CHRONIC ARSENIC TOXICITY

Environmental Health, Natural History and Chemical Assessment

José A. Centeno

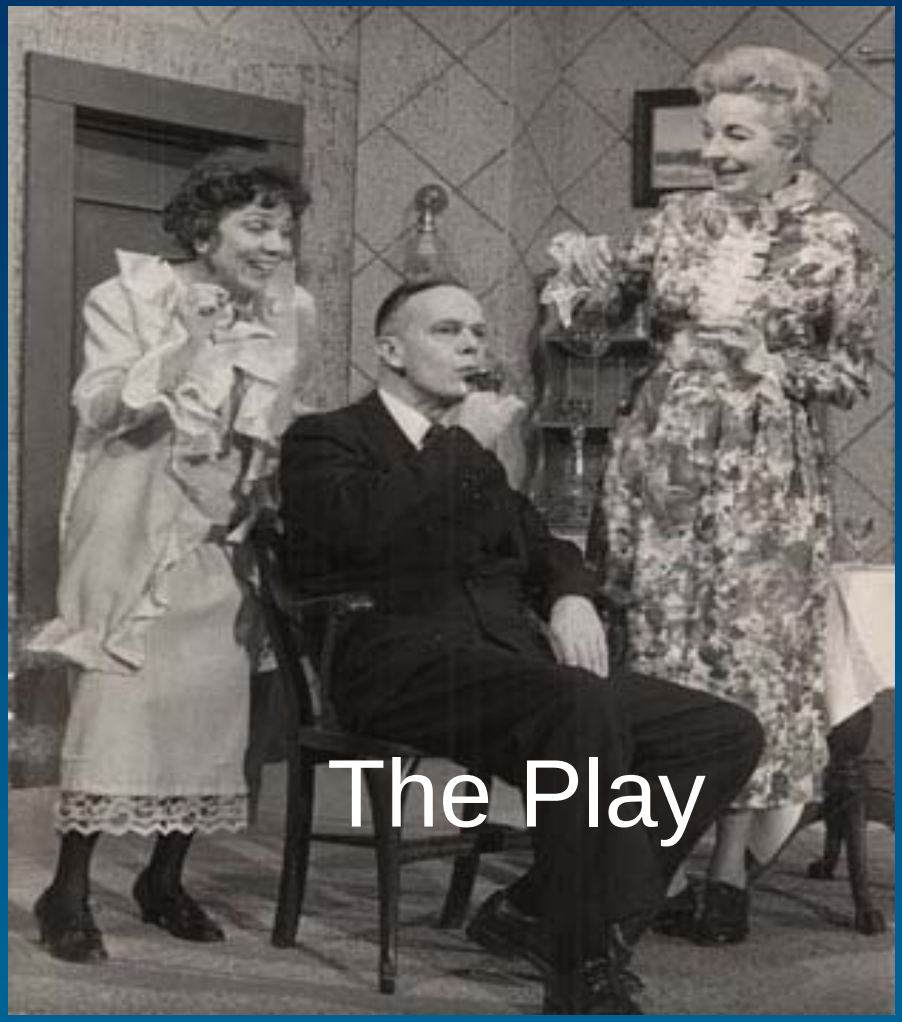
U.S. ARMED FORCES INSTITUTE OF PATHOLOGY

Washington, DC

Email: centeno@afip.osd.mil

Outline

- Introduction
  - Natural History and Global Impact
- Toxicology and Health Effects of Arsenic
- Importance of Speciation
  - Review of Modern Techniques
- Concluding Remarks : Future Directions



By Joseph Kesselring

Arsenic

Natural History

- Well known poison for use as a suicidal and homicidal agent (eg, 19th Century as “Golden Age” of As poisoning)
- Medicinal uses since antiquity to the early 20th century
- Lewisite used in WW I as a vesicant
- Subsequent development of BAL as an antidote
- Primary uses in agriculture, forestry, animal husbandry, wood preservation, pigments, semiconductor industry (arsine gas)
- Natural occurrence is common in water and soil
- Inorganic arsenical compounds are of greatest concern

Pre-Columbian Mummies with Chronic Arsenic Poisoning

Site: Camarones Valley
Northern Chile

Age: Inca period
(15th Century)

Findings:

Hyperpigmentation
Squamous Cell Carcinomas

**80% of modern inhabitants in this region had skin lesions*

Gerszten E, Allison MJ, Sawyer DR. Paleopathology 2, 2000.

Arsenic Poisoning Through the Ages

- **The 19th Century can be regarded as the "golden" age of arsenic poisoning:**

  - ✓ **1881-1882: Goeie Mie ('Good Mary') of Leiden, The Netherlands, poisoned at least 101 friends and relatives. Distributed As_2O_3 in hot milk to her victims after opening life insurance policies in their names.**

Goeie Mie's toll

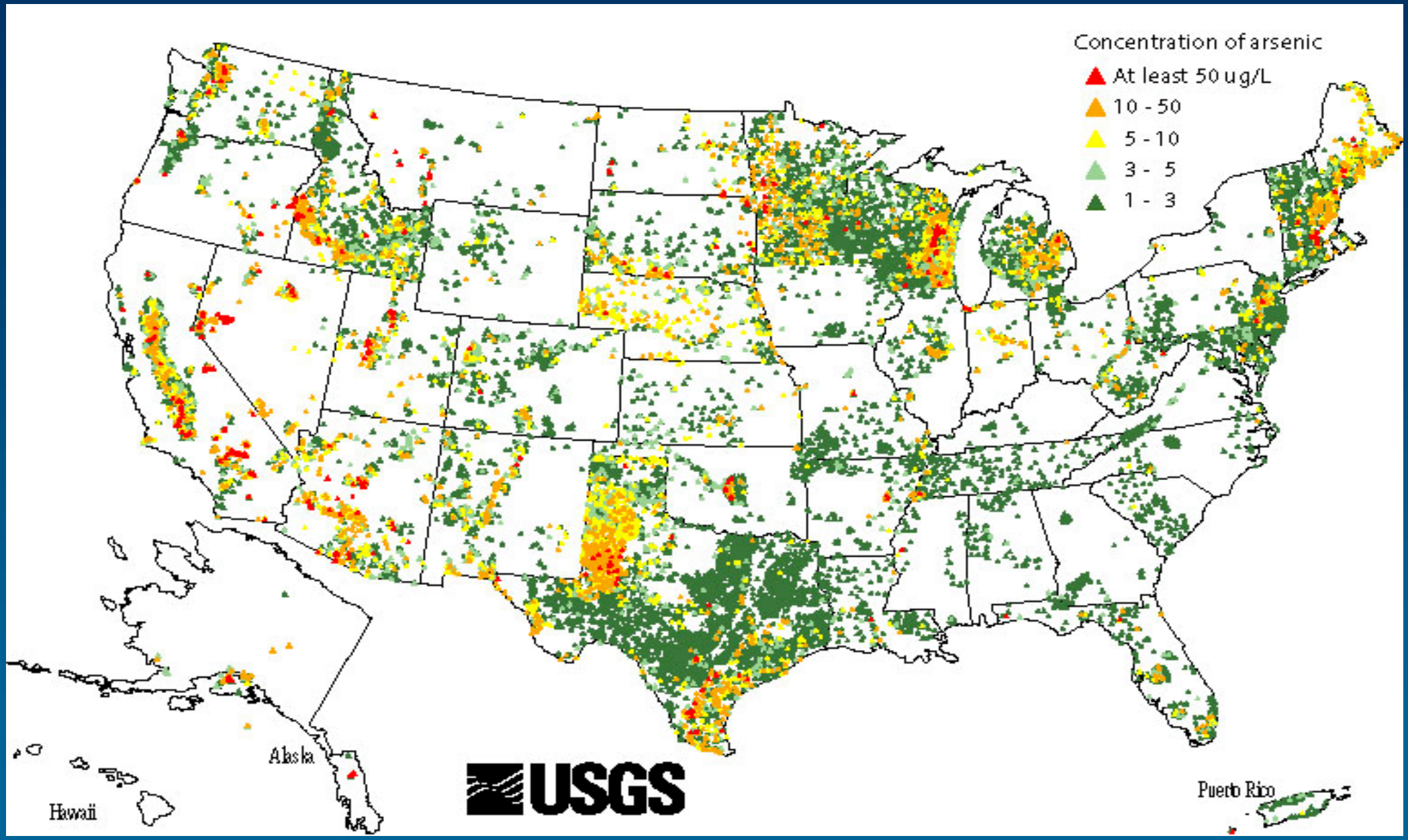
- 102 people poisoned (1867 –1884)
- 45 persons seriously ill, often with neurological sequelae
- 27 persons died, 16 of whom were relatives

ARSENIC EXPOSURE : GLOBAL IMPACT

PEOPLE AFFECTED BY NATURALLY CONTAMINATED WATER and SOIL

W. Scarpeli, Brazil

O.Selinus et al. “Essentials of Medical Geology”


Concentration of arsenic
At least 50 ug/L
10 - 50
5 - 10
3 - 5
1 - 3
USGS
Alaska
Hawaii
Puerto Rico

