PUBLIC STAKEHOLDER WORKSHOP
TO INFORM EPA'S UPCOMING IRIS

TOXICOLOGICAL REVIEW OF INORGANIC ARSENIC

SESSION 2:

APPROACHES FOR HAZARD IDENTIFICATION FOR iAs – NON-CANCER AND CANCER

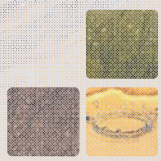
Tuesday, January 8 &
Wednesday, January 9
RTP, North Carolina

HOSTED BY EPA'S NATIONAL CENTER
FOR ENVIRONMENTAL ASSESSMENT

Noncancer and Cancer Effects–Identifying health effects and susceptible populations for hazard ID

2.1 What data are available to identify noncancer effects following iAs exposure (oral or inhalation)?

Lead Discussants: Mirek Styblo, Tim Wade



Toxicological Review of Inorganic Arsenic

Mirek Styblo

Toxicological Review of Inorganic Arsenic

SESSION 2: Approaches for hazard identification for iAs

Noncancer and Cancer Effects:

2.2. What data are available to identify noncancer effects following iAs exposure (oral or inhalation)?

- ***Arsenic and Diabetes Mellitus***

NTP
National Toxicology Program

NTP Workshop: Role of Environmental Chemicals in the Development of Diabetes and Obesity

Breakout Group on
Arsenic

Dana Loomis (chair)
Elizabeth Maull (rapporteur)

Crabtree Marriott Hotel
January 11-13, 2011

NTP
National Toxicology Program

Conclusions:

1. Existing human data provide limited to sufficient support for an association between arsenic and diabetes in populations with relatively high exposure levels (≥ 150 µg As/L in drinking water).
2. The evidence is insufficient to conclude that arsenic is associated with diabetes in lower exposure (< 150 µg As/L drinking water), although recent studies with better measures of outcome and exposure support an association.

Maull et al., EHP, 120:1658–1670 (2012)

NTP
National Toxicology Program

Conclusions:

1. The animal literature as a whole was inconclusive; however, studies using better measures of diabetes-relevant end points support a link between arsenic and diabetes.
2. The animal studies implicate several pathways by which arsenic may influence pancreatic β-cell function and insulin sensitivity
3. The extent of the existing literature was insufficient to consider obesity as an outcome.

Maull et al., EHP, 120:1658–1670 (2012)

What data are available to identify cancer effects of iAs exposure (oral or inhalation)?

David Thomas

2.2 Carcinogenicity of arsenicals – Animal data

- Transplacental models – iAs^{III} & MAs^{III}
  - spectrum of tumor sites
  - relation between metabolic transformation and carcinogenicity
  - mode of action as carcinogen (epigenetic effects)
- Whole life and restricted exposure models
  - combined *in utero* and postnatal exposure
  - urinary bladder tumor models with methylated arsenicals
  - evidence from mice with altered methylation capacity (distribution/retention and carcinogenicity)

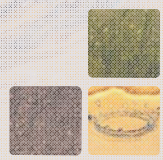
How can these data be used to assess human exposures and health effects?

Commonalities in dosimetry and mode of action

Noncancer and Cancer Effects–Identifying health effects and susceptible populations for hazard ID

2.3 What data are available to identify differences in susceptibility to the effects of iAs?

Lead Discussants: Craig Steinmaus, Mike Waalkes



What data are available to identify differences in susceptibility to the effects of iAs?

Mike Waalkes

Periods of Susceptibility to Inorganic Arsenic in Rodents

- For a long time it proved difficult to induce tumors with inorganic arsenic in adult rodents
- 2012 IARC Volume 100C states:
  - "There is *sufficient evidence* for in experimental animals for carcinogenicity of inorganic arsenic compounds"
    - In the synthesis of rodent data it states: "Early life transplacental and perinatal exposure to sodium arsenite appears to be a time of particular sensitivity in terms of carcinogenesis"

Periods of Susceptibility to Inorganic Arsenic in Rodents

- Perinatal exposure studies with inorganic arsenic in mice
  - Have produced lung tumors in adulthood
    - Multiple studies & labs
    - Human target site
  - And liver tumors in adulthood
    - Multiple studies & lab)
    - Possible human target site

  And adrenal, ovary and uterine tumors

Periods of Susceptibility to Inorganic Arsenic in Rodents

- Beyond cancer
  - Perinatal exposure has also potentially been associated with:
    - Accelerated atherosclerosis
    - Neurotoxicity
    - Altered DNA methylation
    - Impaired thymic function
    - Disrupted lung function
  - Additional work is needed to help define early life events and susceptibility

In Vitro Models: Susceptibility to Arsenic-Induced Transformation and Biomethylation

- Cancer target relevant cells:
  - Vary in ability to biomethylate inorganic arsenic, Including:
    - Skin cells – poor biomethyltion (BML) capacity
    - Liver cells – efficient BML capacity
  - It is thought that a biomethylation product likely causes DNA damage and not inorganic arsenic

