Inorganic Arsenic Assessment: Concerns Regarding IRIS Draft Protocol

Arsenic Science Task Force (ASTF)
Meeting with EPA/ORD
February 5, 2020

Thank you for taking the time to meet with us. The Arsenic Science Task Force consists of arsenic stakeholders, who are working together to support the production of scientific information on arsenic.

Agenda

Introductions

- Purpose of the meeting
- Discussion topics
    - Concerns with Arsenic Protocol and 2019 NAS Report
    - Review of points raised in August 2019 ASTF letter
    - Arsenic mode of action
    - Low dose assessment
- Next steps

The Enigma of Arsenic & History of Studying the MOA

- Arsenic is naturally occurring and ubiquitous at low levels everywhere

- In certain world locations, there are high levels of arsenic in soil and water

- An abundance of epidemiological studies of demonstrating adverse health effects at high levels of exposure

- The challenge of the assessment is to determine what happens at low doses

- Until 1994 there were no positive arsenic cancer studies with animals
  - In 1994 bladder tumors were observed in rats fed with dimethylarsinic acid (DMA) by Fukushima and in EPA Guideline studies

- Studies of DMA mode of action were published by Cohen (1999)

- EPA/OPP agreed (circa 2002) that DMA has a threshold, but at that time there was insufficient information for the mode of action of inorganic arsenic

For those of you who are not familiar with the history of arsenic research, the background helps to understand the existing data base. Low levels of arsenic are found everywhere (in soil, water and food). It is exposure to high levels of arsenic that has caused disease in populations in certain parts of the world where very high levels occur naturally. Unlike other chemicals for which adverse effects are found in animals and extrapolated to humans, researchers were historically unable to trigger tumors in animals, since high levels of arsenic needed to cause cancer were lethal.

In 1994 bladder cancer was observed in rats exposed to dimethylarsinic acid (DMA) a compound of arsenic, and at that time research of arsenic mode of action was made possible.

Issues with the IRIS Protocol

Science
- EPA has decided to not include mode of action in the arsenic risk assessment
- There is a failure to recognize that arsenic dose-response has a threshold
- Reliance on Bayesian modeling and ignoring underlying science at low doses
- Use of physiological based pharmaco-kinetic (PBPK) modeling for calculating low doses

Process
- No release of the evidence tables – which studies are being relied upon?
- Failure to provide information on model averaging

Due to limited time the focus will be on MOA and dose-response

The ASTF expressed concerns in written comments with several problems (science and process) on the IRIS Draft Protocol prior to the NAS Committee meeting in July 2019. The most important concerns are listed on this slide. Because of the limited time of this meeting, we will focus only on the two most critical science issues: reliance on modeling and mathematical calculations instead of on actual data, for dose-response at low exposures, and the decision to avoid consideration of arsenic mode of action that is clearly known.

We are honored to be able to have with us two leading scientists to elaborate on these two science issues.

Dr. Samuel Cohen

Dr. Samuel Cohen is a Professor in the department of Pathology and Microbiology, at the University of Nebraska Medical Center as a practicing surgical pathologist and arsenic researcher. In 2020 he was elected a Fellow of the American Association for the Advancement of Science.

He is the recipient of numerous awards, including, the Society of Toxicology Merit Award, and recently, the 2020 Mildred Christian life time achievement from the Academy of Toxicological Sciences.

Dr. Cohen has studied carcinogenesis over 50 years and specifically arsenic over 25 years, and is the author of numerous arsenic publications. He is a worldwide expert on mode of action.

Importance of Understanding Mode of Action for Toxicological Assessment

Samuel M. Cohen, M.D., Ph.D.
Havlik-Wall Professor of Oncology
Department of Pathology & Microbiology
University of Nebraska Medical Center
Omaha, NE 68198-3135
scohen@unmc.edu

Presentation to the U.S. Environmental Protection Agency
Washington, D.C.
February 5, 2020

UNMC
BREAKTHROUGHS FOR LIFE

Applying statistical analysis without a basis in biology (mode of action, MOA) can lead to misleading interpretation, whether related to causality or dose response.

Association vs. Causality

- An alcoholic one night drinks too much scotch and soda and wakes up the next morning very sick.

- On the second night, he drinks bourbon and soda and wakes up the next morning sick.

- On the third night, he drinks rye whiskey and soda and wakes up the next morning sick.

- He then declares, "I have to stop drinking that soda; it's making me sick."

Let me begin with a tongue-in-cheek example…. The correlation between soda and morning sickness is 100%, but without biologic basis it is meaningless. There are many similar real life examples.

Real Life Example: Cervical Cancer

Time Magazine, August 2, 1982
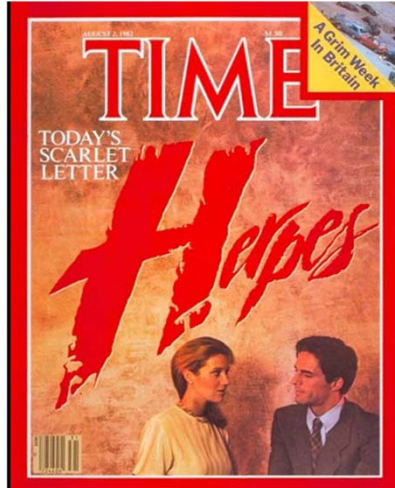
The Nobel Prize in Physiology or Medicine 2008

Harald zur Hausen
Prize share: 1/2

Françoise Barré-Sinoussi
Prize share: 1/4

Luc Montagnier
Prize share: 1/4

The Nobel Prize in Physiology or Medicine 2008 was divided, one half awarded to Harald zur Hausen "for his discovery of human papilloma viruses causing cervical cancer", the other half jointly to Françoise Barré-Sinoussi and Luc Montagnier "for their discovery of human immunodeficiency virus."

In 2008 Dr. zur Hausen was awarded Nobel Prize for his discovery (in the early 1980s) that cervical cancer is caused by the human papilloma virus (HPV).

We now know that HPV, not Herpes, causes cervical cancer.

Real Life Example: Cervical Cancer

- Herpes virus causes cervical cancer ?
  - Strong correlation observed between herpes virus and cervical cancer
  - Was considered the cause in 1970s and 1980s
  - No mode of action (MOA) to explain how
  - Zur Hausen (1980s): the cause is human papillomavirus (HPV)
  - Strong correlation between Herpes (the soda) and HPV infections
  - Was actually evaluating sexual activity

  - **STATISTICAL CORRELATION DOES NOT MEAN CAUSALITY**
    - **BIOLOGICAL RELEVANCE IS REQUIRED**

In the 1970s and 1980s cervical cancer was believed to be due to Herpes virus, based on strong correlation between the two, but there was no plausible MOA.

The discovery of the real cause (HPV), was enabled by understanding of the MOA.

Strong correlation with Herpes virus (the soda) and cervical cancer, was in fact correlation with sexual activity.

Only with the understanding of the biology (MOA) the real cause of cervical cancer became clear.

Biologic plausibility is key guidance in Bradford Hill criteria for epidemiology.