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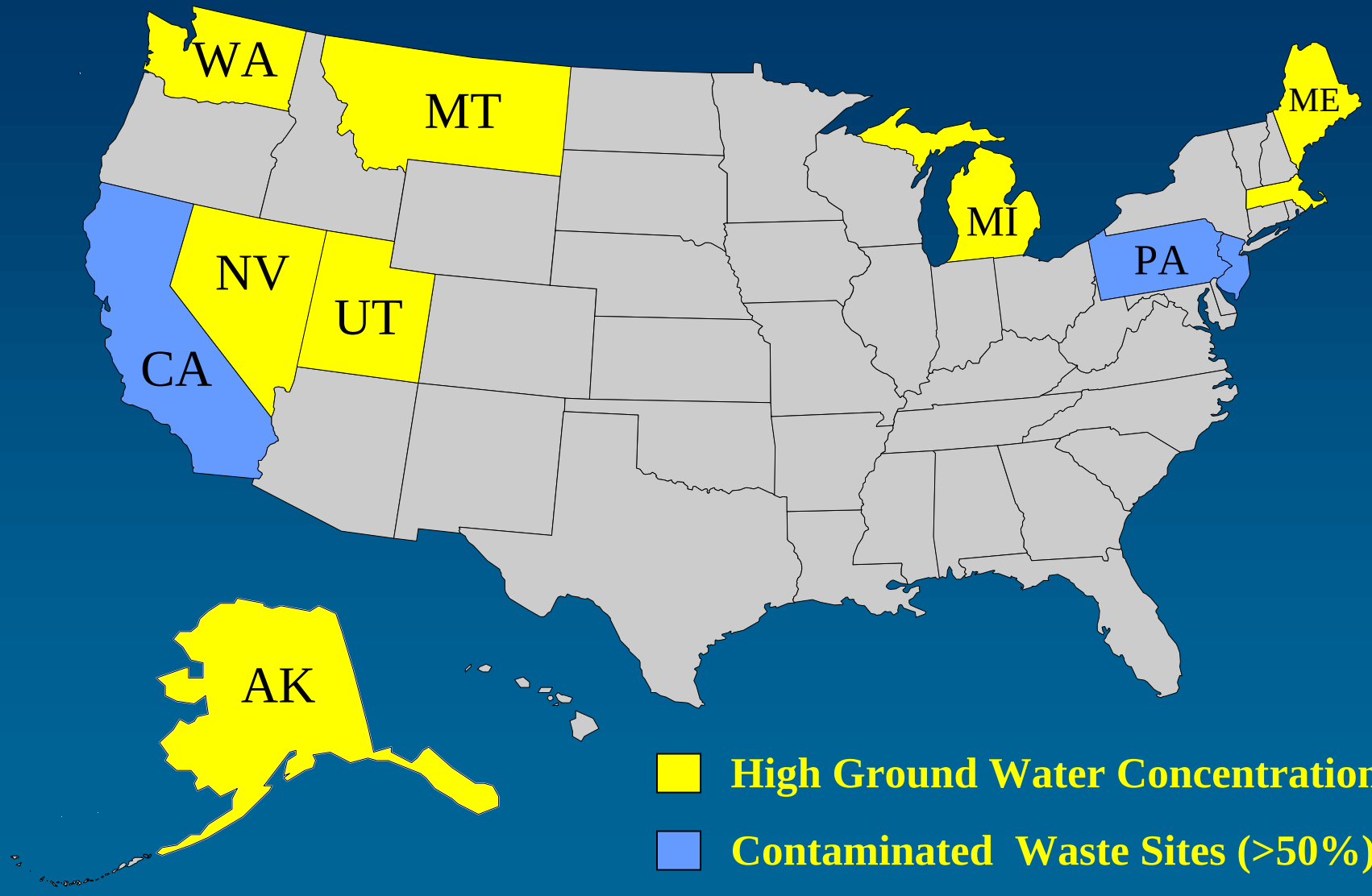
States with Highest Arsenic Levels



Groundwater arsenic calamity in Bangladesh

Ratan Kr. Dhar, Bhajan Kr. Biswas, Gautam Samanta, Badal Kr. Mandal, D. Chakraborti†, Shibtosh Roy*, Abu Jafar*, Ariful Islam*, Gulshan Ara*, Saiful Kabir*, A. Wadud Khan, S. Akther Ahmed** and S. Abdul Hadi****

School of Environmental Studies, Jadavpur University, Calcutta 700 032, India
*Dhaka Community Hospital, Malibagh, Dhaka 1219, Bangladesh
**National Institute for Preventive and Social Medicine, Mahakali, Dhaka 1212, Bangladesh

CURRENT SCIENCE, Vol. 73, No. 1, July 10, 1997, pp. 48-59.

Bangladesh victim of world's worst mass poisoning

Arsenic-tainted water blamed for sickness, death

By Paul Salopek
Chicago Tribune

NOWAPARA, Bangladesh — When the mysterious sores first appeared on Anil Chadra Das' work-toughened hands, the grizzled rice farmer, long hardened against the aches and pains of life in rural Bangladesh, just ignored them.

But the lesions didn't go away. Instead, the small purplish scabs

lard Chappell, an environmental expert at the University of Colorado who recently visited the affected areas in India as the director of an international task force on arsenic poisoning. "You just shake your head in amazement at this one."

The cast of characters in the emerging health disaster includes armies of quack doctors who prey on the poisoning victims, knowing

"My parents told me to leave home when I got sick. They said the spirits of our house were displeased," said Howladar.

Experts say the arsenic beneath Bangladesh's fertile river deltas was probably deposited eons ago after washing down from bodies of ore in the Himalayas. As long as the arsenic compounds — called

mained inert.

But with the advent of intensive irrigation in the 1970s, the aquifers have dropped, exposing the poisons to oxygen for the first time.

Once oxidized, arsenic sulfides become water-soluble.

And like tea seeping from a bag, they percolate from subsoils into dropping water tables with every monsoon flood.

Or so the leading theory goes.

"Nobody knows, exactly," says

who has followed arsenic poisoning outbreaks in Taiwan, Chile, Mexico and the western United States. Most of those incidents have sickened a few thousand people.

One reason the world's worst arsenic epidemic has been so muted, Gibb said, is that — contrary to its sinister, suicide-capsule reputation — arsenic poisoning can be undramatic, even stealthy.

Even wells fouled with 200 times the World Health Organization's

have been found near Suma's village, will not kill outright.

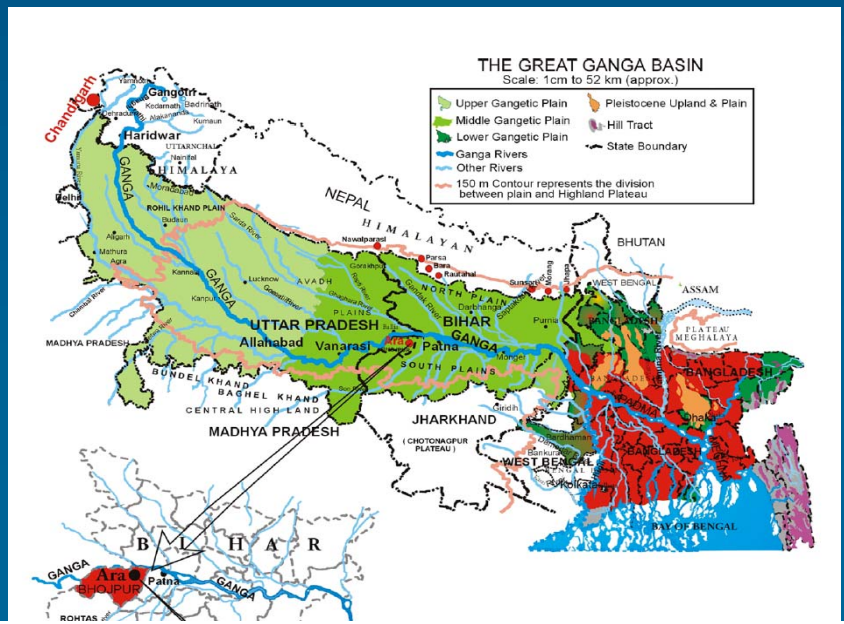
Instead, a buildup of the lethal chemical over months or years causes a wide array of increasingly debilitating ailments, from lesions on the hands and feet to organ cancers, neural disorders, deafness and, possibly, even diabetes.

"Just wait. You'll start seeing patterns of cancers pop up in this region in a few years," said Gibb. "This is a major tragedy exactly

THE DENVER POST, Thursday, January 16, 1997

Arsenic Contamination of Groundwater in India and Bangladesh

- First reported in mid- 1980's
- Tube wells screened in 3000 villages in West Bengal
- Of 18 state districts, 9 are affected
- In Bangladesh, 50 of 64 districts are affected (As levels **<10 - > 10,000 µg/L**)
- 150 million people may be at risk in Bangladesh and in West Bengal alone



Arsenic ($^{33}As_{75}$)

- **Arsenic** (is a *metalloid*; it complexes not only with other metals, but also C, H, O):
  - occur naturally
  - exhibit both metallic and nonmetallic properties
- **Major groups of arsenic compounds:**
  - inorganic arsenic : As_2O_3, As_2S_3, $NaAsO_2$, Na_2HAsO_4
  - liver metabolites ("de-toxification" mechanism) : monomethylarsonate (MMA^{5+}; MMA^{3+}?) & dimethylarsinate (DMA^{5+}; DMA^{3+}?)
  - organic arsenic : Arsenobetaine, Arsenocholine, trimethylarsenic tetramethylarsonium cation
  - arsine gas (AsH_3)
- **Toxicity:** Arsine gas > Inorg (As^{3+}) > Organic (As^{3+}) > Inorg. (As^{5+}) > Organic (As^{5+}) > As^0