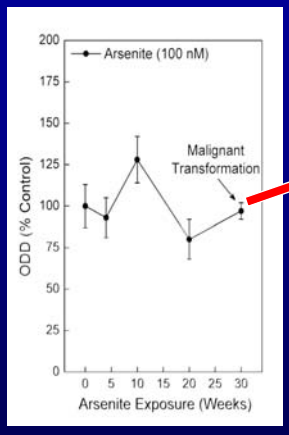
Transformation Method

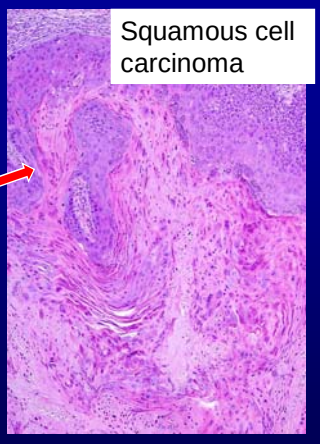
Good cells gone bad

- Continuous arsenical exposure
  - Up to several months
  - Periodically assess for malignant transformation
    - MMPs, invasion, colony formation, genes
      - Usually xenograft studies in mice
    - ODD by the immuno-spin trapping method
      - Measures *in situ* radicals
        - » No isolation artifacts
        - » Low background

Target Relevant Cells Can Show no DNA Damage

Skin keratinocytes (HaCaT) treated with iAs until signs of transformation dictate xenograft study (MMPs, invasion, colony formation, genes, etc.)

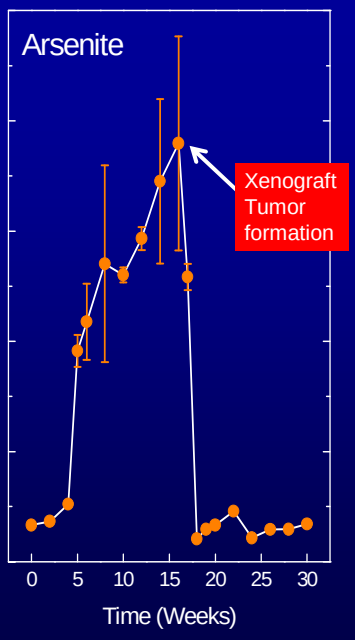




- Skin cells can also be transformed by iAs:
  - Without DNA Damage & can also be poor iAs BML

DNA Damage *In Vitro*

- DNA Damage with iAs
  - Inorganic arsenic BML proficient liver cells
    - Transform much more quickly than skin



In Vitro Models: Susceptibility to Arsenic-Induced Transformation and Biomethylation

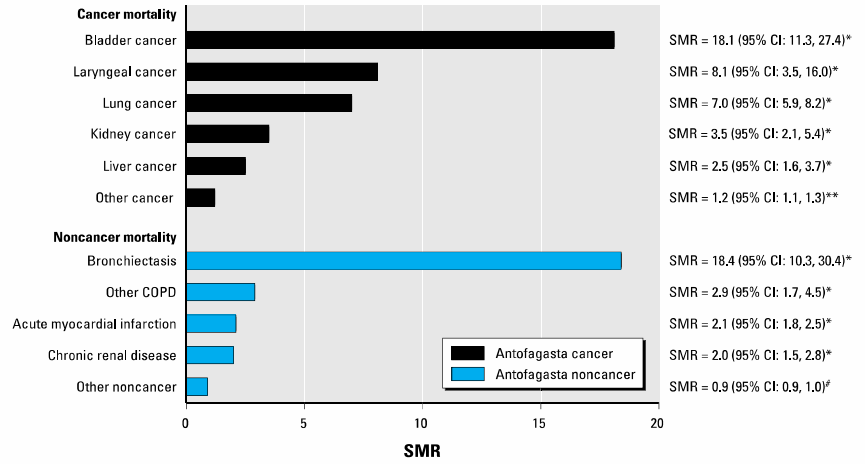
- In arsenic cancer target relevant cells:
  - BML makes a difference
    - Makes cells more sensitive to inorganic arsenic genotoxicity
    - And them undergo malignant transformation much more rapidly

What data are available to identify differences in susceptibility to the effects of iAs?

Craig Steinmaus

IN UTERO AND CHILDHOOD EXPOSURE

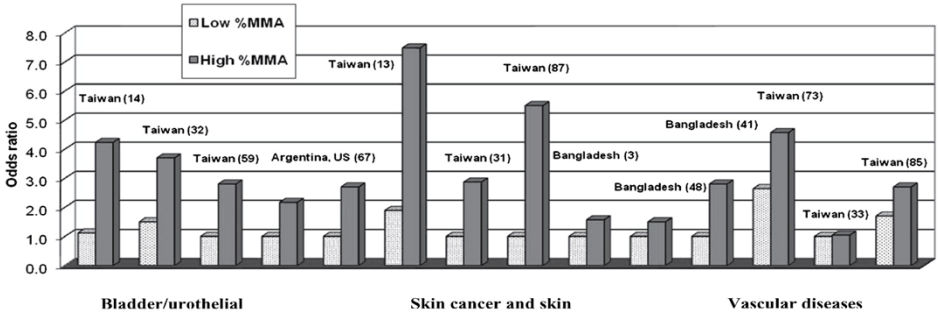
Standardized mortality ratios (SMR) in young adults following in utero or childhood exposure to arsenic, northern Chile *Smith et al. 2012 EHP 120:1527-31*



Confirmation in recently completed case-control study*

OTHER SUSCEPTIBILITY FACTORS WITH ABUNDANT HUMAN DATA

- ✓ Smoking synergy
- ✓ Diet
- ✓ Genetic variants: many
- ✓ Arsenic metabolism: InAs ⇒ MMA ⇒ DMA

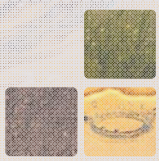


Smith AH, Steinmaus CM. 2009.
Annu. Rev. Public Health. 30:107–22

Mode-of-Action Evidence–Noncancer and cancer effects

2.4 What data are available to identify mode(s) of action for the health effects of iAs (e.g., key toxicokinetic/toxicodynamic events; inter-species or inter-individual differences)?

