcinogens, diet, genetic variation, metabolism
- Effects low-level exposure is major question and challenge for epi studies
- Aggregate exposure; concern over misclassification of exposure

Key Themes – Session 2
MOA

- Consider a MOA analysis as an organizing principle
- Lack of speciation data at target tissues
- Key biological processes/chemical stressors that influence MOAs
- Multiple MOAs and multiple targets – dose plays important role in MOA
- Aggregate exposure; concern over misclassification of exposure
- Complex metabolism

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Key Themes – Session 3
Relevant Factors for D-R

- Arsenic speciation and importance of chemical nomenclature
- Aggregate exposure (e.g., diet, contamination) vs. "sole source" exposure
- Exposure in U.S. population
- Uncertainty in the exposure – life stage, dose
- Misclassification of exposure in studies
- Biomarkers of exposure (proteomics, epigenetics, transcriptomics) – utility for D-R?
- Bioavailability/bioaccumulation

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Key Themes – Session 3
Approaches to D-R

- Multi-tumor modeling – feasible in time-frame?
- PBPK – challenge of animal-human extrapolations, metabolism, and polymorphisms
- Factors impacting D-R: MOA/TK, iAs speciation, exposure estimation, susceptible populations (e.g. age, ethnicity)
- Treat iAs speciation as a mixture for D-R?
- Uncertainty – probability distribution/sensitivity analyses, MOA organization

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Key Themes – Session 3
Extrapolation Approaches to D-R

- Need extrapolations to U.S. population, high dose to low dose, and interspecies/in vitro extrapolations – consider value added
- Human PBPK model – simplify exposure to "total load" of iAs?
- Low dose extrapolation is to a lower response level; a dose-response characterization approach

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Key Themes From Sessions 1-3

Mike Waalkes

Key Themes From Sessions 1-3

- Early life as a critical period of susceptibility to inorganic arsenic
  - Remarkable concordance between human and rodent data in terms of carcinogenesis
    - Sensitivity and sites
    - Cannot be ignored
  - Basis not known
  - Other diseases need further exploration

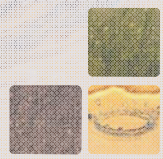
Key Themes From Sessions 1-3

- The role of biomethylation in inorganic arsenic
  - Susceptibility factor
- Poor tissue and target site dosimetry for inorganic arsenic in humans
  - We do not know what gets where with regards to target cells
    - Or what is generated where
    - Urinary metabolites poor substitute

Identifying Client Needs: Draft planning and scoping summary discussion

What are client needs for the Toxicological Review of iAs?

- Recommendations for revisions to the planning and scoping summary to adequately address stakeholder input.



EPA ARSENIC WORKSHOP

PRESENTATION TO U.S.
ENVIRONMENTAL PROTECTION AGENCY
RESEARCH TRIANGLE PARK, NC

JANUARY 8-9, 2013

SAMUEL M. COHEN, M.D., PH.D.
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MODE OF ACTION

- Metabolism and kinetics
- Genotoxicity vs. non-genotoxicity
- Cellular responses
- Critical review of the literature
- Cancer and non-cancer

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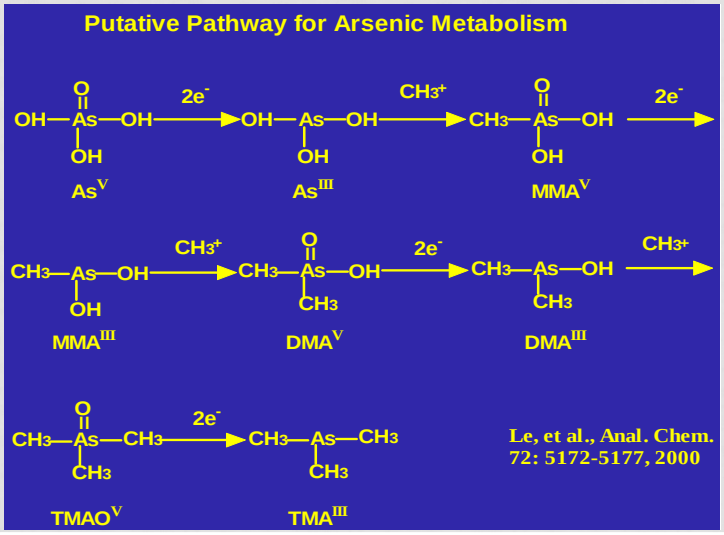
CLASSIC PATHWAY FOR ARSENIC METABOLISM