Arsenic Compounds

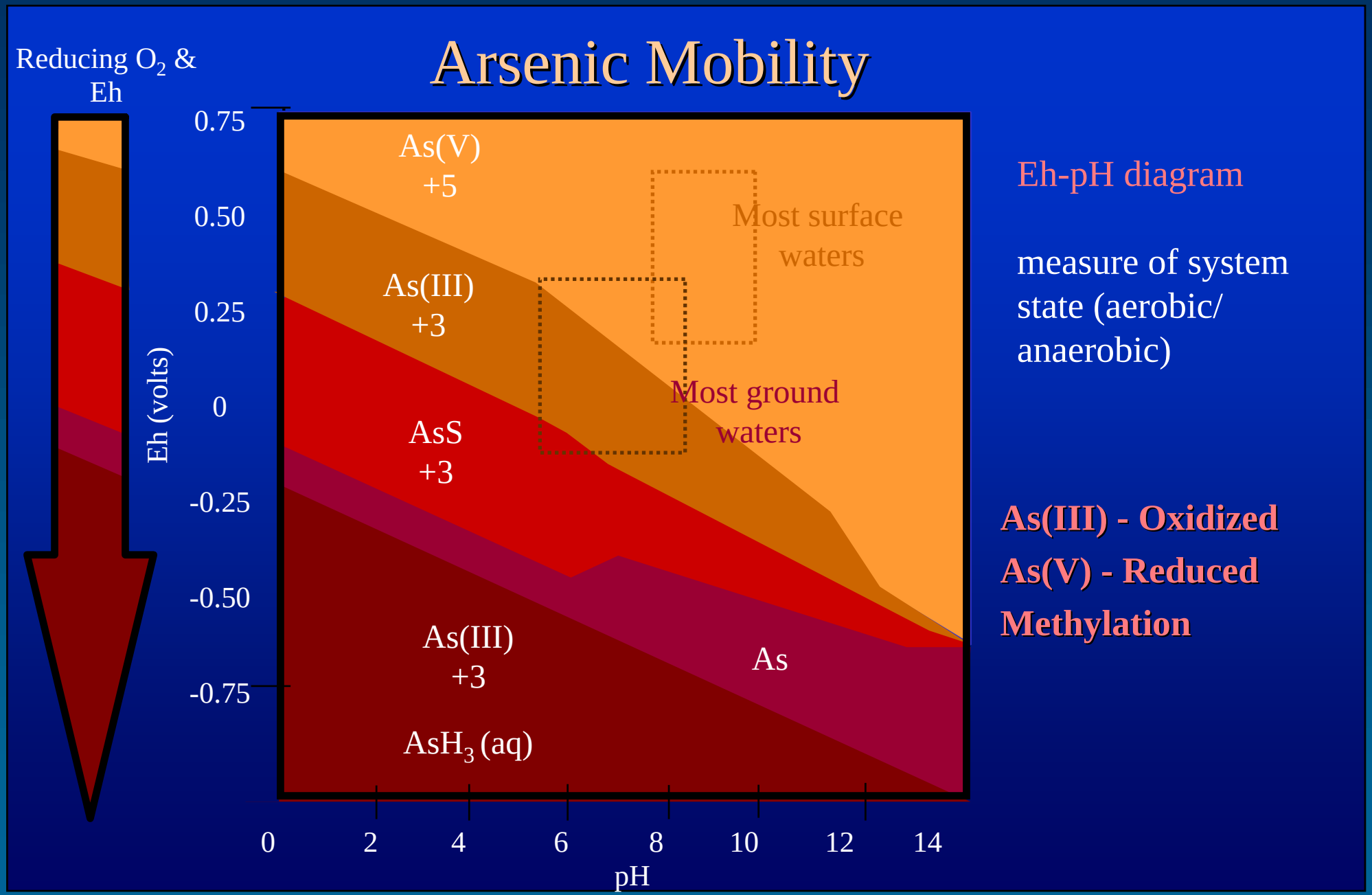
Toxicity Data

Arsenic Compound	LD_{50} (mg/kg)	Animal
Inorganic arsenite [As(III)]	4.5	rat
Inorganic arsenate [As(V)]	14-18	rat
MMAA - **monomethylarsonic**	1,800	mouse
DMAA - dimethylarsinic acid	1,200	mouse
TMAO - trimethylarsine oxide	10,600	mouse
AsB - arsenobetaine (marine)	> 10,000	mouse
AsC - arsenocholine (marine)	6,000	mouse

LD_{50} - concentration at which 50% of a population dies.

Low LD_{50} - more toxic **High LD_{50} - less toxic**

Arsenic Mobility



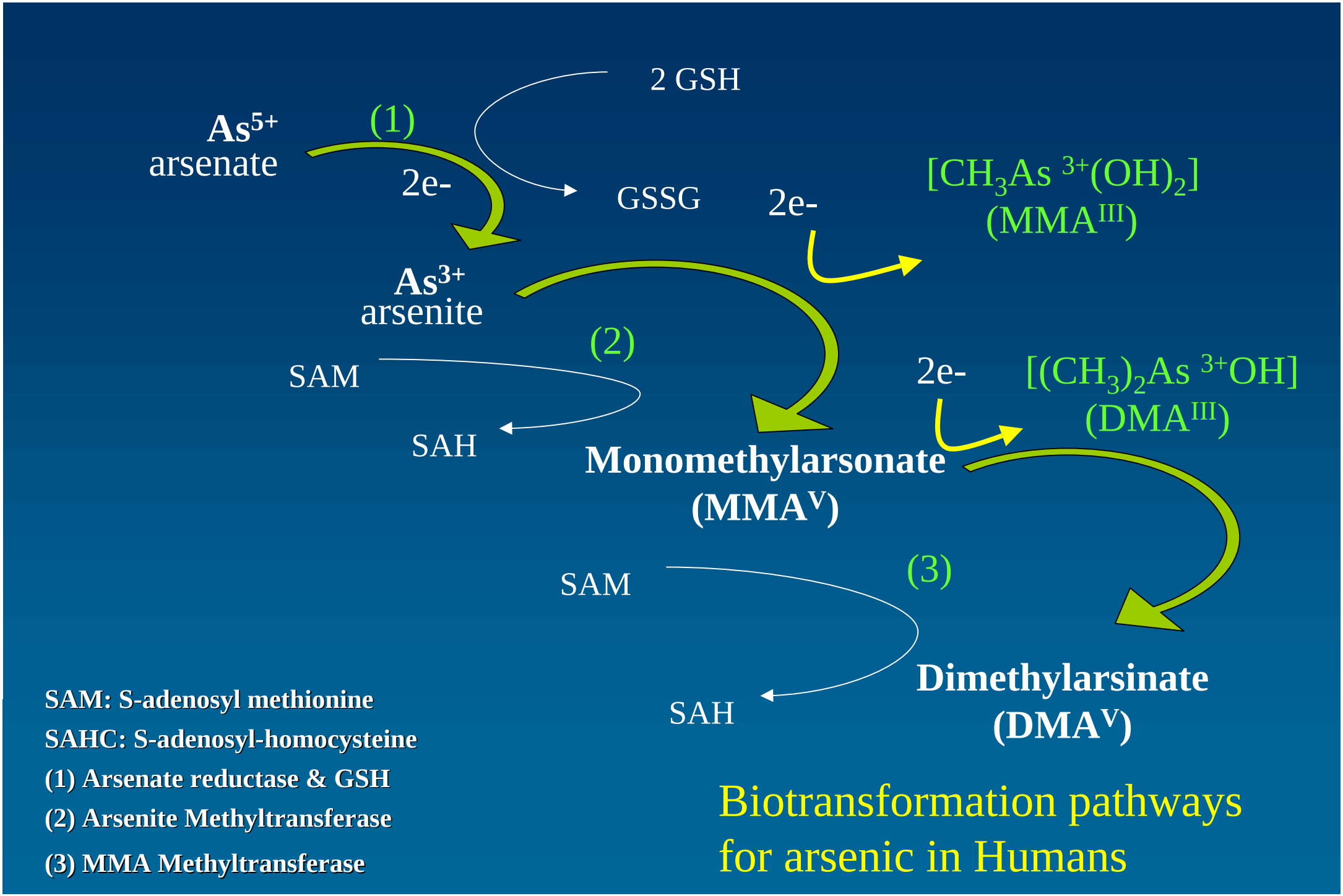
Eh-pH diagram

measure of system state (aerobic/ anaerobic)

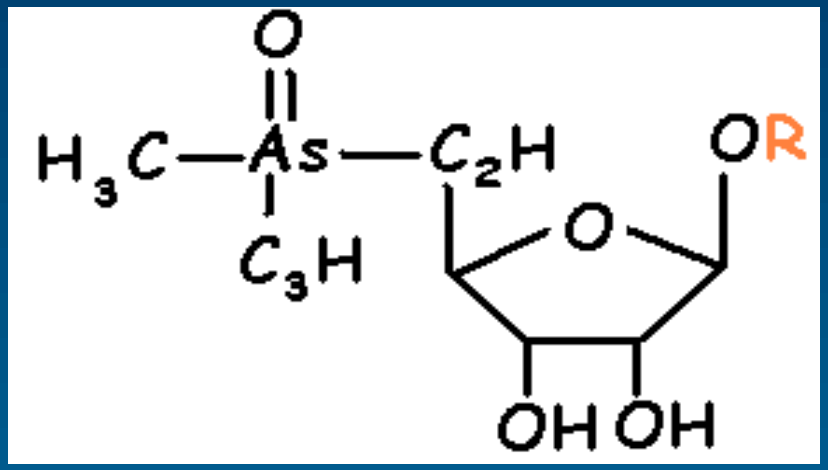
As(III) - Oxidized

As(V) - Reduced

Methylation


2 GSH
As5+
arsenate
(1)
2e-
GSSG
2e-
[CH3As 3+(OH)2]
(MMAIII)
As3+
arsenite
(2)
SAM
SAH
2e-
[(CH3)2As 3+OH]
(DMAIII)
Monomethylarsonate
(MMAV)
SAM
(3)
SAH
Dimethylarsinate
(DMAV)
SAM: S-adenosyl methionine
SAHC: S-adenosyl-homocysteine
(1) Arsenate reductase & GSH
(2) Arsenite Methyltransferase
(3) MMA Methyltransferase
Biotransformation pathways
for arsenic in Humans

Arsenic Structures



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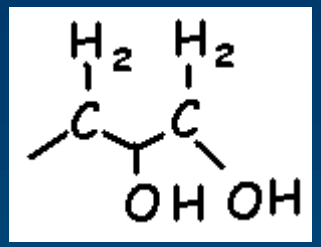
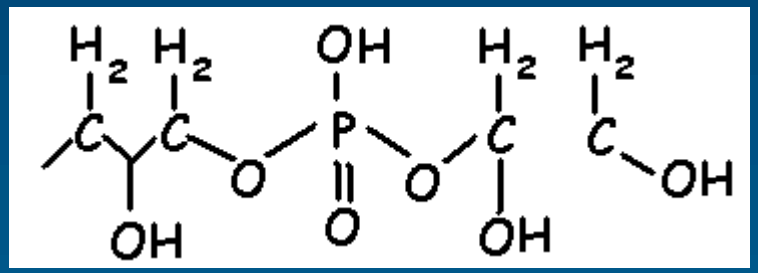
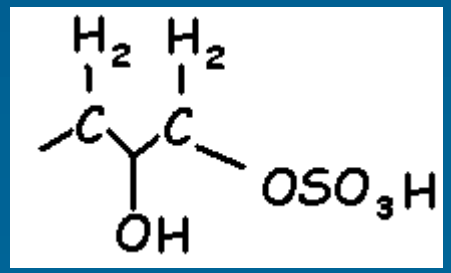