Real Life Example: Coffee

- ## Coffee causes bladder cancer ?

  - 1971, Cole in The Lancet: Coffee drinking causes cancer
  - No MOA

THE LANCET, JUNE 26, 1971
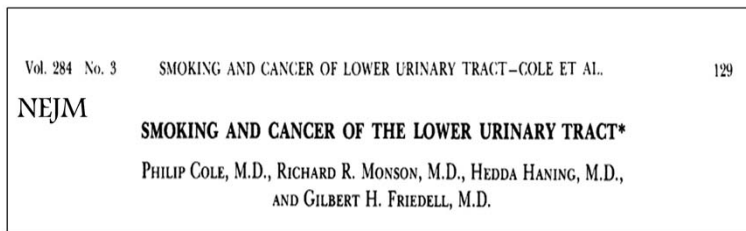
Preliminary Communication

COFFEE-DRINKING AND CANCER OF THE LOWER URINARY TRACT

PHILIP COLE

Another example: In 1971, Phil Cole published article in Lancet claiming coffee caused bladder cancer. This, obviously, caused a major uproar. There was no plausible MOA.

Real Life Example: Coffee

- Coffee causes bladder cancer ?
  - 1971, Cole in NEJM: cigarette smoking caused bladder cancer
    - MOA: Aromatic amines, possibly nicotine
  - Re-examined coffee study controlling for cigarette smoking
    - No effect of coffee ("the soda")
    - Cigarette smoking now known to be confounder

| Vol. 284 No. 3 | SMOKING AND CANCER OF LOWER URINARY TRACT—COLE ET AL. | 129 |

NEJM

SMOKING AND CANCER OF THE LOWER URINARY TRACT*

PHILIP COLE, M.D., RICHARD R. MONSON, M.D., HEDDA HANING, M.D.,
AND GILBERT H. FRIEDELL, M.D.

STATISTICAL CORRELATION DOES NOT MEAN CAUSALITY
BIOLOGICAL RELEVANCE IS REQUIRED

Later in 1971, the same Cole reported in the New England Journal of Medicine that cigarette smoking caused bladder cancer. There was strong correlation and he had biologically plausible MOA: cigarette smoke contained aromatic amines, known human bladder carcinogens.

When he re-analyzed his coffee study, now controlling for cigarette smoking, he found no effect of coffee.

Interestingly, subsequent large prospective epidemiology studies, controlling for cigarette smoking, showed that coffee decreases overall risk of cancer, including several specific cancers such as bladder cancer.

Statistics and Dose Response

- Waalkes group: Mouse lung tumors at low dose

- "positive" at 50 and 500 ppb, but not at 5,000 ppb

- Did not take into account historical controls

- Haseman (NTP) rule for common tumors: significance at $p < 0.01$! $p < 0.05$ frequently arise by chance and includes high false positive rate (Haseman, 1983)

- $p < 0.01$ has been adopted by OECD, FDA

- Bayesian analysis, ignoring biology, "validated" the same erroneous results

Similar misinterpretations occur in dose-response studies if not based on biology:

Waalkes group (NCI, then NTP) claimed increased lung tumors in mice at exposure of 50 & 500 ppb arsenic in drinking water, but not at 5000 or 6000 ppb, and increased again at 12,000 and 24,000 ppb.

The finding was "validated" by Bayesian statistics.

This finding doesn't make sense biologically.

The researchers did not take into account historical background control rates, and did not use the more appropriate statistic of $p < 0.01$ for common tumors as originally described by Joe Haseman at NTP in 1983 and later adopted by the OECD and by the FDA.

Statistics and Dose Response

Haseman: Journal of Biopharmaceutical Statistics, 8(1), 45-49 (1998):

"Additional factors that should be taken into account include (1) whether or not the effect was dose-related, ... (4) historical control data for the tumor in question, including overall background tumor rate and range of values previously observed in similar control groups."

- Proper interpretation of the data requires science
- When incorporating historical controls and Haseman rule, there is no effect until dose ≥ 24,000 ppb.
- Bayesian analysis of arsenic epidemiology studies without MOA is similar to Bayesian analysis of animal study without appropriate science.

Haseman explained the need for comparisons with historical controls, and the need for rational dose response.

Waalkes group did not address either.

The same incorrect conclusion is repeated with Bayesian analysis as with standard statistics, because neither takes into account the biology.

Note that the Bayesian analysis of the Waalkes studies was done by Druwe and Burgoon, the same authors who performed the Bayesian analysis of arsenic epidemiology for EPA, as shown on posters presented at the NAS protocol review meeting.

Ignoring Historical Controls

Lung Tumor Incidences in Control Mice in Waalkes Studies

Study	% incidence in control
▪ Tokar et al., 2011	34%
▪ Tokar et al., 2012	42%
▪ Tokar et al, 2012	20%
▪ Waalkes et al., 2014	22%

- **Historical controls from literature 8.8 – 61.1%**
- Nohara et al., Toxicol Sci, 129: 293-304, 2012
  - No increased incidences of lung tumors using Waalkes protocol

The control incidences in four of Waalkes studies range from 20% to 42%.

The controls incidences of this mouse strain in the literature, range 8.8% to 61%

Of critical importance is that Waalkes' results were not reproducible, not by Nohara et al. 2012, and even not in one of the studies from Waalkes lab (Ahlborn et al., 2009).

Importantly, the results were not reproducible even at p< 0.05

Ignoring Haseman Rule of p<0.01 for significance

Waalkes et al., Arch Toxicol, 88: 1-11, 2014:

Arsenic ppb Males	Adenomas %	p	Carcinomas %	p
0	14		8	
50	27	0.184	27	0.037
500	38	0.024	19	0.112
5000	15	0.415	15	0.166
Trend		**0.233**		**0.487**
Females				
0	11		5	
50	25	0.043	5	0.492
500	21	0.143	8	0.346
5000	11	0.473	5	0.490
Trend		**0.136**		**0.463**

The table presents the results of one of Waalkes' studies.

Increased adenomas in males at 500 ppb (not in 5000 ppb) and carcinomas at 50 ppb, but only at $p < 0.05$, not $p < 0.01$. The authors associated the tumors with the exposure to arsenic.

There is no effect in females. There is no significant trend in either gender.

The tumors are clearly not treatment related!

The same incorrect conclusion are made with standard statistics as with Bayesian analysis.

Postulated Modes of Action for Arsenic Carcinogenesis

- **IRIS Protocol:**
- Arsenic toxicity may be attributed to several complex modes of action, therefore mode of action cannot be considered:
- **Current State of Science:**
- MOA is known and has a threshold
- Alternative modes of action have been suggested but:
    - Most have been disproven
    - Others have higher thresholds and could be lethal
- **Arsenic: NOT DNA Reactive**
- The only linear mode of action – DNA Reactivity - does NOT occur (Nesnow et al., 2002)

IRIS is claiming they don't need MOA because there are sufficient low dose data from epidemiology studies. However, the data to which they are referring are results of modeling, not actual findings.