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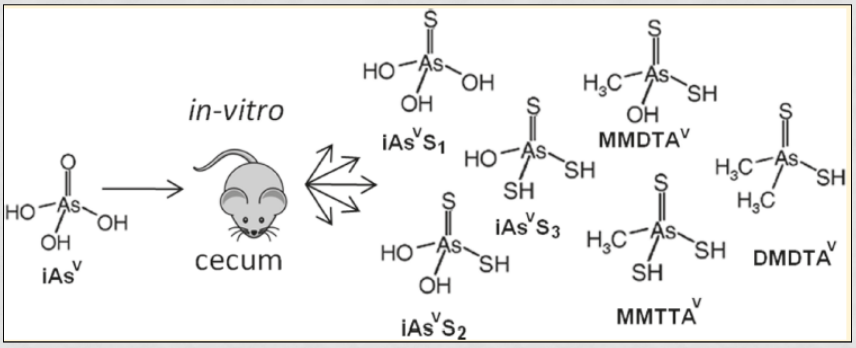
THIOLATED ARSENICALS



Pinyayev et al., Chem. Res. Toxicol., 24:475-7, 2011.

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TOXICITY OF ARSENIC METABOLITES IN UROTHELIUM IN VITRO, EXPRESSED AS LC_{50} VALUES (μM)

Cell Line	As^{III}	As^{V}	MMA^{III}	MMA^{V}	DMA^{III}	DMA^{V}	TMAO	$DMMTA^{V*}$
MYP3 (Rat)	0.75	3.4	0.42	3000	0.38	600	1600	1.3
ITI (Human)	8.3	34.6	0.9	2700	1.0	230	14000	1.4

* $DMMTA^{V}$ - Dimethyl monothiol arsenic acid

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RECENT DEVELOPMENTS IN RESEARCH OF ARSENIC METABOLISM

- As^{III} methyl transferase (As3Mt):
  - Glutathione in reaction mixture has little effect on methylation
    - Strongly favors classical pathway (Does not support Hayakama's proposed pathway)
  - DMA is poor substrate for human enzyme
    - Explains lack of TMAO formation in humans
- Thiol analogs:
  - Formed primarily by a chemical reaction of oxyarsenicals with H_2S
  - Primarily from GI bacteria, but also in tissues
  - Rapidly transported into cells and rapidly converted chemically to trivalent oxyarsenicals
    - Explains their high cytotoxicity compared to pentavalent oxyarsenical analogs

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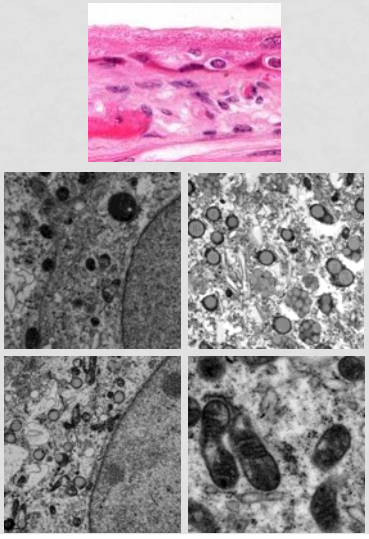
INTRAMITOCHONDRIAL GRANULES IN MOUSE UROTHELIUM