Glycerol -ribose



Phosphate-ribose



Sulfonate-ribose

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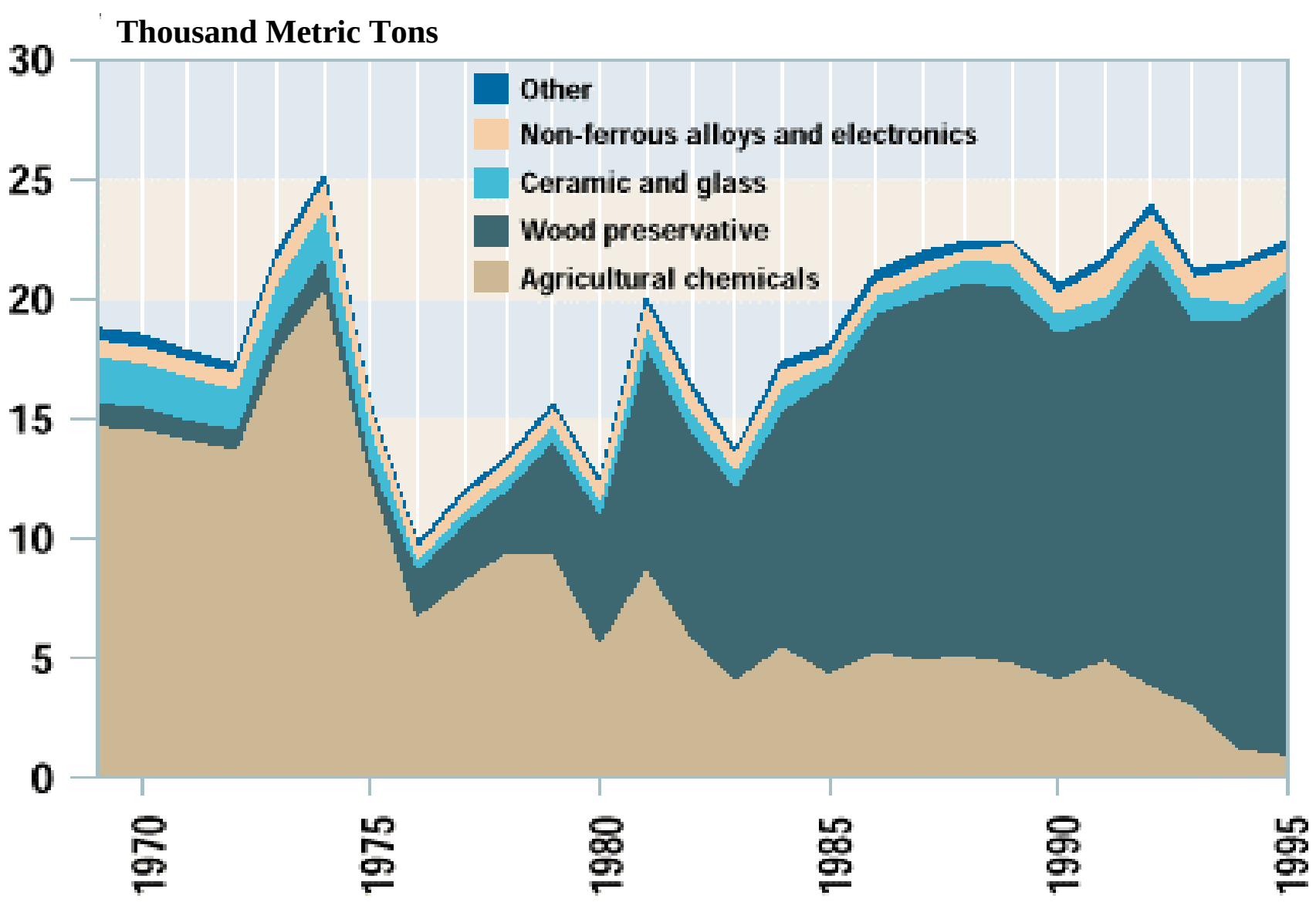
Potential Sources of Arsenic Exposures

- Natural (geological) (As^{III} and As^{V})
- Drinking water (As^{V})
- Foodstuff (Inorganic and organic As)
- Industrial
  - Smelting of sulfidic ores (As^{III})
- Pesticides (As^{V})
- Medical drugs and therapy (*Trisenox*, As_2O_3)
- Cr-Cu-As Pressure-Treated wood

Use of Arsenic in United States



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Potential Sources of Exposure: Cr-Cu-As Treated Wood

TOXIC PLAYGROUNDS

Forts and castles made of arsenic-treated wood last for years, but should kids be playing on them?

By JEFFREY KLUGER

IF THERE'S ONE THING WOOD KNOWS HOW to do, it's rot. Expose lumber to the elements, and within as few as five years, sun, rain, termites and fungus can reduce it to pulp. That's why builders were so enthusiastic in the 1970s when the lumber industry introduced pressure-treated boards—ordinary planks and posts injected with an extraordinary preservative known as CCA that can extend the life of wood fivefold, eliminating repairs and saving millions of trees annually. What got less attention at the time is the fact that CCA stands for chromated copper arsenate—a form of arsenic. And that's turned out to be a problem.

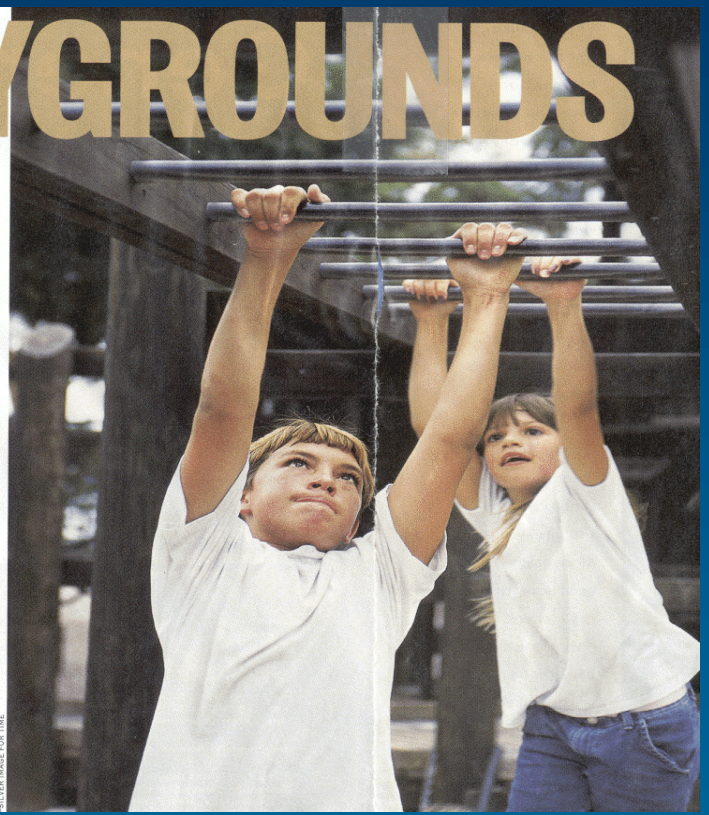
Though CCA is infused deep into the fibers of wood under very high pressure, the poison—which keeps the insects away—now seems to be leaching out. It's bad enough if decks, docks and maybe even a few picnic tables begin sweating arsenic, but the toxin was also widely used in children's playgrounds, where over the past couple of decades thousands of whimsical wooden forts and castles have been built on sites that once housed metal swings and

threat of poison in playgrounds, but in government circles, the alarm bells being sounded by consumer groups have reached the point where officials feel they have to act. Last week the Environmental Protection Agency announced that starting in the fall, CCA-treated lumber sold in the U.S. will contain a warning label, and stores will be provided with stickers and signs for their displays. At the same time, the Consumer Product Safety Commission agreed to ask for public comments on petitions that could lead to an outright ban of CCA. In Florida, dozens of playgrounds have been shut down, and Governor Jeb Bush has ordered a state-run wood-treatment plant to switch to another preservative. While adults wrestle with the politics of the problem, however, it's kids who may be paying the ultimate price.

98% of the wood sold for outdoor use in the U.S. is treated with chromated copper arsenate. In Florida alone, that adds up to nearly 30,000 tons of arsenic

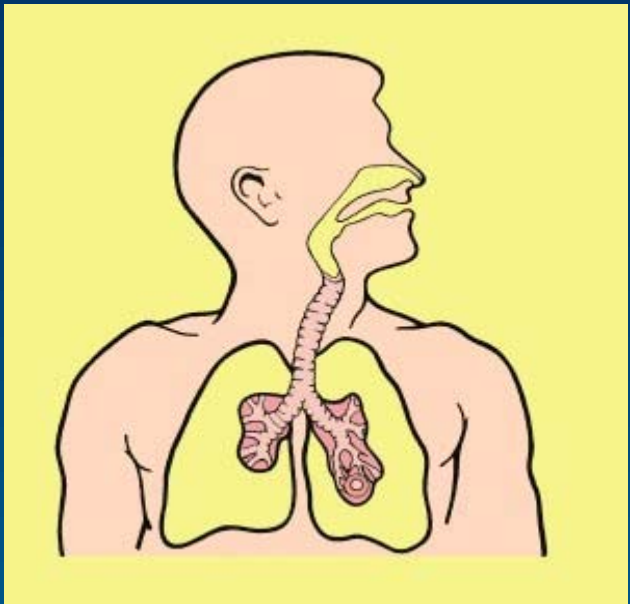
For scientists investigating arsenic, the numbers are sobering. Ninety-eight percent of outdoor wood sold in the U.S. is treated with CCA. In Florida alone, nearly 30,000 tons of arsenic are believed to be at large. Investigators testing soil in the state's playgrounds have found arsenic levels far higher than hazardous-waste ex-

SILVER IMAGE FOR TIME



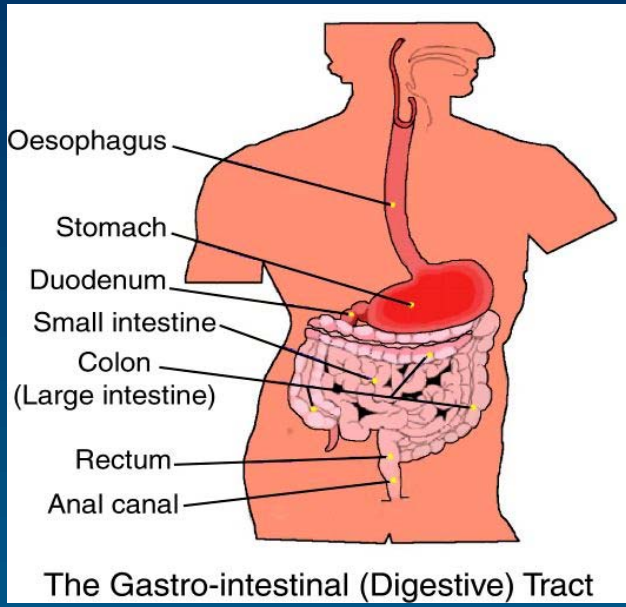
98% of the wood sold for outdoor use in the U.S. is treated with chromated copper arsenate. In Florida alone, that adds up to nearly 30,000 tons of arsenic