There are no statistically significant increases at low dose: how can this possibly be rationally modeled? (See also Tsuji's presentation).
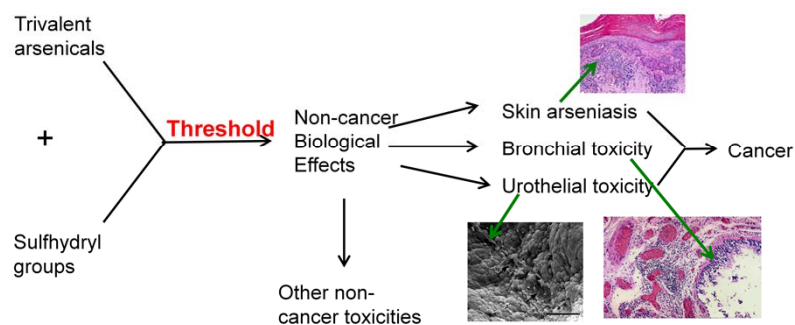
The MOA for arsenic is known and involves a threshold.

Many alternative MOAs have been suggested for arsenic, but these are either not supported by data, or not relevant to humans, or the effect occurs only at lethal doses.

The only possible MOA that does not have a threshold is DNA reactivity, but Nesnow (at EPA RTP labs) showed this does not happen for arsenic and cannot occur even theoretically , since the arsenicals are anions, cannot react with anionic nucleotide sites in DNA.

The MOA for arsenic is the one with the lowest threshold of all the proposed MOAs. It is described in the next slide

Arsenic Mode of Action

Trivalent arsenicals + Sulfhydryl groups → **Threshold** → Non-cancer Biological Effects → Skin arseniasis, Bronchial toxicity, Urothelial toxicity → Cancer; Other non-cancer toxicities

If exposure is below threshold for any key event, adverse event will not occur.
Tsuji et al., Crit Rev Toxicol, 2019

The MOA for arsenicals involves reaction of arsenicals with sulfhydryl groups in critical cellular proteins which differ by cell type.

This MOA is a threshold effect since these proteins are constantly being synthesized; An effect will be produced only when the rate of production of the protein can't keep pace with the protein destruction (inactivation) by the arsenicals.

The biological effects of arsenicals are all non-cancer effects.

If effect is in epithelial cells, there will be cell death leading to regenerative proliferation, ultimately tumors.

Non-cancer, precursor epithelial effect has been demonstrated in humans in skin, lung, bladder.

The use of safety factors for non-cancer effects, as routinely done in toxicology and risk assessment, will be protective for cancer.

See Cohen et al., 2013; Tsuji et al., 2019.

Mode of Action for Arsenic Carcinogenesis

- Strong evidence for a threshold from *in vitro, in vivo* and epidemiology studies
    - *In vitro* threshold > 0.1 μM
    - In rodents threshold> 2 ppm in drinking water, which generates tissue concentration > 0.1 μM
    - In humans threshold> 100 ppb in drinking water, which generates tissue concentration > 0.1 μM

    - **ARSENIC CARCINOGENESIS DOSE-RESPONSE IS NON-LINEAR AND HAS A THRESHOLD**

This MOA was first demonstrated in rats with DMA-induced bladder cancer, but subsequently shown for inorganic arsenic in rats and mice, either in diet or drinking water. DMA effect was accepted as threshold by OPP based on MOA.

Quantitative evidence for the mode of action:

- *In vitro* effects occur when trivalent arsenicals reach a concentration >0.1 μM.

- *In vivo* effects occur when tissue level (or urine) reaches > 0.1 μM.

- In rats and mice, oral dose has to be > 2ppm to reach >0.1 μM at tissue level to get biologic response.

To reach a level of > 0.1 μM in humans, requires about 100 ppb in drinking water, using very conservative assumptions for the calculations.

Epidemiology studies support a threshold in humans of about 100 ppb in drinking water.

Dr. Joyce Tsuji

Dr. Joyce Tsuji is a Principal Scientist at Exponent. She is a Board-certified in toxicology and a Fellow of the Academy of Toxicological Sciences. Dr. Tsuji serves regularly on National Academy Committees, and was a peer reviewer of the 1999 NAS report on arsenic in drinking water, and the 2019 NAS report of the arsenic protocol.

Dr. Tsuji has 30 years of experience in risk assessment and biomonitoring of arsenic in the environment and food. She has authored numerous publications on arsenic toxicology, epidemiology and biomonitoring.

Low Doses of Arsenic in Epidemiological Studies: Critical Details in Assessing Dose Response

Joyce S. Tsuji, Ph.D., DABT, FATS
Exponent
Bellevue, WA

Meeting with U.S. EPA, Washington, DC
February 5, 2020

Overview of Topics: Low-Dose Arsenic Exposure

- Extrapolation of epidemiological data down to background exposures

- Inaccuracies in dose-response modeling of epidemiological data

- Effects of bias, misclassification, and confounding on epidemiological data

- Wealth of scientific evidence regarding the nature of the dose-response relationship that is ignored by modeling

Increased Risks in Epidemiological Studies are not Close to U.S. Background Exposures

- ▶ U.S. EPA (2019) states that epidemiological study exposures associated with a 20% increase in relative risk* are very close to U.S. background exposures

- ▶ The relative risk estimation was based on selected datasets with positive model fits

- ▶ The benchmark dose modeling is insensitive to the equivocal nature of the data at low doses and ignores:
  - ▶ Lack of statistical significance
  - ▶ Inconsistent dose response in the actual data

- ▶ Evaluation of study bias and confounding was insufficient

Source: Hobbie et al. (2019); U.S. EPA (2019)   *for bladder and lung cancer and circulatory system diseases   3

The 2019 IRIS protocol document states that exposures in epidemiological studies associated with a 20% increase in relative risk above background are very close to U.S. background exposures. This statement appears to imply that the epidemiological studies are showing that U.S. background arsenic exposures are associated with a 20% increase risk of lung, bladder, and cardiovascular disease. However, IRIS's relative risk estimation is based on the use of benchmark dose modeling of selected datasets from studies that could be fit with modeling. This modeling can produce a positive dose-response regardless of whether relative risks in the epidemiological studies are statistically significantly increased at low doses, and can even show a consistently increasing dose-response with exposure despite data with an inconsistent dose-response. The evaluation of the suitability of epidemiological studies for risk assessment also should include more detailed attention to study bias and confounding, and particularly the effect of exposure misclassification in some studies.