Lead Discussants: Vasken Aposhian, Doug Wolf



Mode of Action Evidence – Noncancer and cancer effects

What data are available to identify mode(s) of action for the health effects of iAs?

H. Vasken Aposhian

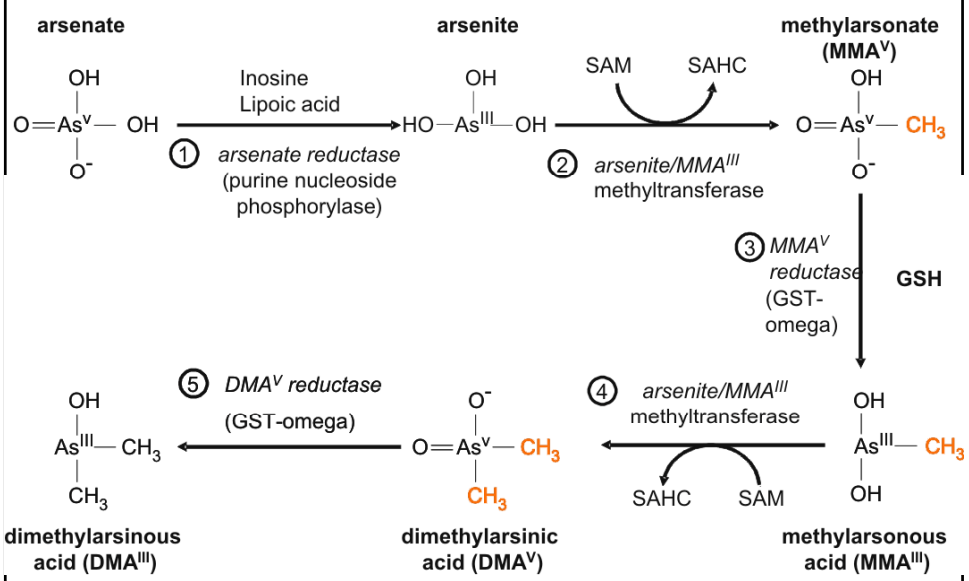
Mode of Action Evidence – Noncancer and cancer effects

What data are available to identify mode(s) of action for the health effects of iAs?

(e.g., What are the key toxicokinetic and toxicodynamic events? Have inter-species or inter-individual differences been identified?)

Lead Discussants: H. Vasken Aposhian, Doug Wolf

Biotransformation



This may seem more complicated and to some extent it is.
There is another more complicated scheme that not everyone agrees with

Inter-species differences

- Extrapolating arsenic results and data from rat experiments must be viewed with caution.

  Cohen, Vahter and ……
- Marmoset monkey, chimpanzee and guinea pig do not methylate arsenic.

  Vahter, Aposhian and ……..
- Humans are about 10X more sensitive than animals to inorganic Aresenic

Inorganic As Metabolism

Leading investigators

Thomas, D.

Styblo, M.

Cohen, Sam

Aposhian, V.

Others

DMA(III)
Dimethylarsinous acid

- Some unpublished data regarding toxicity.
- Suggest contacting Bill Cullen, UBC
- Very dangerous

Inter-individual differences

- Genetic polymorphisms:

"….AS3MT genetic variation may play an important role modulating such risk in northern Mexico, especially among children."

Sampayo-Reyes et al., 2010; Marcos, Barcelona

"…persons who excrete a higher proportion of MMA have a greater risk of skin lesions after data are adequately controlled for…"

Christiani grp., 2011

"phenotypic anchoring"

studies that are designed to relate specific alterations in gene expression profiles to specific adverse effects of environmental stresses defined by conventional parameters of toxicity such as clinical chemistry and histopathology.

- "…differences in urinary arsenic metabolites, genetic factors related to arsenic metabolism, and their joint distributions modulate arsenic toxicity."

Ahsan, Graziano, ++++ (2007)" skin lesions

Apologies to those researchers and studies I have not mentioned

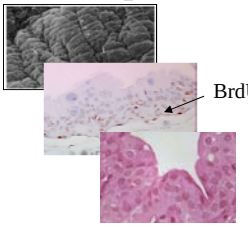
Some Thoughts Around Mode of Action and Identification of Key Events

Cacodylic Acid: Key Events an example of a mode of action

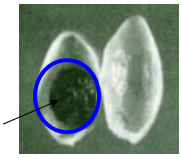
DMA^{III} ← DMA^{V}

6 hours	Urothelial Cytotoxicity
1 Week	Regenerative Proliferation
8-10 weeks	Hyperplasia
104 weeks	Tumors