Inhalation



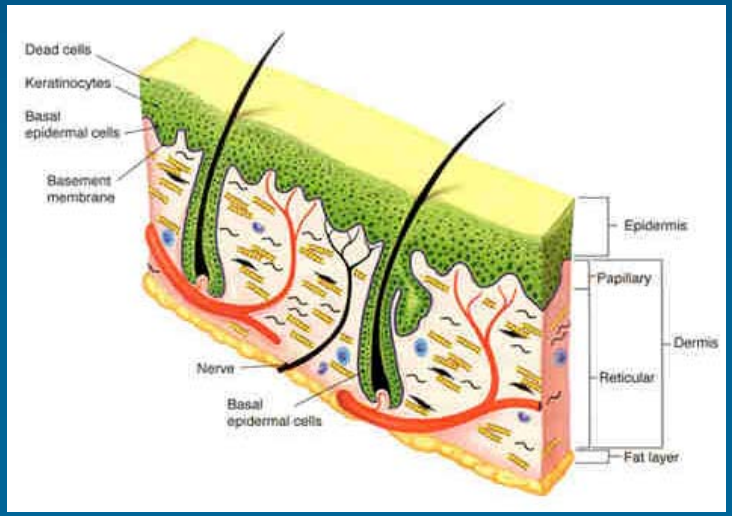
Arsenic Exposure Pathways

Ingestion



The Gastro-intestinal (Digestive) Tract

Dermal contact



Arsenic Exposure

HEALTH EFFECTS

Major evidence that Arsenic is a Human Carcinogen

Exposed group	Increased Cancer Risk
Smelter workers	Lung cancer
Arsenical pesticide workers	Lung cancer
Patients treated with arsenic-containing medicinals (mostly for psoriasis)	Skin cancer
Arsenic in drinking water: Taiwan, Chile, Argentina, Mexico, China, Bangladesh, India (Bengal region), Japan, etc.	Skin cancer
Follow-up study on Taiwan group	Bladder, lung, liver, and possibly kidney, and colon

Significant effects seen in multiple drinking water studies

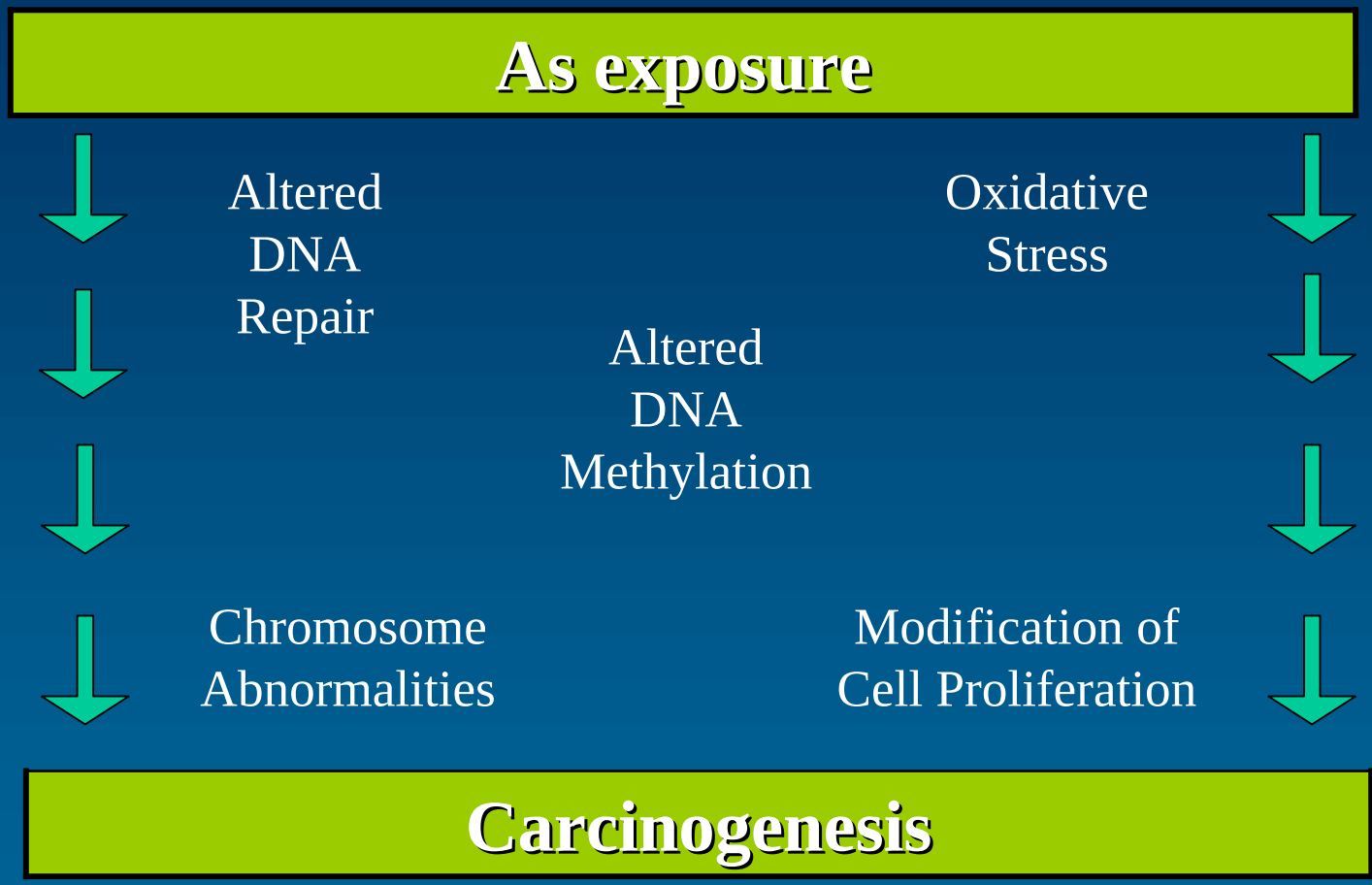
ARSENIC PARADOX

Arsenic (arsenite, arsenate) is a well-established human carcinogen, but until recently has been negative in all well-designed animal carcinogenicity bioassays.

In vivo carcinogenesis by inorganic arsenic before 2001

- Inorganic arsenic consistently failed to induce tumors in 4 species of animals at reasonable doses.
- Only extremely harsh conditions (high doses intubated into lung) gave some tumors. Most mice died.
- Arsenite also failed as an initiator and as a promoter in 2 stage carcinogenesis.
- Arsenite is a comutagen. Could it be a cocarcinogen?

Potential Modes of Action for Arsenic Carcinogenesis



Health Effects Associated with Arsenic Exposure

- Cancer: skin, lung, bladder, liver, kidney
- Cardiovascular disease
- Peripheral vascular disease
- Developmental effects
- Neurologic & neurobehavioral effects
- Diabetes Mellitus
- Hearing loss
- Portal fibrosis of the liver
- Lung fibrosis
- Hematological effects (e.g., anemia)

Tchounwou PB, Patlolla AK, Centeno JA.
Toxicologic Pathology 31:575-588 (2003).

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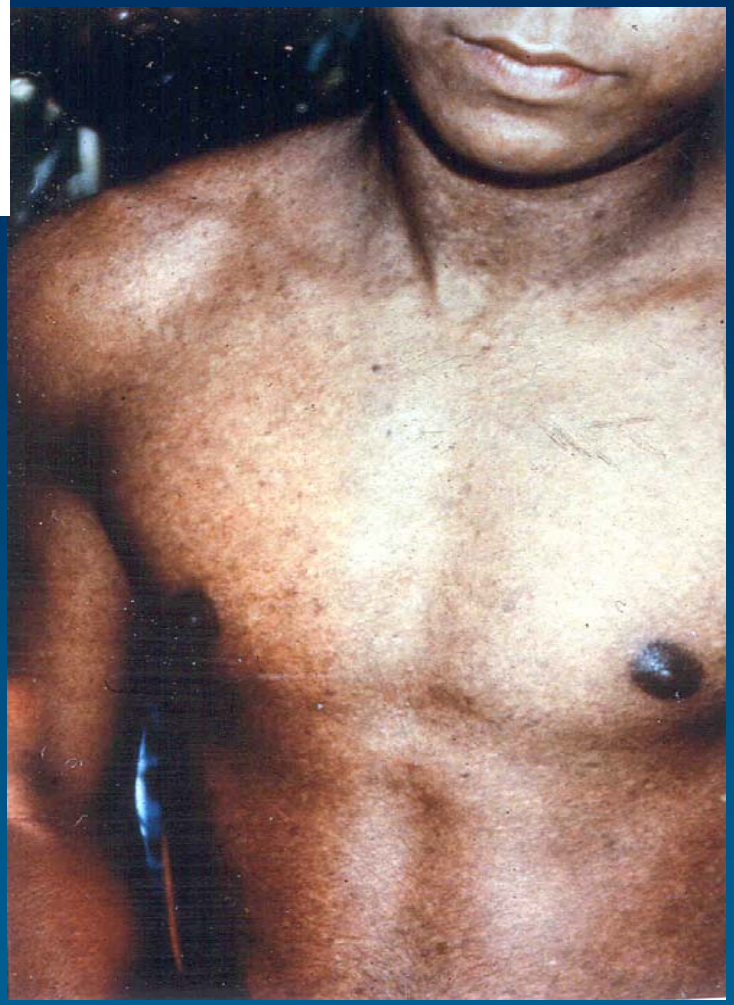
Arsenic-Induced Hyperpigmentation



As-Exposure from Drinking Water (West Bengal, India)

As-Exposure From coal use (Guizhou Province, China)

Centeno JA, et al. 2000 (ISBN:1-881041-68-9)

Basal Cell Carcinoma

Centeno JA, et al. *Environ Health Perspec* 110, 883-886 (2002)

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Dermatological Manifestations: Dose-Response Relationships

1. At high dose exposure (e.g. 0.04 mg/kg/d) hyperpigmentation may appear with 6 months to 3 years.

2. At lower exposure rates (e.g. 0.01 mg/kg/d) hyperpigmentation may appear within 5 to 15 years.

3. Hyperkeratoses usually follows the initial appearance of hyperpigmentation with a period of years.

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Gastrointestinal Manifestations of Chronic Arsenic Exposure

1. ***Noncirrhotic portal hypertension*** **is a rare, but relatively specific effect that may occur after years of arsenic ingestion at a concentration of 0.01 mg/kg/d. Diagnosis is by liver biopsy.**

   Hepatic or splenic enlargement **has been reported, but is not an invariable finding. Hepatic size may be measure by palpation, but is often inaccurate and is ultimately qualitative.**

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Gastrointestinal Manifestations of Chronic Arsenic Exposure
(cont.)