U.S. Background Inorganic Arsenic Exposure is Lower than in Most Epidemiological Studies

Exposure Source	Average Lifetime Dose (µg/kg-day)
Drinking water at 100 ppb	2.9
Drinking water at 10 ppb	0.29
90th Percentile diet and water	0.15
Mean diet and water	0.08
Reasonable maximum exposure to arsenic in soil at 1 to 100 ppm	0.00022 to 0.022

Source: Tsuji et al. (2007); CDM (1996)

U.S. background exposures from food and water (Tsuji et al. 2007), the dominant sources of exposure for the U.S. population, are lower than exposure at the arsenic drinking water standard (10 ppb) (EPA Office of Water calculations assuming 2 L/day and 70 kg). EPA risk assessment of reasonable maximum exposure for arsenic in soil (CDM 1996—Anaconda, MT risk assessment) estimates even lower exposure for soil levels of arsenic in the 1 to 100 ppm range. Most of the epidemiological data on arsenic involve exposures to arsenic in drinking water greater than 10 ppb, and very few relative risks are statistically significantly elevated at exposures equivalent to approximately <100 ppb in drinking water (dose at 100 ppb is based on EPA Office of Water calculations). Therefore, U.S. background exposures to food and water, as well as soil arsenic exposures at most sites, will be well below levels associated with statistically significant increases in health risk.

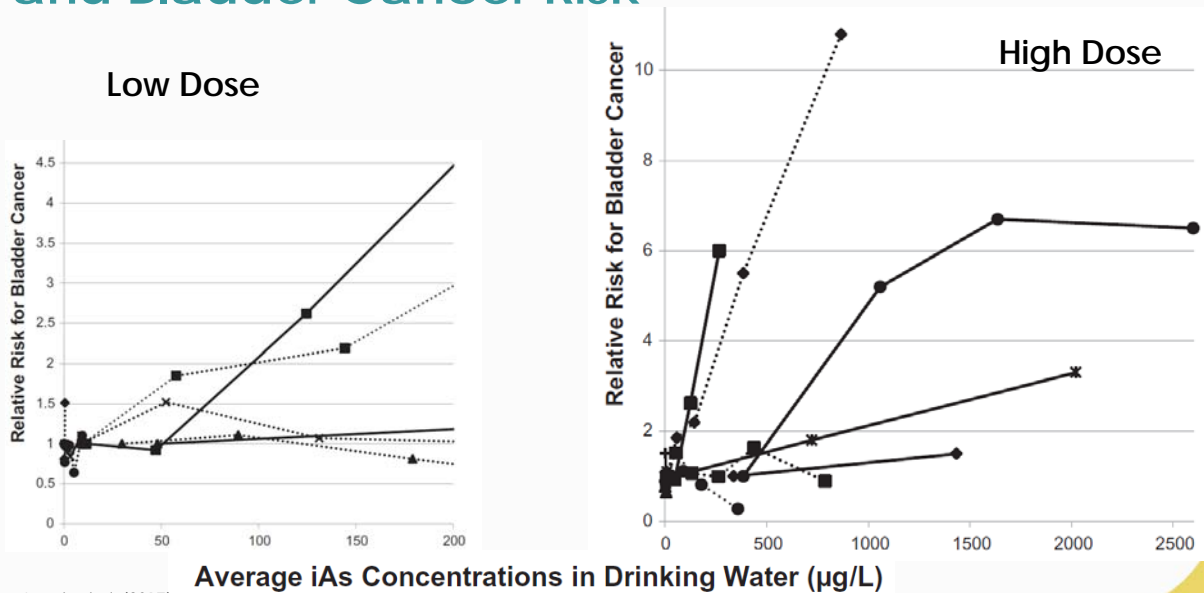## Modeling is Insensitive to Lack of Dose-Response Trend at Low Doses

FDA Dose-response Modeling of NE Taiwan Lung Cancer Risk
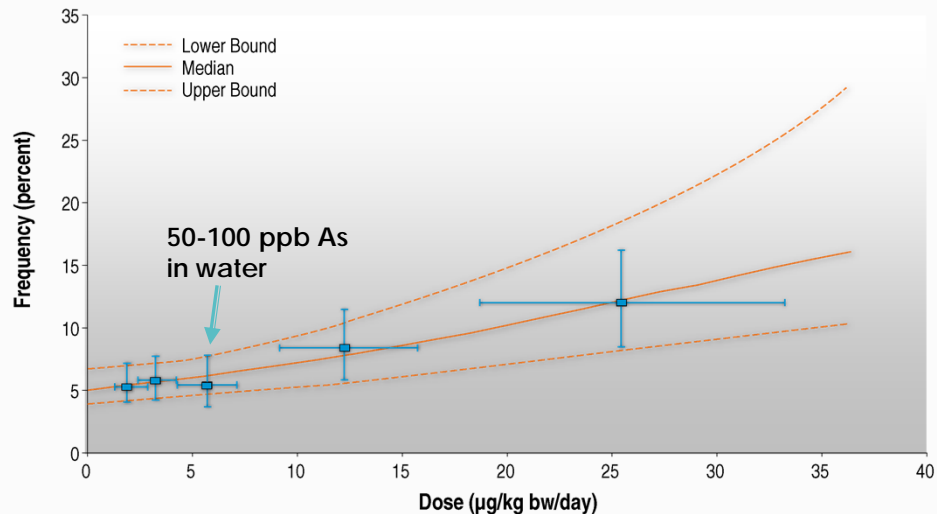
Source: FDA (2013)

Dose-response modeling that attempts to fit the full range of the arsenic data is insensitive to the lack of dose-response relationship and lack of statistically significant risk at low doses (below 100 ppb in drinking water in NE Taiwan). This example shows the individual study exposure category ranges and calculated midpoints by FDA in their risk assessment of arsenic in juice. The modeling artificially produces a positive dose-response relationship in this range, even though the observed low dose data show no such trend.

Data from Epidemiological Studies of Arsenic and Bladder Cancer Risk

Low Dose

High Dose

Relative Risk for Bladder Cancer

Average iAs Concentrations in Drinking Water (μg/L)

Source: Lynch et al. (2017)

In evaluating the dose-response relationship of arsenic and bladder cancer based on multiple studies, considerable heterogeneity is present, with an overall increase in bladder cancer risk at high doses that dwarfs the association at low doses (figure on the right). Expanding the low-dose region (figure on the left) shows a less consistent increase in risk with increasing dose.

Meta-Regression Modeling of Arsenic Risk is Driven by Data in High-Dose Region

- Sponsored by the Texas Commission on Environmental Quality
- Analysis of multiple epidemiological studies
- Smoothing of dose response relationship
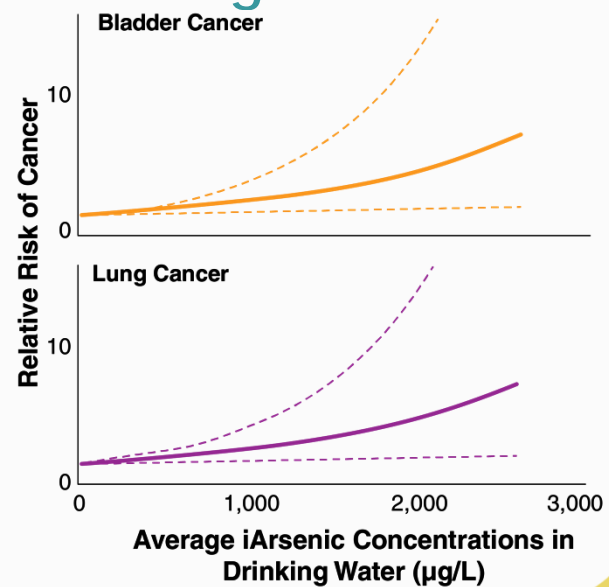
Source: Lynch et al. 2017. Environ. Int. 106:178-206

In spite of the lack of clear dose response at low doses in epidemiological studies, as seen in the previous figure at <100 ppb), meta-regression modeling of these data, however, **artificially** produces **a positive** dose-response relationship with a positive slope down to low doses. See also Rhomberg et al. (2011a,b) regarding the effect of exposure misclassification in bias toward a positive more linear response despite an underlying threshold relationship in epidemiological data.