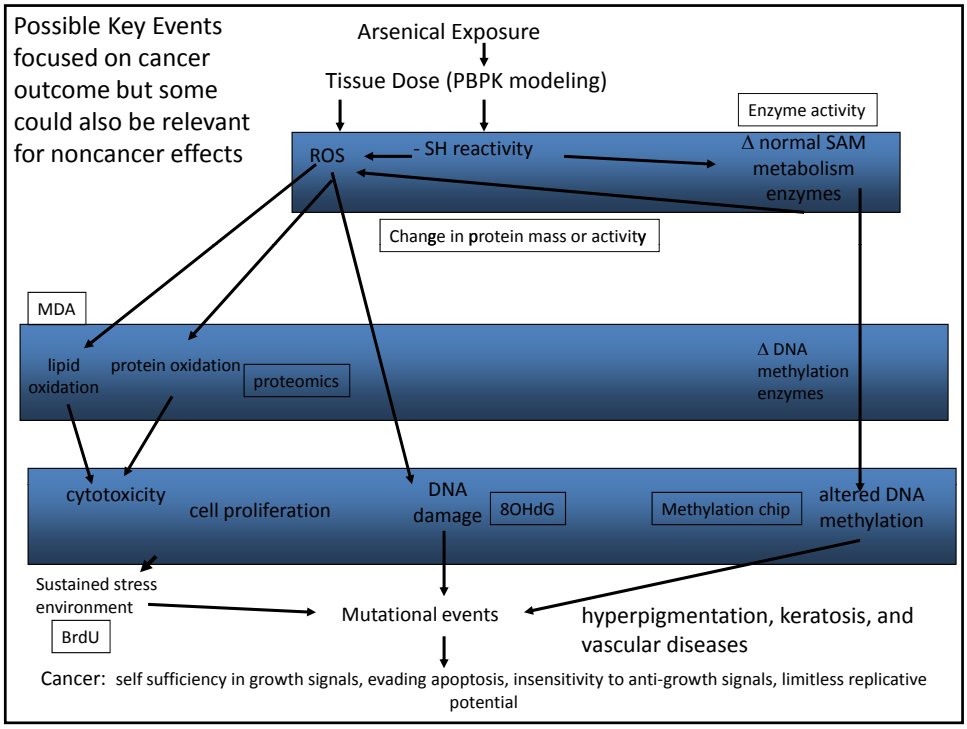
Urinary bladder from a female F344 treated with 100 ppm DMA^{V}



BrdU labeling



Urinary bladder tumors



Mode of Action Evidence – Noncancer and cancer effects

What data are available to identify mode(s) of action for the health effects of iAs?

What are the key toxicokinetic and toxicodynamic events?

Have inter-species or inter-individual differences been identified?

Have sufficient key events been identified to describe mode(s) of action for the noncancer effects?

Is there significant overlap of key events to harmonize the risk assessment across all adverse effects (cancer and noncancer)?

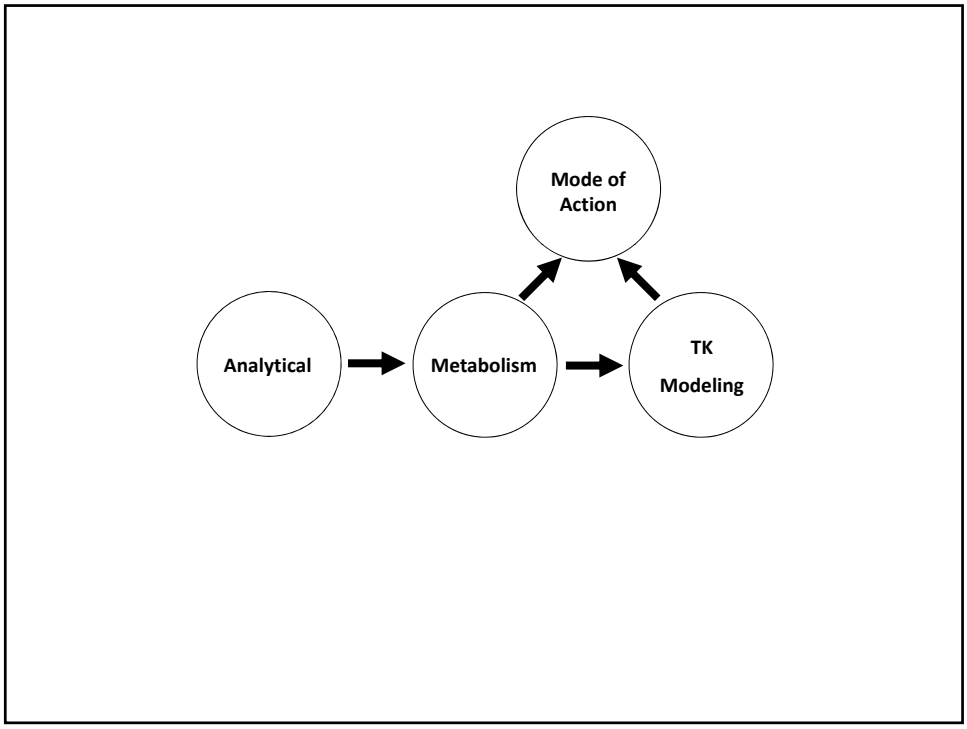
What data are available to identify mode(s) of action for the health effects of iAs?

Doug Wolf

2.4 Mode of action evidence – Noncancer and cancer effects

- *"mode of action"* is defined as a sequence of key events and processes, starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation
- *"key event"* is an empirically observable precursor step that is itself a necessary element of the mode of action or is a biologically based marker for such an element
- Mode of action is contrasted with "*mechanism of action*," which implies a more detailed understanding and description of events, often at the molecular level, than is meant by mode of action
- The toxicokinetic processes that lead to formation or distribution of the active agent to the target tissue are considered in estimating dose but are not part of the mode of action as the term is used here.

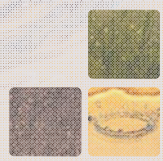
Guidelines for Carcinogen Risk Assessment, p. 1-10, Risk Assessment Forum U.S. EPA, 2005


Mode of Action
Analytical
Metabolism
TK Modeling

Mode-of-Action Evidence–Noncancer and cancer effects

2.5 What data are available to identify common toxic moieties and mode(s) of action for multiple health effects (noncancer and cancer) from iAs exposure?