2. Relationship of arsenic to cirrhosis is uncertain.

3. Relationship of arsenic to *hepatocellular carcinoma* is possible, but is an inconsistent findings in epidemiological studies. Viral infection may be a factor in hepatocellular carcinoma from studies on arsenic-exposed endemic areas (Taiwan).

4. *Liver angiosarcomas*: Case reports only; no population studies. However, it is a long-period of exposure and highly associated with arsenic exposure.

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Cardiovascular Manifestations of Chronic Arsenic Exposure

1. Peripheral vascular insufficiency has been linked to chronic arsenic exposure, most notably in Taiwan.

   Early symptoms: subjective complaints of coldness and numbness in the extremities (usually the feet). Raynaud's syndrome has also been reported.

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Arsenic Exposure: Other Health Effects
Recent Environmental Studies

- Chiou et al. (*Env Health Perspec 1999*) reported a dose response relationship between the prevalence of cerebrovascular disease and ingested inorganic arsenic in 8102 men and women.

After adjusting for demographics, smoking, EtOH, personal history and family history, the authors found an increased prevalence of CVD especially infarction.

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Arsenic : Other Health Effects

Recent Studies (cont.)

Cerebral Infarction Risk Ratios

- 1.0 X at 0.0 ppb As
- 3.4 X at 0.1 - 50 ppb As
- 4.5 X at 50.1 - 299.9 ppb As
- 6.9 X at > 300 ppb As

Chiou et al. (*Env Health Perspec 1999*)

Arsenic Exposure: Other Health Effects
Recent Environmental Studies

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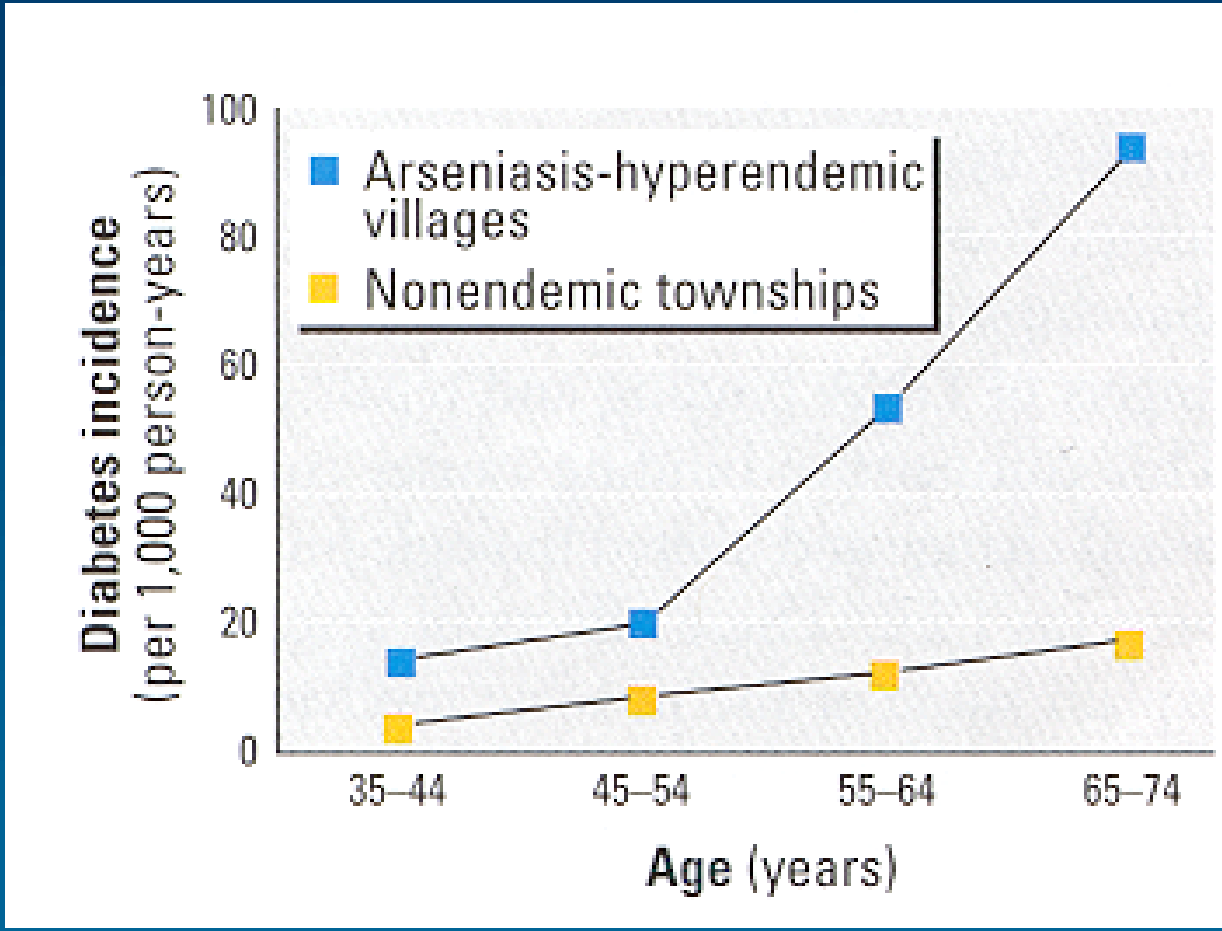
Other Health Effects: Incidence of Diabetes Mellitus and its Possible Association with Arsenic Exposure (Taiwan Study)



Tseng C-H, et al. *Env Health Perspec* 2000;108:847-851

Arsenic Exposure

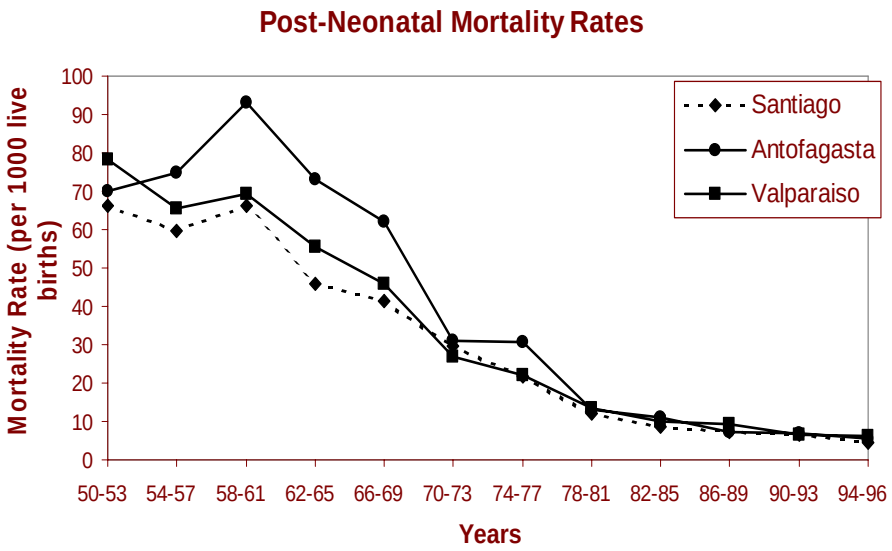
Environmental Case Studies

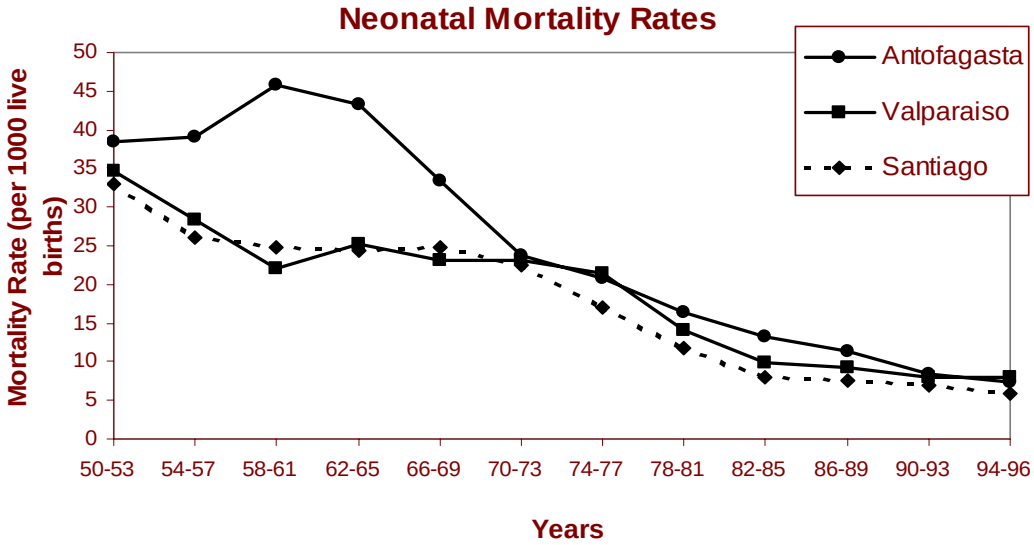
Average Arsenic Levels in Antofagasta[1]

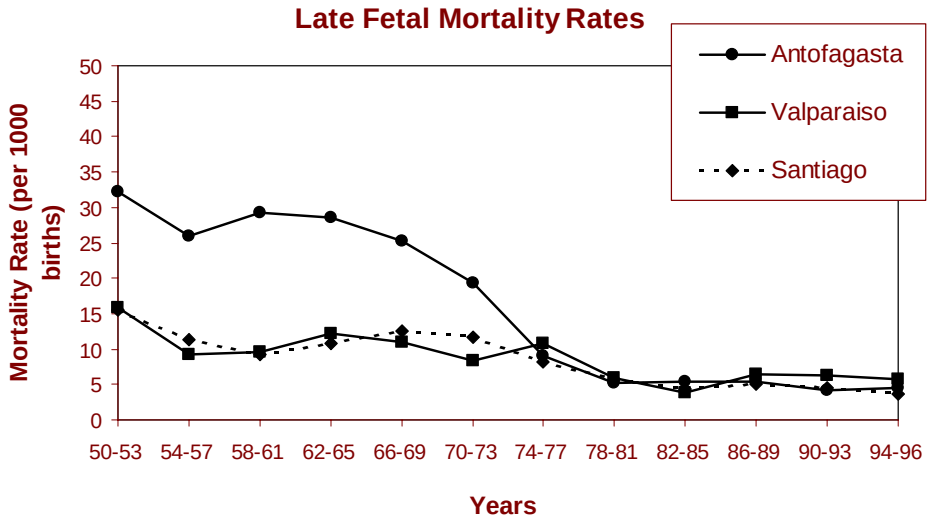
Year	Concentration (ug/L)
1950-1957	90
1958-1970	860
1971-1979	110
1980-1987	70
1988-1996	40

[1]Data represents an average of existing arsenic water measurements (Pederos 1994)

Retrospective evaluation of infant mortality in Antofagasta (Chile), a high arsenic exposure community, compared to infant mortality rates in low arsenic areas







Hopenhayn-Rich, et al. 1999

Results From Mortality Analysis

- Overall decrease over time in fetal, neonatal and postnatal mortality.