EPA Bayesian Hierarchical Meta-Regression Modeling: Bladder Cancer

Figure 2. Extra Lifetime Bladder Cancer Risk due to Oral iAs Exposure, using MLE Dose Estimates
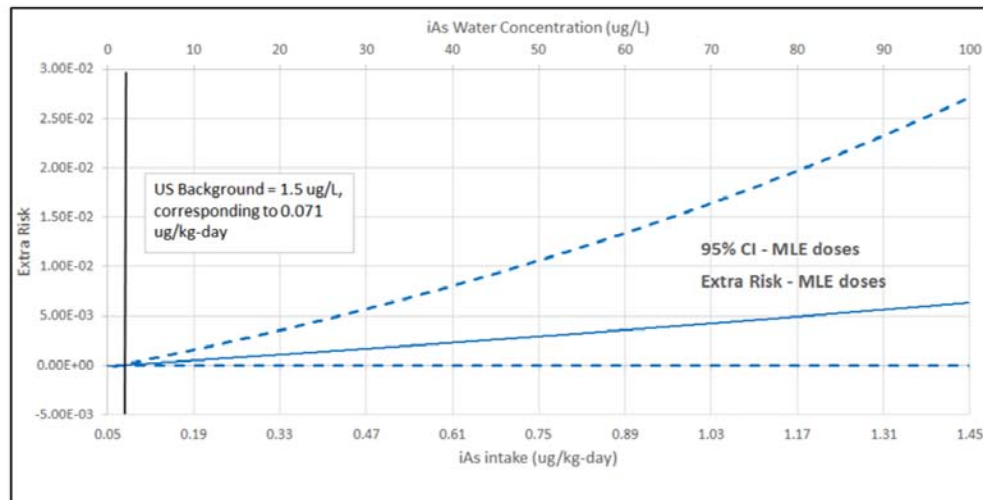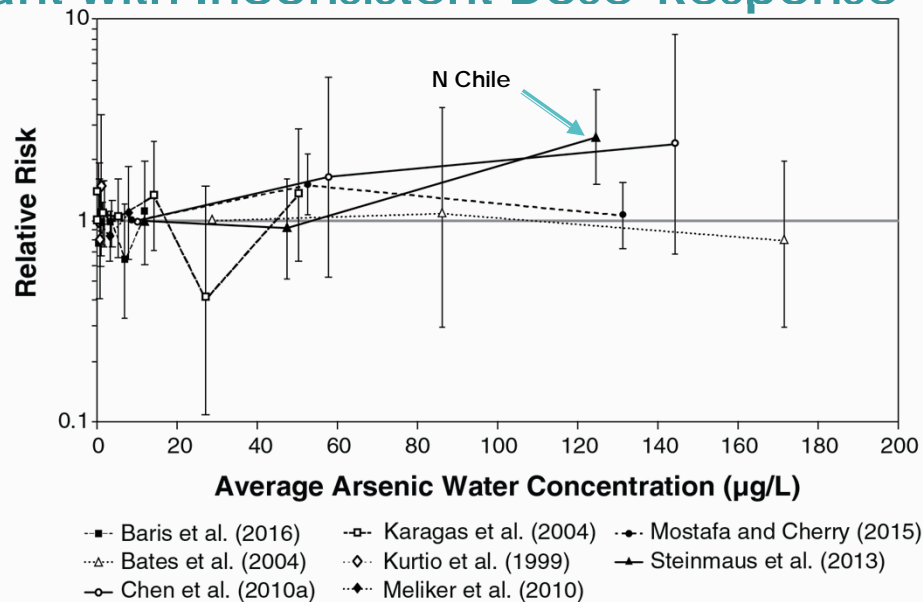

Source: Allen et al. (2019)

EPA Bayesian Hierarchical Meta-Regression Modeling presented in a poster in 2019 also shows a positive dose-response in risk through the low-dose range up to 100 ppb. The relative risks actually observed in the underlying studies as presented in the poster, however, show much less consistency in dose-response and little to no statistically significant increase in risk.

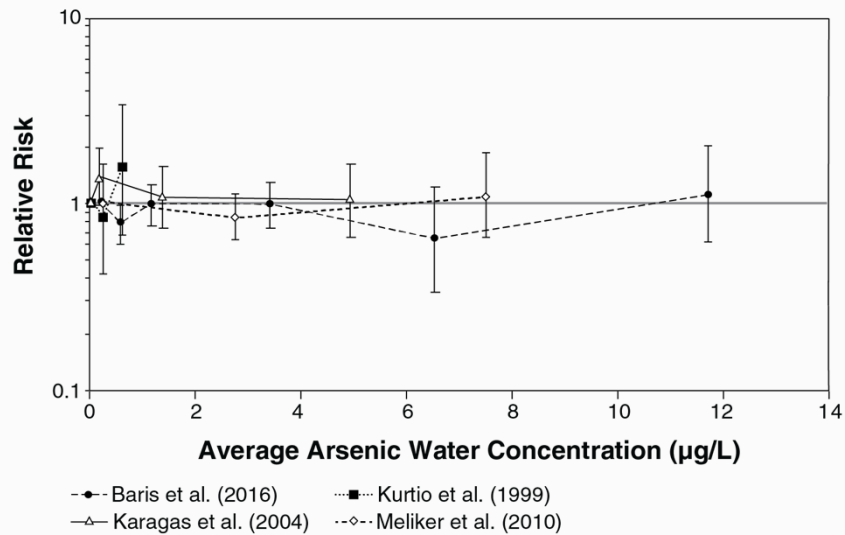Low-Dose Risks are Statistically Non-Significant with Inconsistent Dose-Response

Bladder Cancer

N Chile

Source: Tsuji et al. (2019)

- ■- Baris et al. (2016)
- △- Bates et al. (2004)
- ○- Chen et al. (2010a)
- □- Karagas et al. (2004)
- ◇- Kurtio et al. (1999)
- ◆- Meliker et al. (2010)
- ◆- Mostafa and Cherry (2015)
- ▲- Steinmaus et al. (2013)