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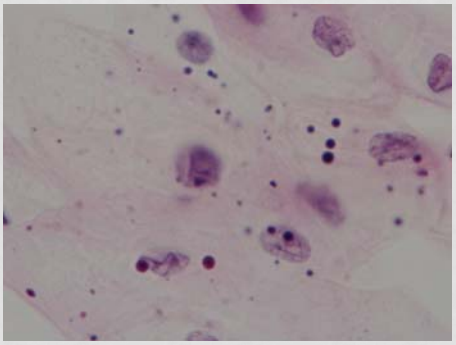
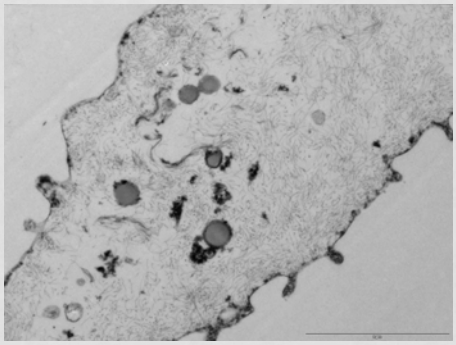
INTRACYTOPLASMIC GRANULES IN UROTHELIAL CELLS OF PML PATIENTS TREATED WITH As_2O_3





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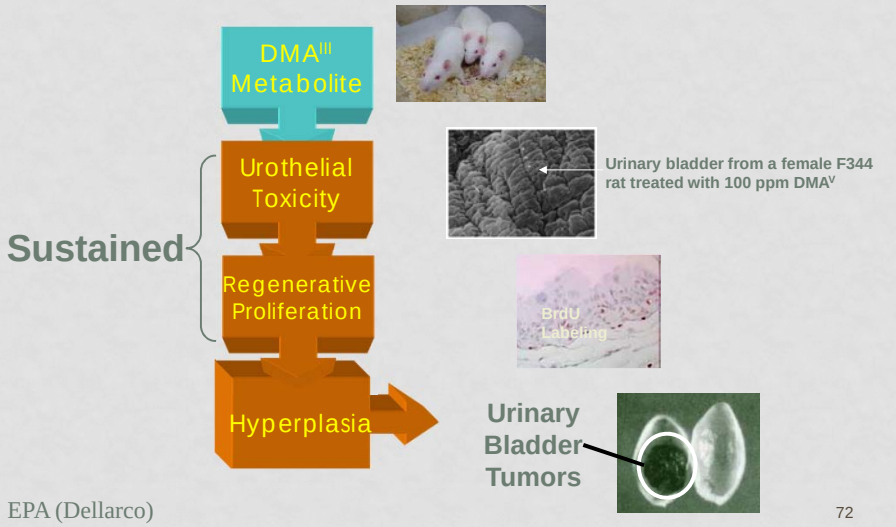
MODE OF ACTION OF DMA^V



EPA (Dellarco)

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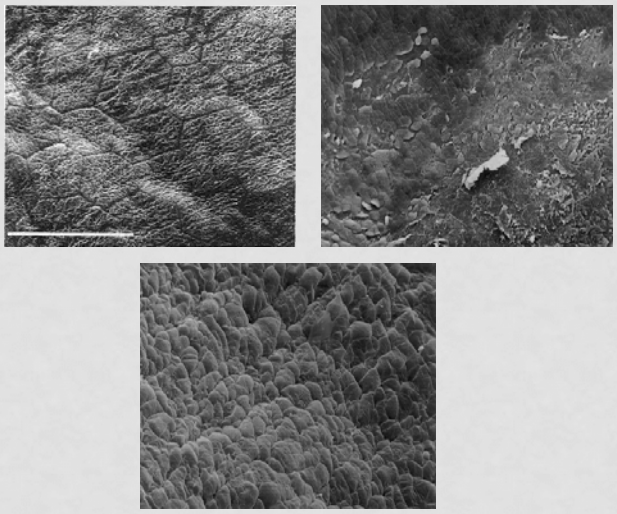
UROTHELIAL CYTOTOXICITY AND PROLIFERATION INDUCED BY DMA^V IN RATS



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GENE EXPRESSION IN DIFFERENT HUMAN CELL TYPES INDUCED BY ARSENICALS

HBECs (bronchial)	HEK001 (skin)	1T1 (bladder)
Cell death & survival	Cell death & survival	
Cell growth & proliferation	Cell growth & proliferation	
Cell-cell signaling	Cell-cell signaling	
Cell movement	Cell movement	
Cell development	DNA replication & repairs	
Small molecule bioschemistry	Cell cycle	
	Cell assembly & organization	
	Lipid metabolism	

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RELEVANCE TO HUMANS

Clinical Manifestations and Arsenic Methylation After a Rare Subacute Arsenic Poisoning Accident
Y. Xu, Y. Wang, Q. Zheng, B. Li, X. Li, Y. Jin, X. Lv, G. Qu, and G. Sun.
Toxicol. Sciences, 10: 278-284, 2008.