Lead Discussants: Rory Connolly, Mirek Styblo



Mode-of-Action Evidence – Noncancer and cancer effects

2.5 What data are available to identify common toxic moieties and mode(s) of action for multiple health effects (noncancer and cancer) from iAs exposures?

Rory Conolly

January 8, 2014

Mode of action

- A sequence of key events linking the dose with the health effect
  - Intended to be pragmatic, get away from potentially infinite detail of the mechanism of action
  - Pragmatism: Should inform us about susceptibility and the shape of the dose-response curve
    - E.g., cytolethality ➔ threshold
    - Adaptive response ➔ nonlinearity

Dosimetry (PK)

- Mode of action discussions sometimes downplay the importance of exposure – tissue dose relationships
  - Unfortunate!
- ILSI key event dose-response framework
  - Unifies PK and MOA

iAs: A vast amount of data, but…

- How much is <u>useful</u> for risk assessment?
- kinds of data are useful?
  - Depends on intended use of the data
    - exposure
    - Hazard id
    - Dose-response assessment

When are data useful?

- Useful when can be used with minimal uncertainty
  - i.e., directly relevant to the intended use
- Relevance inversely proportional to need for extrapolation
  - In vitro to in vivo
    - Transformed cells in culture to people
  - High to low dose
  - Estimated exposure vs measured
  - Etc.

Literature evaluation

- Cull based on relevance!
  - Most of the literature not useful for risk assessment
    - Sam Cohen point this morning
- Critically evaluate when use of data would reduce uncertainty relative to what you will do as a default
  - Comparative uncertainty analysis

Factors that encourage moving away from the default

- Understand biological relevant of the test system
- Understand dosimetry
- Availability of dose-response and time-course datasets

Questions / issues

- Characterization of heritable effects
- Concentration filtering of effects
- Don't forget about PK!
- Integrate health risk assessment with analysis of sustainability
- If epi is of sufficient quality then how much additional research is needed?

What data are available to identify common toxic moieties and mode(s) of action for multiple health effects (noncancer and cancer) from iAs exposure?

Mirek Styblo

Toxicological Review of Inorganic Arsenic

SESSION 2: Approaches for hazard identification for iAs

Mode-of-Action Evidence –Noncancer and cancer effects

2.5. What data are available to identify common toxic moieties and mode(s) of action for multiple health effects (noncancer and cancer) from iAs exposure?

Lead Discussants: Rory Connolly, Mirek Styblo

- ***The Diabetogenic Effects of iAs - MOA***

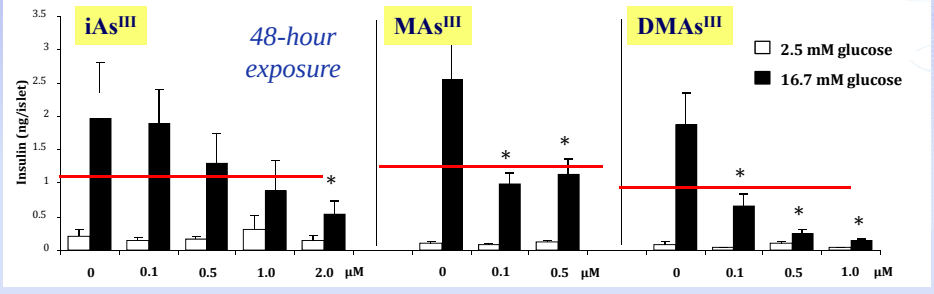
NTP
National Toxicology Program

Potential Mechanism:

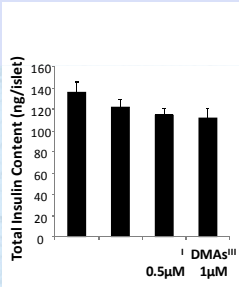
1. Influence of inorganic arsenic on glucose-stimulated insulin secretion in pancreatic β-cells.
2. Influence of trivalent arsenicals on glucose uptake in adipocytes and skeletal muscle cells.
3. Inhibition of adipogenic and myogenic differentiation

Maull et al., EHP, 120:1658–1670 (2012)

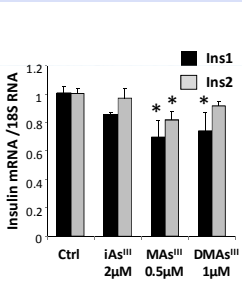
Trivalent Arsenicals Inhibit Glucose Stimulated Insulin Secretion by Isolated Murine Pancreatic Islets