This figure demonstrates the nature of the underlying data in the low-dose region. Most of these studies were included in the modeling in the previous slides (Allen et al. 2019; Lynch et al. 2017). This figure is from a study funded by TCEQ to take a closer look at the epidemiological studies at low doses less than 100-150 ppb, excluding studies without individual-level exposure data and without individual correction for confounding by smoking. This figure shows relative risks and confidence limits for bladder cancer associated with average water concentration exposure (see Tsuji et al. 2019 for details). Relative risks greater than 1 indicate higher risks relative to the lower-dose control, while those less than 1 indicate lower risks relative to the lower-dose control. The confidence limits if exclusively above or below 1 indicate whether increases or decreases, respectively, are statistically significant. The only statistically significant risk is at an average arsenic exposure concentration >100 ppb in a study from northern Chile, which, as will be discussed below, likely involves exposure misclassification from past high exposures. The lack of increased risk at 10 ppb or even 50 ppb gives reassurance that the drinking water standards are not associated with an increased risk according to the epidemiological studies.
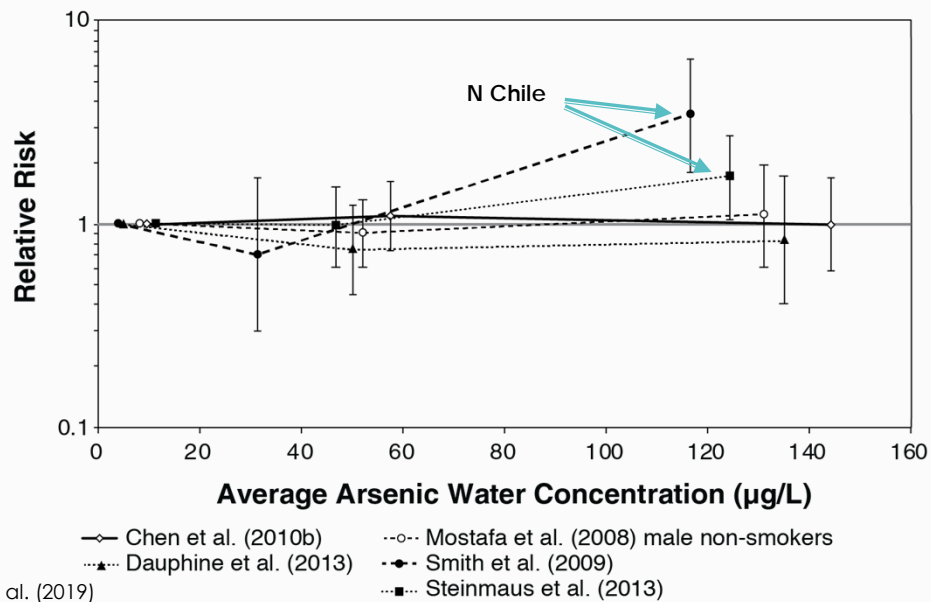
Lack of Dose Response at <12 ppb in Drinking Water (Bladder Cancer)

Source: Tsuji et al. (2019)

Examination of the few studies with data at the lower end of the low-dose range similarly shows no dose-response and no statistically significant increase in risk with exposure.

Low-Dose Lung Cancer Risk

Source: Tsuji et al. (2019)

Studies of lung cancer risks at low doses likewise show an absence of a dose-response relationship and no statistically significantly increased risks, except for two studies from Northern Chile at average exposures above 100 ppb.

High Past Exposures in Northern Chile are Obscured by Average Water Exposure Levels

Example from Antofagasta

Source: Steinmaus et al. (2013)
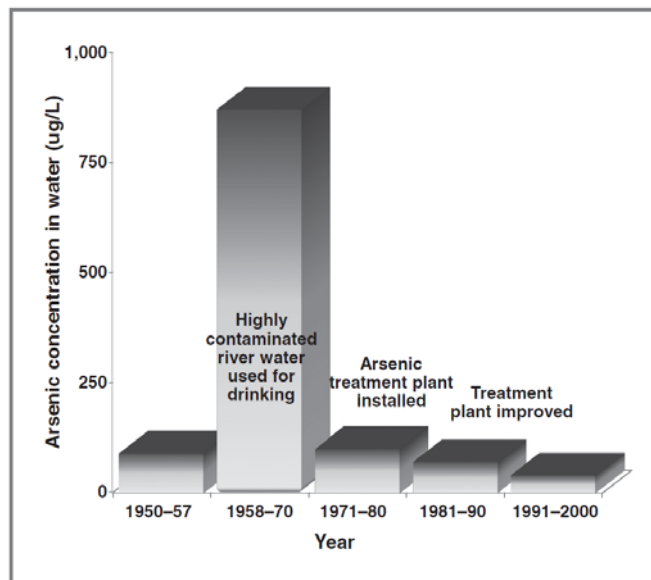
Studies from Northern Chile in particular are highly likely to involve exposure misclassification when lifetime average, current, or cumulative dose metrics are used. For several towns, in particular Antofagasta, one of the largest, the drinking water before 1971 had very high arsenic concentrations (e.g., 860 ppb), considerably in excess of those causing cytotoxicity and other adverse effects. Levels decreased substantially afterwards. Even if people lived in a town with high water concentrations only for a short period of time, the magnitude of this exposure may have resulted in an increase in future risk of disease. Thus, their much lower average exposure concentration would misrepresent their past higher exposures and increased risks as a result.

Low-Dose Arsenic Risks for Never Smokers: Bladder Cancer

- Reduces residual confounding from smoking
- The effect of smoking on disease is complex and statistical correction is often incomplete
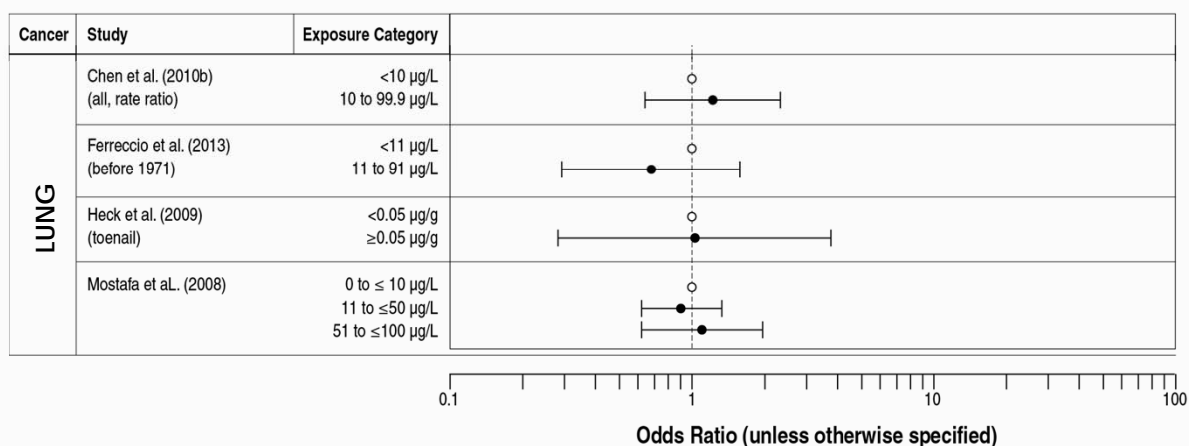- Doesn't eliminate all tobacco sources (e.g., betel quid)
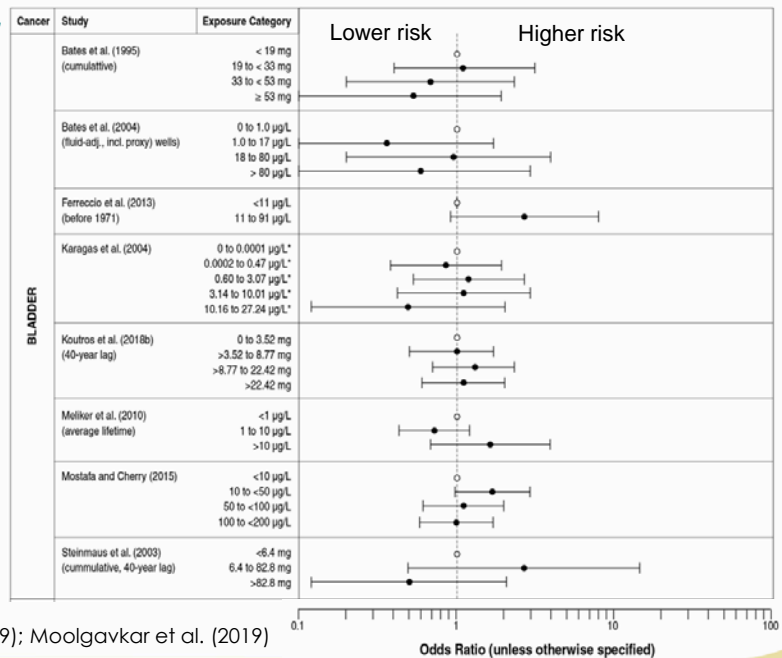
Source: Tsuji et al. (2019); van Osch et al. (2019); Moolgavkar et al. (2019)