- Increased fetal and infant mortality in Antofagasta during the period of high arsenic in drinking water.

Chronic Arsenic Exposure from Drinking Water and Reproductive Effects

Study being conducted in Chile:

- **Retrospective evaluation of infant mortality in Antofagasta, a high arsenic exposure community, compared to infant mortality rates in low arsenic areas**
- **Both Valparaiso and Antofagasta have centralized water distribution systems.**
- **The arsenic concentration in the drinking water in Antofagasta is about 40-50 µg/L; the arsenic concentration in Valparaiso is < 1 µg/L.**

Prospective Study (Continued)

- Prospective evaluation of birth outcomes in a cohort of pregnant women from Antofagasta and Valparaiso, the control community.
- Birth outcomes will examine low birth weight, preeclampsia, prematurity, and gestational diabetes.
- Maternal urinary arsenic levels and newborn hair arsenic concentrations will be measured as indicators of arsenic exposure.
- Collect additional biological samples (maternal blood, placental tissue, and cord blood) and study methylation patterns during pregnancy.

Clinical Characteristics of 248 patients in West Bengal
(DN Guha Mazumder et al. 1999)

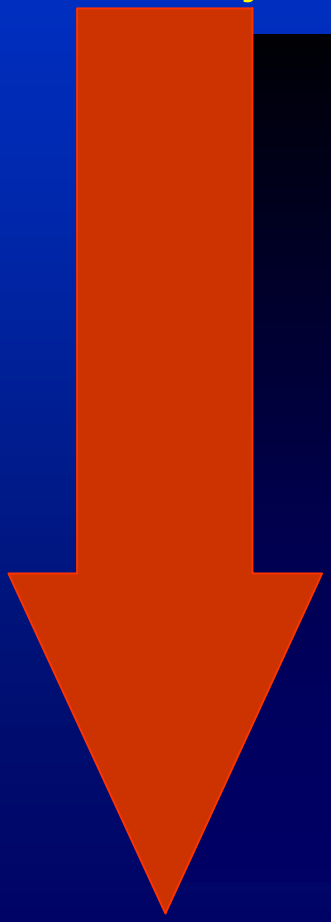
Clinical features	No. of Patients	%
Skin cancer	5	2.02
Kidney cancer	1	0.40
Keratosis (sole and palm)	162	65.3
Rain-drop pigmentation	234	94.4
Blackfoot disease (dry gangrene)	3	1.20
Hepatomegaly	190	76.6
Splenomegaly	73	29.4
Polyneuropathy	74	29.8
Weakness (fatigue)	163	65.7
Cough	154	62.1
Anemia	109	44.0
Dyspepsia	165	66.5

Arsenic Exposure

Analysis and Biological Markers of Exposure

Arsenic Species

Decreasing Toxicity



AsH_3 - arsine (gas)

As(III) - inorganic arsenite

As(V) - inorganic arsenate

MMAA – monometylarsonic (As^{3+})

DMAA – dimetylarsinic acid (As^{3+})

MMAA - monomethylarsonic acid

DMAA - dimethylarsinic acid

TMAO - trimethylarsine oxide

AsB - arsenobetaine (marine) *

AsC - arsenocholine (marine) *

Thus, arsenic speciation studies are critical for accurate toxicological evaluation, bioaccessibility studies, and risk assessment.

As speciation studies

Environmental Analysis:

- As speciation in water (numerous reports)

Biological Analysis:

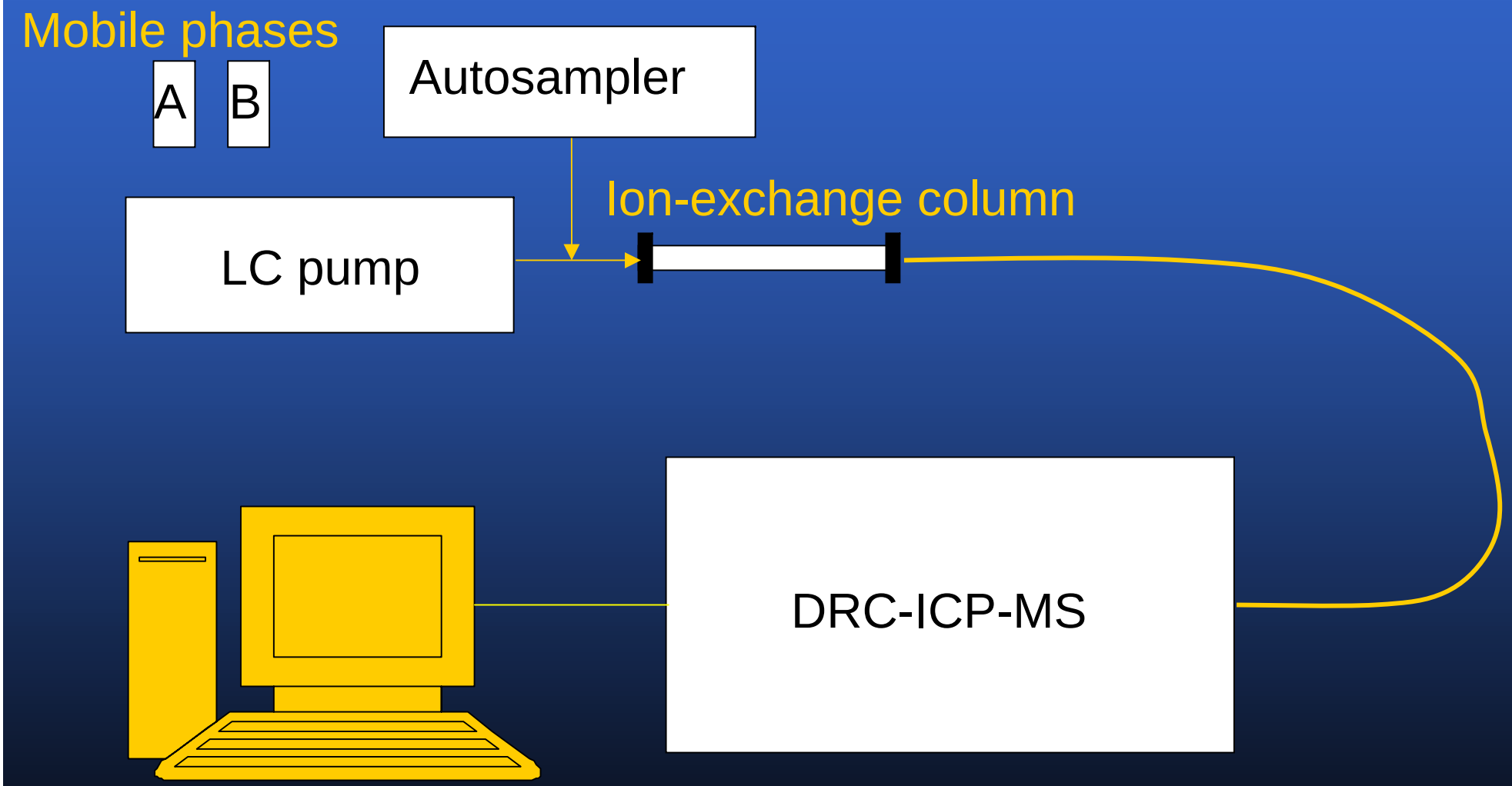
- Urine analysis (numerous reports)
- Body fluids – blood, bile, plasma (Suzuki)
- Hair and nail samples (Suzuki)
- Marine animal samples (several reports, review by McSheehy)
- Tissues?

Overview of As speciation techniques

- **Separation:**
  - Liquid chromatography – most common
    - Reverse phase, ion pair, ion exchange
  - Gas chromatography
  - Capillary electrophoresis
  - Supercritical fluid chromatography

- **Detection:**
  - ICP-MS (element-specific)
  - Hydride generation AA
  - Mass spectrometry
  - Voltametry