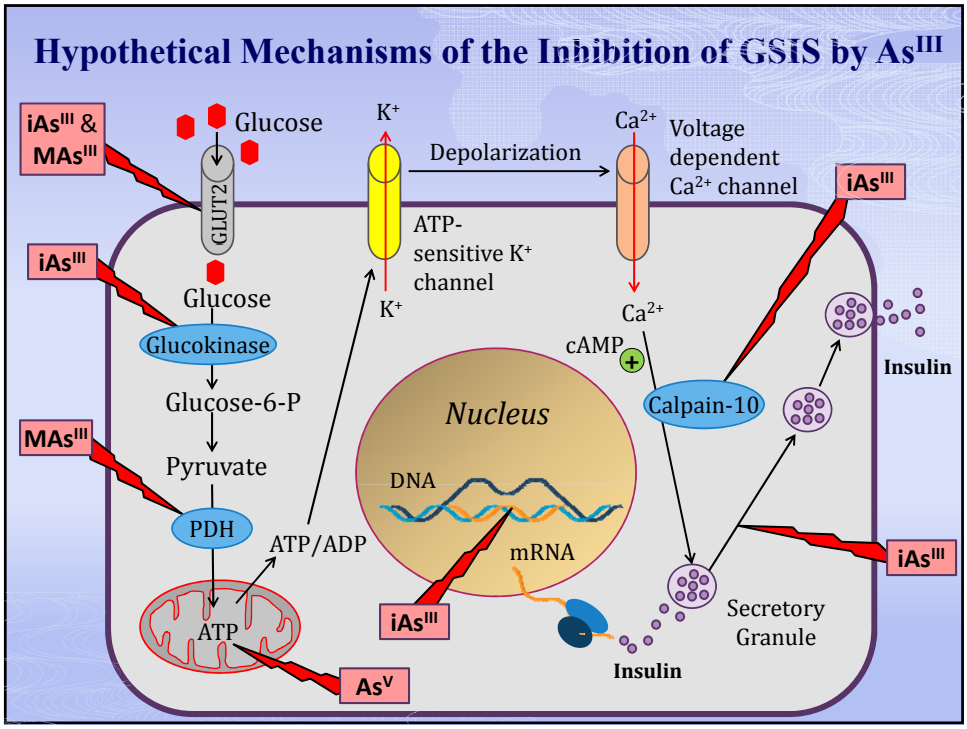
Insulin Content

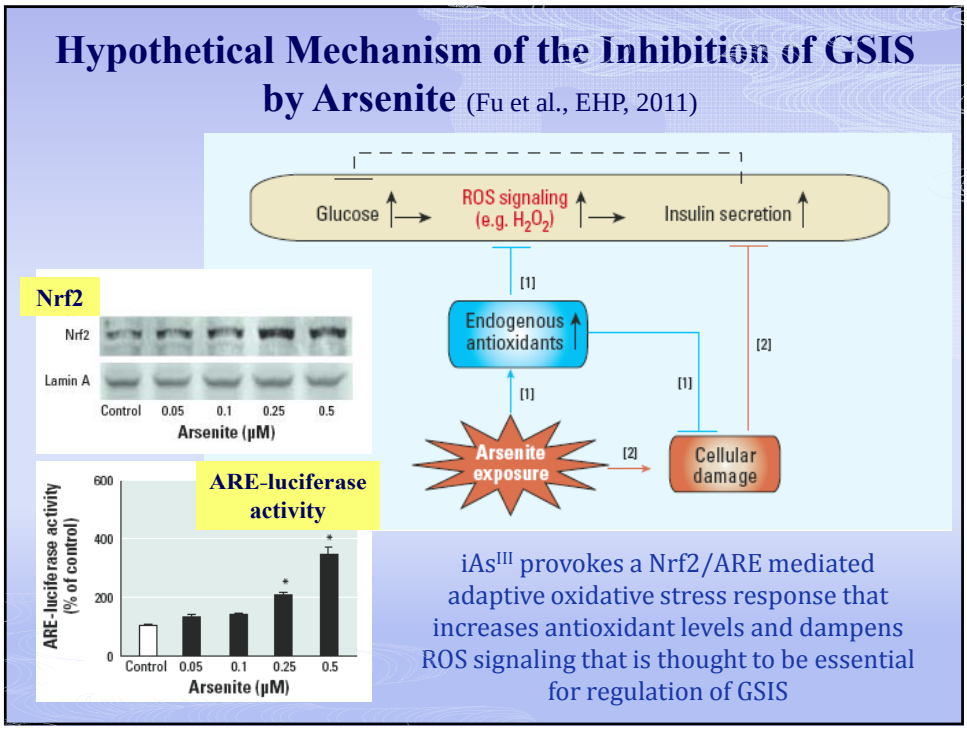


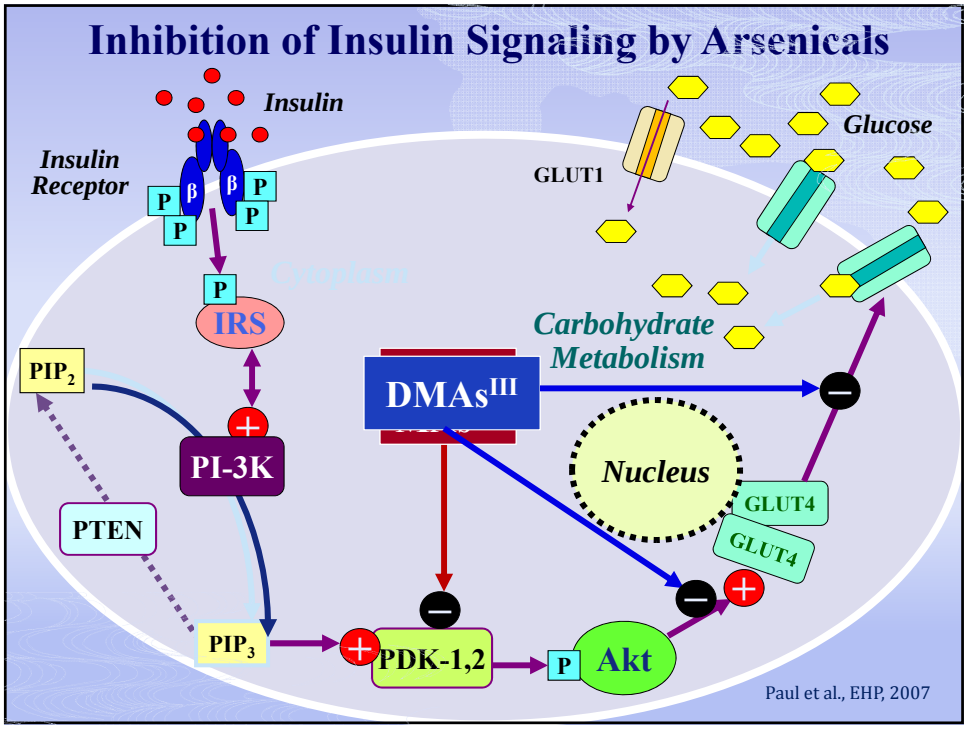
Insulin mRNA



Douillet et al., TAAP 2013

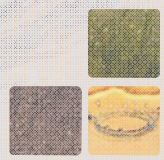

Hypothetical Mechanisms of the Inhibition of GSIS by AsIII
iAsIII & MAsIII
Glucose
GLUT2
K+
Depolarization
Ca2+
Voltage dependent Ca2+ channel
iAsIII
ATP-sensitive K+ channel
Glucose
Glucokinase
Glucose-6-P
Pyruvate
PDH
ATP/ADP
ATP
AsV
Nucleus
DNA
mRNA
cAMP
Calpain-10
Insulin
Secretory Granule

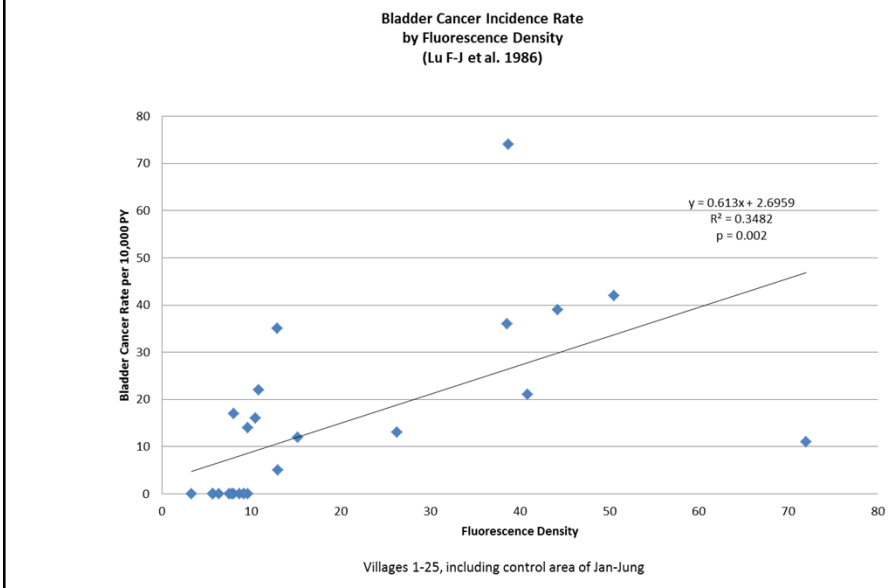

Hypothetical Mechanism of the Inhibition of GSIS by Arsenite (Fu et al., EHP, 2011)
Glucose
ROS signaling (e.g. H2O2)
Insulin secretion
[1]
Endogenous antioxidants