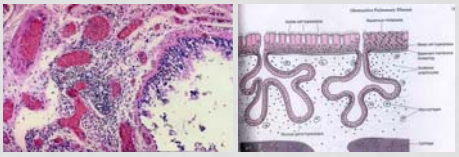
Observations

High exposure

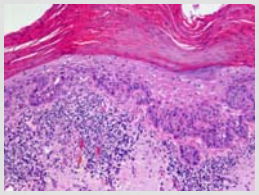
DMA^V & TMAO in urine

Hematuria in 1/3 of exposed group

HUMAN CHRONIC BRONCHITIS



SKIN: ACTINIC KERATOSIS



IN VIVO CYTOTOXICITY OF INORGANIC ARSENIC

- Threshold is 1 ppm elemental
- Rats and mice
  - Males and females
  - Arsenite and arsenate
  - Detection method:
    - Histopathology
    - Labeling index
    - Scanning electron microscopy
    - Genomics

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IN VITRO CYTOTOXICITY OF INORGANIC ARSENIC

- Threshold approximately 0.2 µM
  - Urothelial cells (rats and humans)
  - Bronchial epithelial cells (humans)
  - Keratinocytes (humans and mice)
- Less than 0.2 µM – Adaptive
- 0.2-1.0 µM – Cytotoxicity
- Greater than 10 µM – Lethal

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ARSENIC RESEARCH LITERATURE PRECAUTIONS (1/2)

- Analytical methods
- Arsenic form:
  - As^{III} vs. As^{V}
  - Inorganic vs. methylated vs. other organics
- Concentrations (in vitro) and doses (in vivo):
  - Environmental drinking water concentration of 10 µg/L (0.2 µM) is not systemic or tissue concentration
  - Many claim in vitro studies with "environmentally relevant concentrations of 10 µg/L"
  - Concentrations of trivalents >10 µM in vitro are meaningless; lethal dose

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ARSENIC RESEARCH LITERATURE PRECAUTIONS (2/2)

- Cell types:
  - Must use epithelial cells
  - Lung fibroblasts do not represent cell of origin for lung cancer
  - Malignant cell lines (e.g., osteosarcoma) have limited value without corroboration
- Primary cell vs. established cell lines:
  - Established cell lines have multiple differences compared to normal, especially p53
- In vitro vs. in vivo:
  - Trivalent arsenicals bound to rat hemoglobin
  - Oxidative stress in vitro (Gentry et al., 2010)
  - No oxidative stress in vivo (Clewell et al., 2011) (Suzuki et al., submitted)

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INTERACTION OF ARSENICALS WITH RAT HEMOGLOBIN

In Vitro	*In ViVo*
As^{III} + Hb ⟶ As^{III}—Hb	As^{V} + Hb ⟶ DMA^{III}—Hb
MMA^{III} + Hb ⟶ MMA^{III}—Hb	MMA^{V} + Hb ⟶ DMA^{III}—Hb
DMA^{III} + Hb ⟶ DMA^{III}—Hb	DMA^{V} + Hb ⟶ DMA^{III}—Hb

Feeding As^{V}, MMA^{V} or DMA^{V} to rats produces DMA^{III}—Hb

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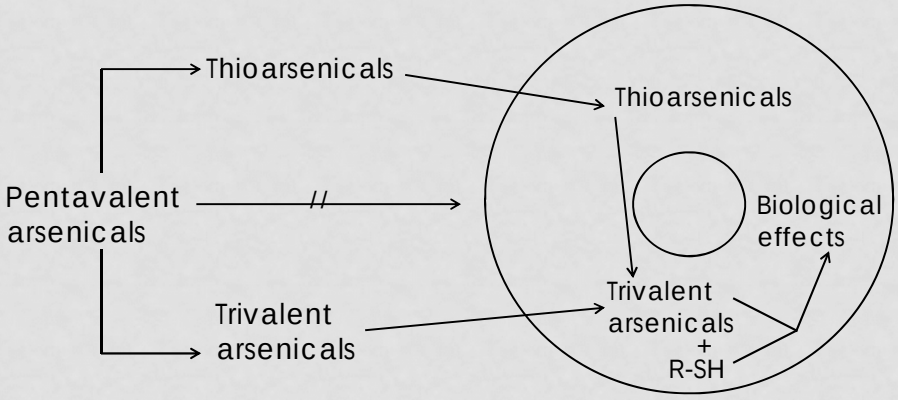
BIOLOGICAL EFFECTS OF ARSENIC