One of the confounding factors with a large impact on lung, bladder, and cardiovascular disease risk is smoking, for which statistical adjustment is often incomplete. Tobacco exposure also reduces folate status, which at higher doses decreases arsenic methylation and detoxification. This is particularly important in studies based in foreign countries with higher arsenic concentrations and without folic acid fortification of foods as in the U.S.

Examining risks at low doses after restricting to never smokers reduces potential residual confounding from incomplete adjustment for smoking, although it does not entirely remove confounding by use of tobacco in other forms such as betel quid. Nevertheless, relative risks of bladder cancer for never smokers are clearly not increased by arsenic exposure at low doses, according to the epidemiological data. Examination of never smokers provides clearer assessment of the independent effects of arsenic exposure with less potential for residual confounding from smoking.

Low-Dose Arsenic Risks for Never Smokers: Lung Cancer

Cancer	Study	Exposure Category	
LUNG	Chen et al. (2010b) (all, rate ratio)	<10 µg/L 10 to 99.9 µg/L	
	Ferreccio et al. (2013) (before 1971)	<11 µg/L 11 to 91 µg/L	
	Heck et al. (2009) (toenail)	<0.05 µg/g ≥0.05 µg/g	
	Mostafa et aL. (2008)	0 to ≤ 10 µg/L 11 to ≤50 µg/L 51 to ≤100 µg/L	

Odds Ratio (unless otherwise specified)

14

Studies of low-dose arsenic exposure and lung cancer risk in never smokers similarly show no dose-response or statistically significantly increased risks.

Importance of Complete Exposure History: Skin Cancer and Skin Lesions

- Skin lesions are potential precursors to skin cancer
- Few studies have a complete arsenic water exposure history and documented skin lesions/cancer as being likely arsenic-related
- Inner Mongolia (Lamm et al. 2007)
  - Skin cancer at >122 ppb
  - Skin lesions at > 40-50 ppb
- West Bengal (Haque et al. 2003)
  - Skin lesions at >100 ppb
  - "The lowest peak arsenic ingested by a confirmed case was 115 /ug/liter"

Skin lesions and skin cancer were a Tier 1 endpoint according to NAS (2013). Most studies, however, involve considerable exposure misclassification because they have not obtained a complete exposure history. The few studies that obtained a full exposure history found that skin lesions and skin cancer occur at higher doses generally in excess of 100 ppb. The increase in the prevalence of skin lesions reported by Lamm et al. (2007) at >40-50 ppb was based on modeling rather than observed data. No skin cancers were observed below 122 ppb. Similarly, in the study by Haque et al. 2003, the lowest peak arsenic exposure level experienced by a confirmed skin lesion case was 115 ug/L.

Consistency of Cellular Arsenic Concentration with Animal and Human Evidence

	Cellular-Level Arsenic (III) Concentration	
	<0.1 to 0.2 µM	≥0.2 µM
In Vitro (human primary cells)	Cells in adaptive state; DNA integrity not affected	Cellular toxicity, protective responses, effects on cell cycle control genes, DNA repair, cell death
Rats	<10,000 ppb in drinking water No bladder toxicity or other effects	≥10,000 ppb in drinking water Bladder cell toxicity and regeneration, tumors
Humans	**<100 to 150 ppb** in drinking water **Lack of clear health effects in populations**	**150 to >1,000 ppb** in drinking water **Increasing noncancer and cancer risk with higher exposures to arsenic in water**

Source: Tsuji et al. (2019)

There is an overall consistency of evidence on cellular concentrations *in vitro* and associated doses *in vivo* that do not result in adverse effects leading to increased risk of disease. Note that drinking water concentrations in rats must be much higher than in humans to reach equivalent cellular arsenic concentrations. This consistency of *in vitro*, animal, and human epidemiological evidence provides confidence on doses that would not be associated with adverse health effects in humans (generally equivalent to <100 ppb in drinking water).

References

- Allen, B. et al. 2019. Bayesian hierarchical meta-regression of epidemiological studies: dose-response modeling and target population predictions. Poster 7. U.S. Environmental Protection Agency Office of Research and Development and National Center for Environmental Assessment.

- CDM 1996. CDM. 1996. Baseline human health risk assessment. Anaconda Smelter NPL site, Anaconda, Montana. Prepared for U.S. Environmental Protection Agency, Helena, MT. CDM Federal Programs. Golden, CO.

- FDA. 2013. Quantitative assessment of arsenic in apple juice. Center for Food Safety and Applied Nutrition. U.S. Food and Drug Administration. Draft. July 1.

- Haque R, Mazumder DN, Samanta S, Ghosh N, Kalman D, Smith MM, Mitra S, Santra A, Lahiri S, Das S, et al. 2003. Arsenic in drinking water and skin lesions: dose-response data from West Bengal, India. Epidemiology. 14:174–182.

- Hobie, K. et al. 2019. Analyzing study-specific estimates of exposure associated with a defined relative risk vs U.S. background exposure (RRBs) for inorganic arsenic (iAs). Poster 4. ICF International, U.S. Environmental Protection Agency Office of Research and Development, and National Center for Environmental Assessment.