[2]
Arsenite exposure
Cellular damage
Nrf2
Nrf2
Lamin A
Control 0.05 0.1 0.25 0.5
Arsenite (μM)
ARE-luciferase activity
ARE-luciferase activity (% of control)
600
400
200
0
Control 0.05 0.1 0.25 0.5
Arsenite (μM)
iAsIII provokes a Nrf2/ARE mediated adaptive oxidative stress response that increases antioxidant levels and dampens ROS signaling that is thought to be essential for regulation of GSIS


Inhibition of Insulin Signaling by Arsenicals
Insulin
Insulin Receptor
β
β
P
P
P
P
P
Cytoplasm
IRS
GLUT1
Glucose
Carbohydrate Metabolism
PIP2
PI-3K
DMAsIII
Nucleus
GLUT4
GLUT4
PTEN
PIP3
PDK-1,2
P
Akt
Paul et al., EHP, 2007

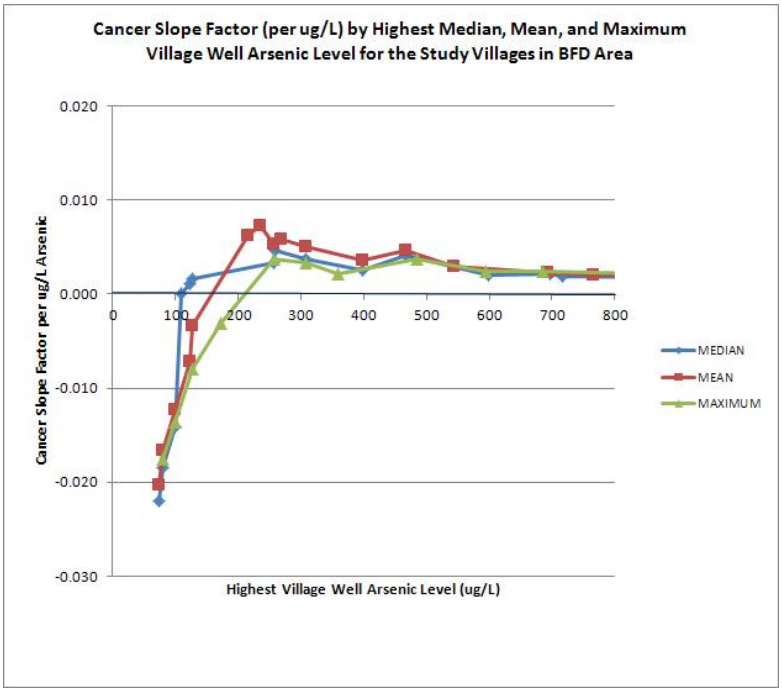
Methods for Identifying, Evaluating, and Synthesizing Literature