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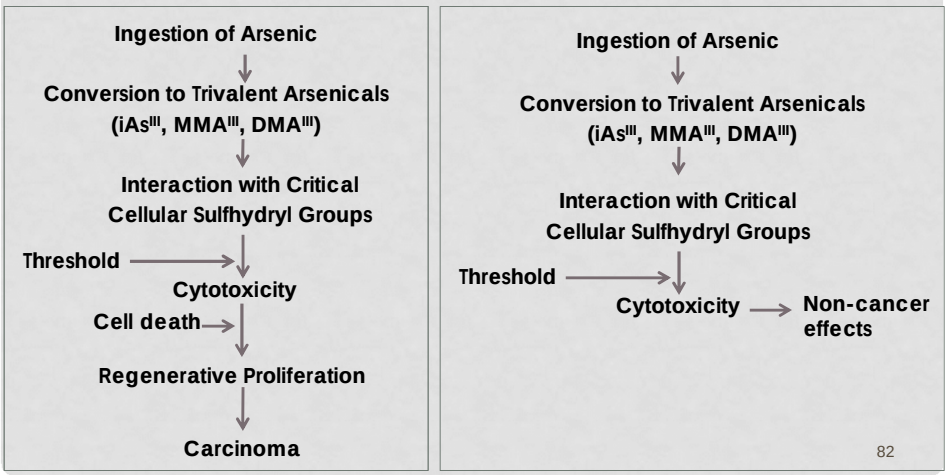
Mode of Action for Inorganic Arsenic: Working Hypothesis



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PUBLIC STAKEHOLDER WORKSHOP TO INFORM EPA's UPCOMING IRIS

TOXICOLOGICAL REVIEW OF INORGANIC ARSENIC

Michele Roberts

Environmental Justice Health Alliance

January 9, 2013

Environmental Justice

Why other sources matter in toxicological assessments

Studies show the need to protect the Bell Curve

- RECENTLY published in Environmental Health Perspectives, "Beyond Uncertainty Factors: Protecting the Bell Curve
- "some individuals have predisposing risk factors that make them uniquely sensitive to the effects of these environmental stressors." (Schmidt, 2012 at: http://ehp.niehs.nih.gov/2013/01/121-a26/)

Health professionals call for broad reform

- Dr. David Bellinger, Harvard Medical School Professor stated: "The assessment of effect modifiers should drive the study design. As it stands now, analysis of potential modifiers is usually something tacked on at the end of the main study …"
- Dr. Bellinger concludes that toxicological reviews *need* to identify the most susceptible people and quantify their added risk in order to make appropriate management decisions.

Social Stressors

- Poverty

  -access to nutritional food

  -access to health care and prenatal care

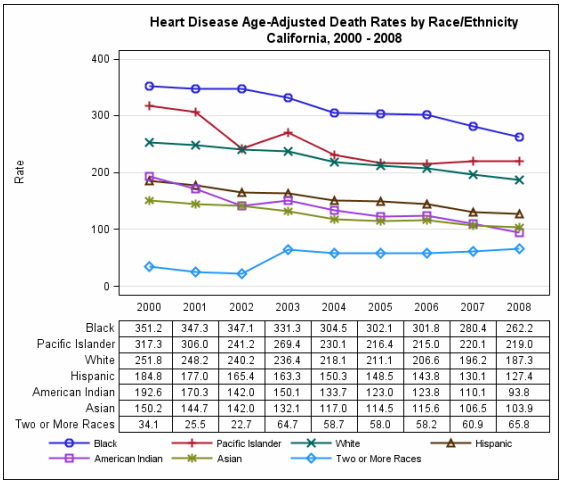
  -housing conditions

- Racism

Arsenic and Cardiovascular Disease

- 2012 systemic review on arsenic exposure and hypertension found an increasing trend in the odds of hypertension with increasing arsenic exposure. (Abhyankar et. al, 2012)
- Abhyankar found that “low” arsenic exposure imposed a more than 50% increase in the risk of hypertension.
- Another study, Zhang et. al (2012) found substantially elevated risk with low exposure to arsenic.