- Lamm SH, Luo Z-D, Bo F-B, Zhang G-Y, Zhang Y-M, Wilson R, Byrd DM, Lai S, Li F-X, Polkanov M. 2007. An epidemiological study of arsenic related skin disorders and skin cancer and the consumption of arsenic-contaminated well waters in Huhhot, Inner Mongolia, China. Human Ecol Risk Asesss. 13:713–746.

- Lynch HN, K Zu, EM Kennedy, T Lam, X Liu, DM Pizzurro, CT Loftus, LR Rhomberg. 2017. Quantitative assessment of lung and bladder cancer risk and oral exposure to inorganic arsenic: Meta-regression analyses of epidemiological data. Environ Int. 106:178–206.

- NAS. 2013. Critical aspects of the EPA's IRIS assessment of inorganic arsenic: interim report. Committee on inorganic arsenic, board on environmental studies and toxicology, division on earth and life studies. National Academy of Sciences. Washington (DC): National Academies Press.

References

▶ Moolgavkar, S.H., E.T. Chang, H.N. Watson, and E.C. Lau. 2018. An assessment of the Cox proportional hazards regression model for epidemiological studies. Risk Anal. 28(4):777–794.

▶ Rhomberg, L. R., J. K. Chandalia, C. M. Long, and J. E. Goodman. 2011a. Measurement error in environmental epidemiology and the shape of exposure-response curves. Crit Rev Toxicol 41(8):651-671.

▶ Rhomberg, L. R., J. E. Goodman, L. T. Haber, M. Dourson, M. E. Andersen, J. E. Klaunig, B. Meek, P. S. Price, R. O. McClellan, and S. M. Cohen. 2011b. Linear low-dose extrapolation for noncancer heath effects is the exception, not the rule. Crit Rev Toxicol 41(1):1-19.

▶ Steinmaus, C.M. et al. 2013. Drinking water arsenic in northern chile: high cancer risks 40 years after exposure cessation. Cancer Epidem Biomarkers Prev. 22(4):623-30.

▶ Tsuji, J.S., L.J. Yost, L.M. Barraj, C.G. Scrafford, and P.J. Mink. 2007. Use of background inorganic arsenic exposures to provide perspective on risk assessment results. Regul. Toxicol. Pharmacol. 48:59–68.

▶ Tsuji, JS, ET Chang, PR Gentry, HJ Clewell, P Boffetta and SM Cohen. 2019. Dose-response for assessing the cancer risk of inorganic arsenic in drinking water: the scientific basis for use of a threshold approach, Critical Reviews in Toxicology. 49(1):36–84.

▶ U.S. EPA. 2019. Updated Problem Formulation and Protocol for the Inorganic Arsenic IRIS Assessment. EPA/635/R-19/049. Integrated Risk Information System. U.S. Environmental Protection Agency.

▶ Van Osch et al. 2019. Modeling the complex exposure history of smoking in predicting bladder cancer. A pooled analysis of 15 case-control studies. Epidemiol. 30: 458–465.

Mode of Action is Essential to Integrate Lines of Evidence

There is a preponderance of evidence and consistency of scientific data (*in vitro*, *in vivo* and epidemiology), ALL supporting a threshold for the dose-response of arsenic, based on mode of action.

The IRIS protocol ignores mode of action in spite of clear directions from the 2013 NAS committee:

"Mode-of-action analyses should be used to inform dose–response modeling with respect to the shape of the curve, particularly in the low dose region....." (Page 6)

"...mode-of-action analysis is an essential step in Integrated Risk Information System (IRIS) assessment..." (Page 71)

As we have heard from the two leading scientists, all the scientific data support the same conclusion, that arsenic dose-response has a threshold, as the mode of action indicates.

The 2013 NAS Committee that reviewed the 2010 draft IRIS assessment also concluded that the mode of action should be considered, but IRIS continues to refuse to consider MOA. IRIS claims there are sufficient data at low doses from epidemiological studies, but those data are **not** actual experimental data. These data are calculated and modelled.

Previous Risk Assessments Guided by Mode of Action

- Chloroform: "not likely to be carcinogenic to humans… under exposure conditions that do not cause cytotoxicity and cell regeneration" (U.S. EPA IRIS 2001)
  - EPA noted equivocal epidemiological data with positive results only at excessively high doses or with confounding factors
- Hexavalent chromium oral reference dose: "derived to protect against cytotoxicity-induced regenerative hyperplasia as a precursor carcinogenic MOA event" (TCEQ 2016)
- Dimethylarsinic acid (DMA) hallmark threshold response accepted (EPA/OPP)

Inorganic arsenic has a similar threshold mode of action!

The mode of action of cytotoxicity and cell regeneration has been used before to determine threshold, in the cases of chloroform, hexavalent chromium and DMA.

Deficiencies in the NAS Committee Review

- Purpose was Protocol review
- Agency presented assessment approach (posters & presentations)
- NAS Committee was not requested to review the publications where low dose is evaluated and where MOA has been discussed.
- Evidence for MOA was dismissed by the NAS Committee.
- Focus of the NAS review was on the risk assessment methodologies

In addition to the concerns we expressed regarding the science, we would like to mention several other points: The purpose of the July meeting was announced as the presentation of the draft Protocol to the NAS Committee. Instead, the data presented clearly showed the assessment was already underway. There was no opportunity for review and comment by the public or by the NAS committee prior to those presentations in July. Some of the methods used were not even described in the draft protocol. The NAS committee, in its review, admitted that they did not look at the publications that had been used for the assessment, but simply relied on the information provided to them by EPA. For example, IRIS said new epidemiological studies published after 2013 that reported exposure data at low doses, obviated the need for use of MOA data. In fact, the publications referenced did not include observed data, but were based on modeled data at low doses.

Importance of an Accurate Assessment

The current protocol will result in an overly conservative assessment with standards that are not scientifically justified, causing unnecessary economic burden, for example:

- **Drinking water**
  - The current 10 µg/L is a compromise based on BAT, lower standard would require a level **not** achievable by most cities.
- **Soil remediation for Superfund**
  - A change in the standard may result in clean up to background.
  - EPA Fund-led and industry-led soil cleanups performed at more than 100 sites will need to be revisited along with sites not cleaned up. This is **huge**!!

Consequences of Excessively Conservative Assessment

Too Hazardous to Consume?

- Wheat flour
- Rice
- Corn meal
- Peanut butter
- Apple juice
- Grapes
- Cucumber

- Lettuce
- Spinach
- Onion
- Carrots
- Sugar
- Dry table wine
- Tap water

Foods with higher inorganic arsenic might become "hazardous" if the cancer slope factor is significantly changed as previously proposed in 2010 by EPA.

Source: Yost et al. (2004);
http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/
TotalDietStudy/ucm184293.htm

These foods, that are currently considered healthy, are just a few examples of foods that could be banned if the final assessment fails to find a threshold dose response based on mode of action .

Our Requests

ORD Leadership Should Direct IRIS to:

1. Critically examine the low dose epidemiology data and to not rely on modelling that involves extrapolation; and

2. Specifically utilize MOA in the inorganic arsenic IRIS risk assessment.

Thank you