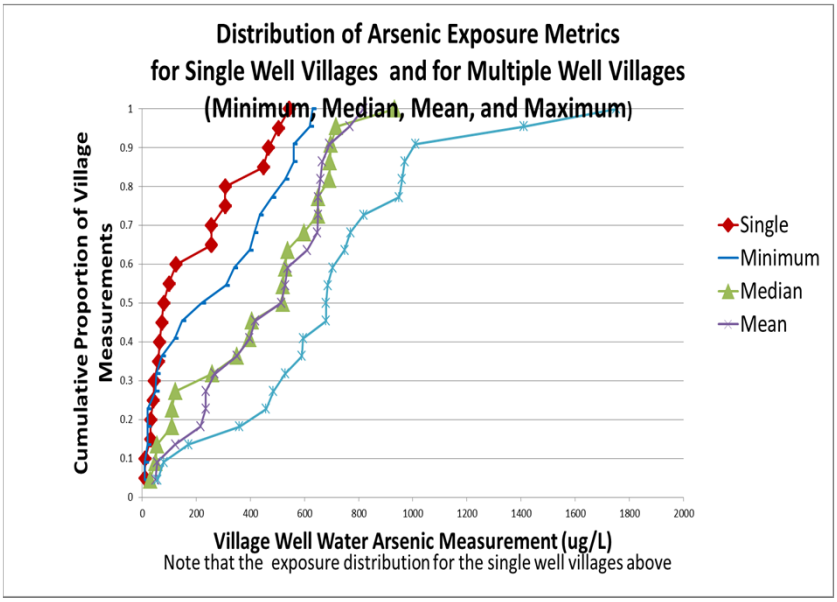
Public Comments

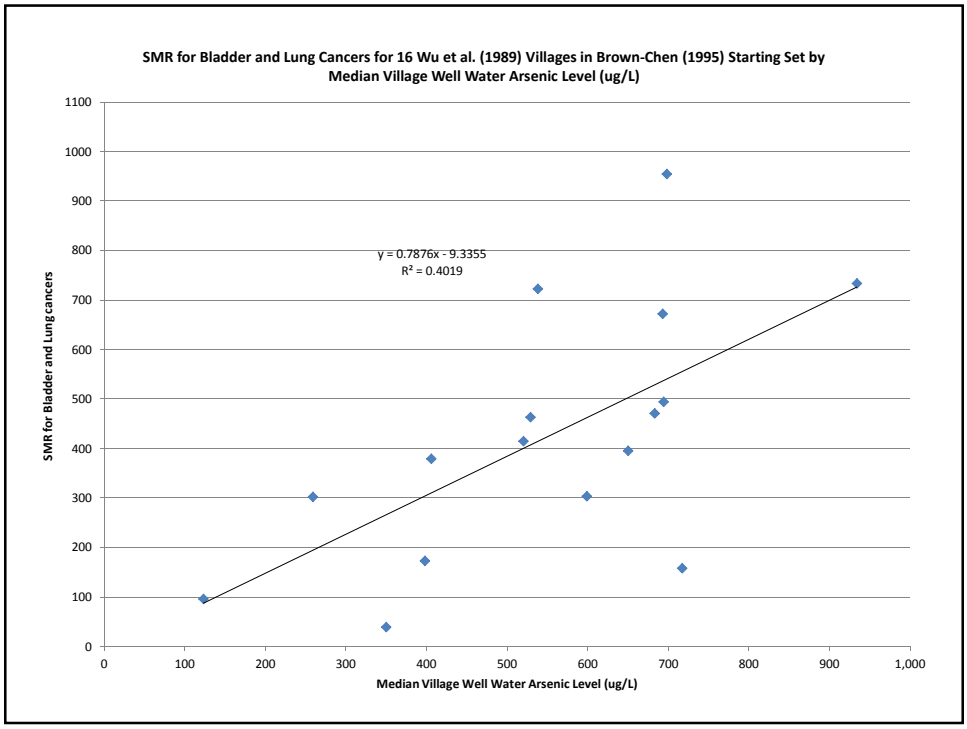




Villages 1-25, including control area of Jan-Jung


Cancer Slope Factor (per ug/L) by Highest Median, Mean, and Maximum Village Well Arsenic Level for the Study Villages in BFD Area
Cancer Slope Factor per ug/L Arsenic
0.020
0.010
0.000
-0.010
-0.020
-0.030
0
100
200
300
400
500
600
700
800
Highest Village Well Arsenic Level (ug/L)
MEDIAN
MEAN
MAXIMUM


Distribution of Arsenic Exposure Metrics for Single Well Villages and for Multiple Well Villages (Minimum, Median, Mean, and Maximum)
Cumulative Proportion of Village Measurements
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
200
400
600
800
1000
1200
1400
1600
1800
2000
Village Well Water Arsenic Measurement (ug/L)
Note that the exposure distribution for the single well villages above
Single
Minimum
Median
Mean


SMR for Bladder and Lung Cancers for 16 Wu et al. (1989) Villages in Brown-Chen (1995) Starting Set by Median Village Well Water Arsenic Level (ug/L)
y = 0.7876x - 9.3355
R² = 0.4019
SMR for Bladder and Lung cancers
Median Village Well Water Arsenic Level (ug/L)
0
100
200
300
400
500
600
700
800
900
1000
1100
1,000