Cardiovascular Disease Among African Americans
Race/Ethnic Group Differences



	2000	2001	2002	2003	2004	2005	2006	2007	2008
Black	351.2	347.3	347.1	331.3	304.5	302.1	301.8	280.4	262.2
Pacific Islander	317.3	306.0	241.2	269.4	230.1	216.4	215.0	220.1	219.0
White	251.8	248.2	240.2	236.4	218.1	211.1	206.6	196.2	187.3
Hispanic	184.8	177.0	165.4	163.3	150.3	148.5	143.8	130.1	127.4
American Indian	192.6	170.3	142.0	150.1	133.7	123.0	123.8	110.1	93.8
Asian	150.2	144.7	142.0	132.1	117.0	114.5	115.6	106.5	103.9
Two or More Races	34.1	25.5	22.7	64.7	58.7	58.0	58.2	60.9	65.8

Scientific Studies call for Reform

- Study by researchers of the University of North Carolina, (Mo et al, 2011) findings suggest the need for an integrative mode of action in humans, with many results supporting numerous other studies that have found chronic arsenic exposure can cause cancer, cardiovascular disease and diabetes.
- Arsenic associated with diabetes mellitus, cerebrovascular disease and other potentially fatal diseases that are more prevalent in African Americans (Meliker et al, 2007).

Conclusion and next steps

- Arsenic exposure can exacerbate cardiovascular disease as evidenced by a large body of science and standard medical tests.
- African Americans are at elevated risks of preliminary and advanced stages of cardiovascular disease and have higher mortality rates from heart disease across the age span, with young and middle aged having more disproportionate elevations in death rates.

Conclusion and next steps

- Evidence of arsenic's cardiovascular toxicity and elevated risk among African Americans is substantial persuasive, and sufficient to rely on in risk assessments of arsenic.
- EPA's evaluation of arsenic must take under consideration and explicitly address the disproportionate health burden exposure imposes on African Americans.
- The elevated risks must be incorporated in quantitative evaluations of noncancer toxicity and be addressed in public policy.

Thank you!

Recommendations

Mike Waalkes

Recommendations

- In general, humans appear more sensitive than rodents to inorganic arsenic
  - Clear with cancer
    - Other diseases (?)
  - Should be considered in review

Recommendations

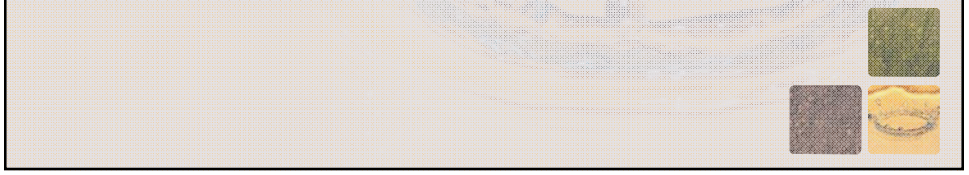
- Since inorganic arsenic is associated with multiple, diverse diseases
  - Review should keep an open mind to the possibility of multiple modes of action

Recommendations

- There is a good evidence that inorganic arsenic as well as various metabolites are toxicologically active
  - Review should keep an open mind to the possibility of multiple species being active

Methods for Identifying, Evaluating, and Synthesizing Literature

Public Comments



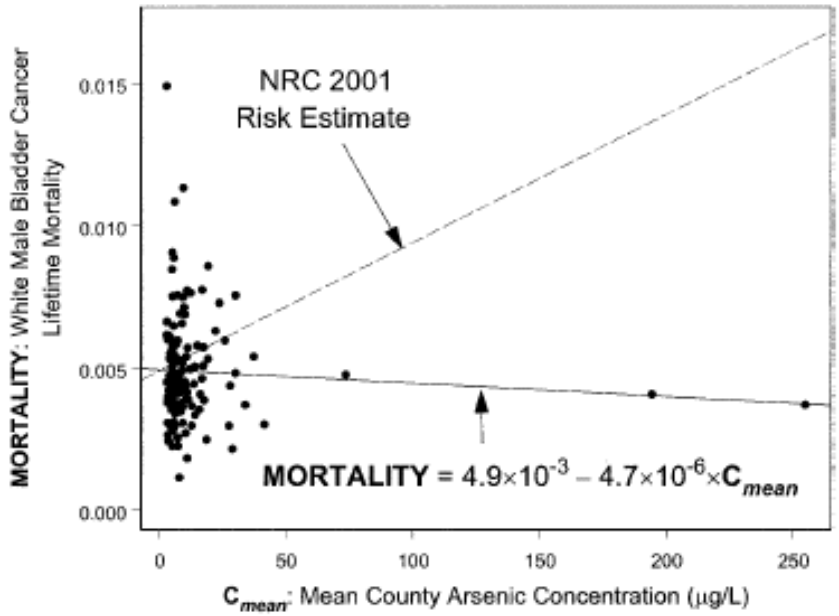
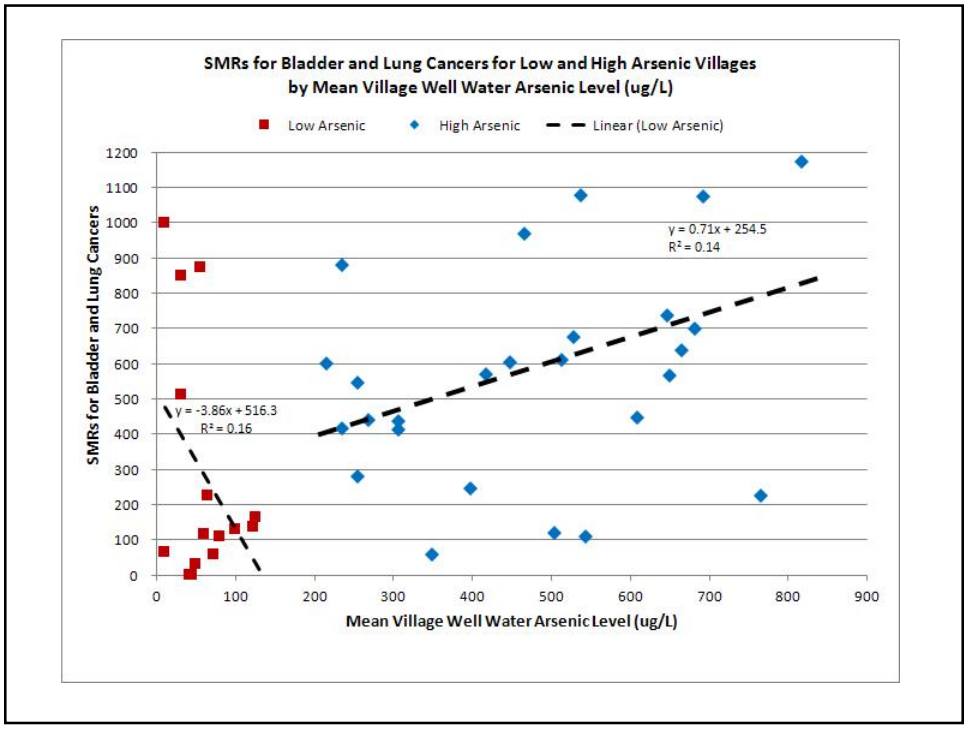


Fig. 3. White male bladder cancer lifetime mortality rate by Mean arsenic concentration (U.S. counties with median arsenic levels of ≥3 µg/L in drinking water).


SMRs for Bladder and Lung Cancers for Low and High Arsenic Villages
by Mean Village Well Water Arsenic Level (ug/L)
Low Arsenic
High Arsenic
Linear (Low Arsenic)
y = 0.71x + 254.5
R² = 0.14
y = -3.86x + 516.3
R² = 0.16
SMRs for Bladder and Lung Cancers
Mean Village Well Water Arsenic Level (ug/L)
0
100
200
300
400
500
600
700
800
900
1000
1100
1200