ICP-MS (with DRC) Instrument setup



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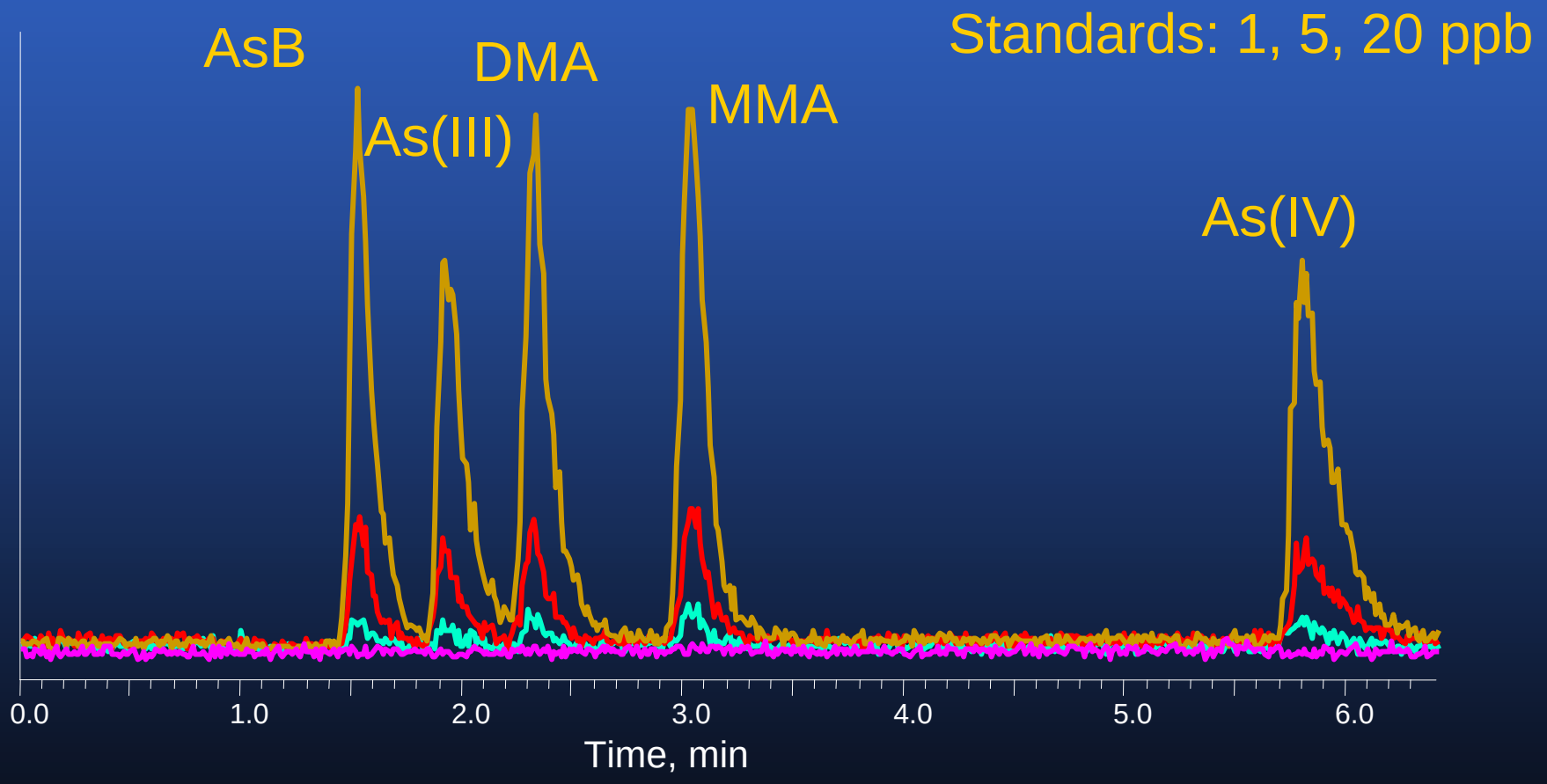
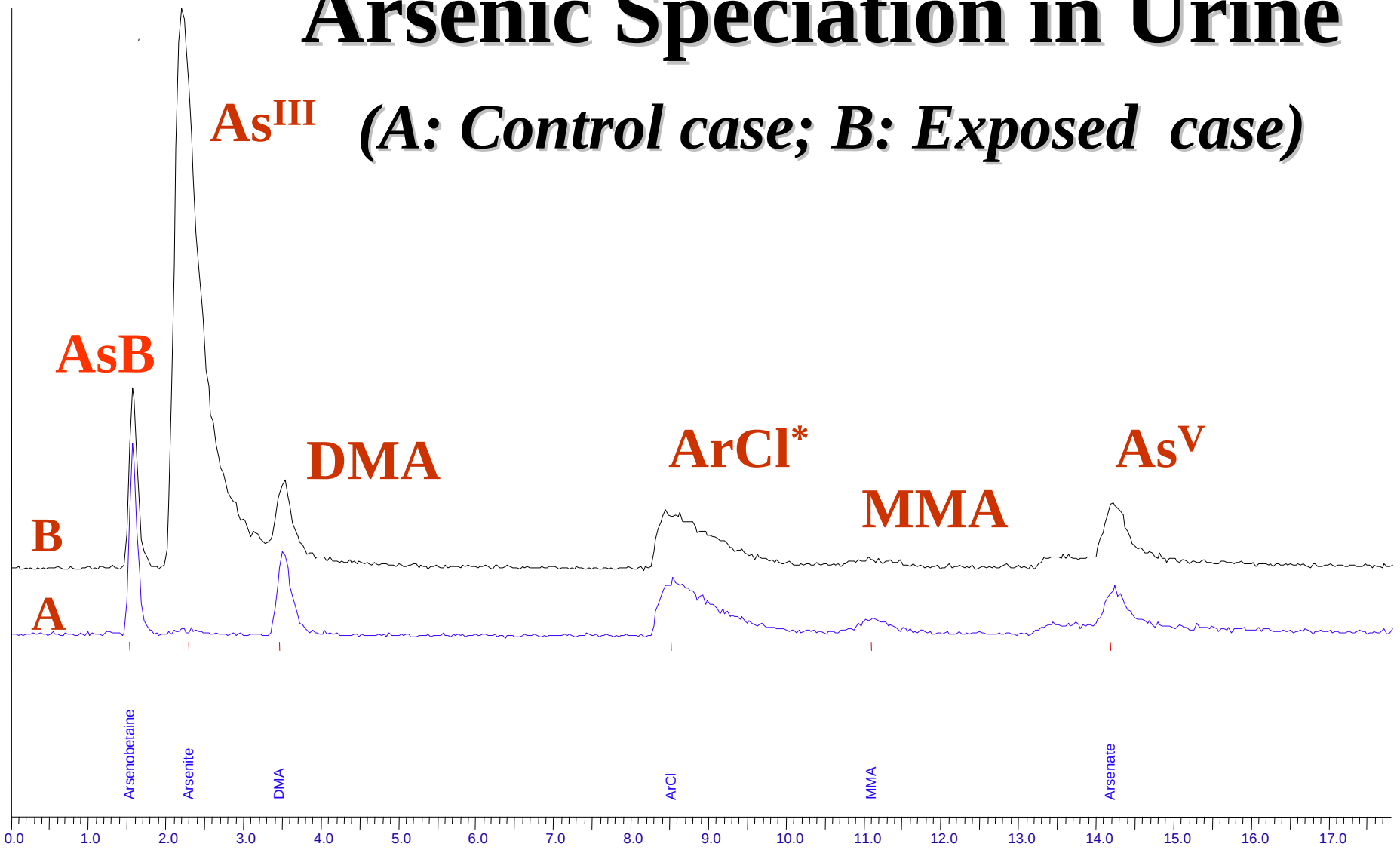
Isocratic HPLC-DRC-ICP-MS Calibration



Arsenic Speciation in Urine



AMINOTRANSFERASES AS BIOMARKERS
Correlation with Total and Speciated Arsenic in Rat Liver

Biomarkers (serum): Alanine aminotransferase (GPT)
Aspartate aminotransferase (GOT)

Rationale:

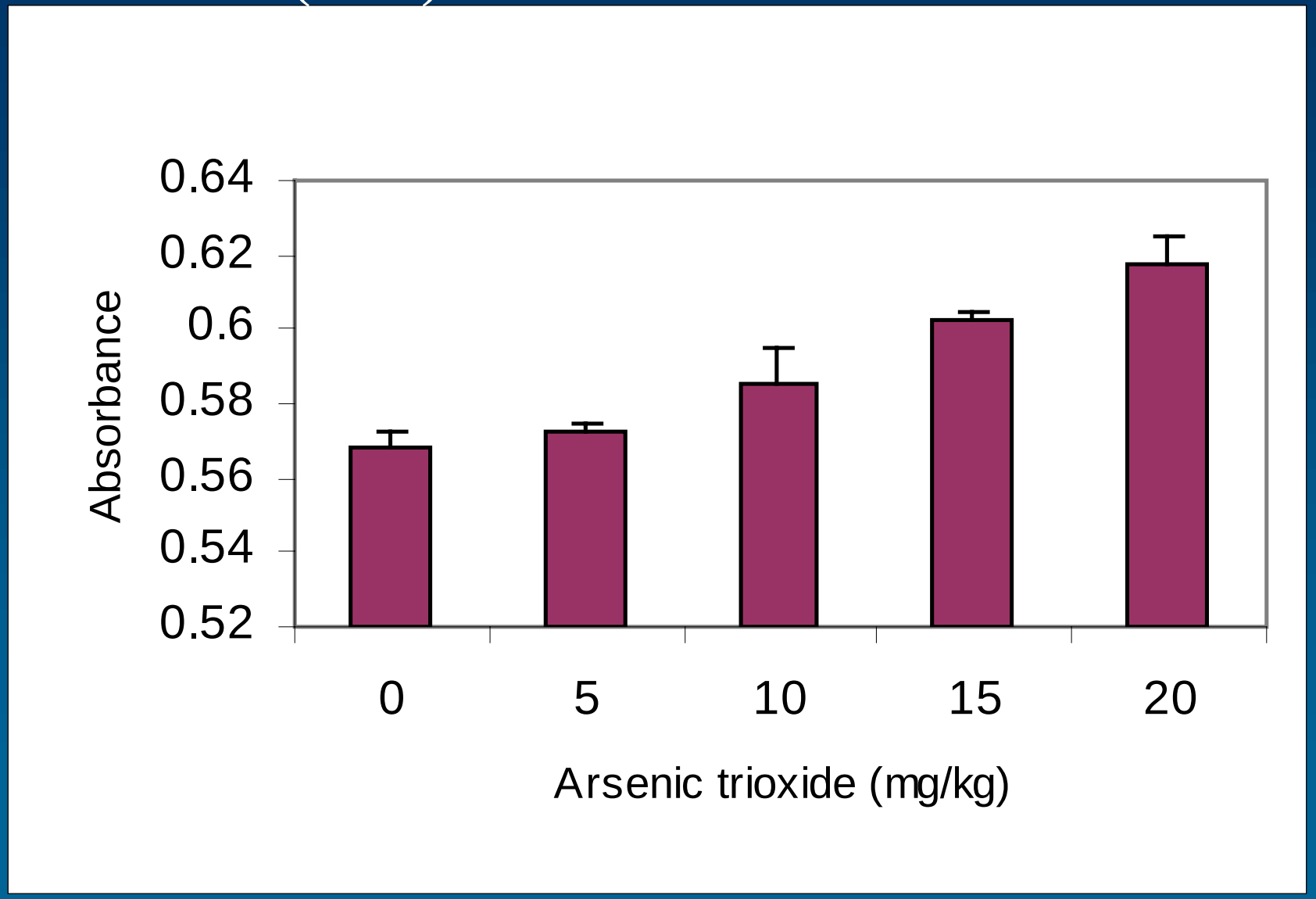
1. GPT and GOT are released from liver when hepatocytes are damaged or destroyed;

2. Serum activities of these enzymes has been reported to increase in cases of hepatocellular damage due to toxic substances;

3. Previously published studies reported elevated levels of serum aminotransferases following arsenic toxicity.

Chemical Analysis: Aminotransferases (GPT and GOT) serum activity
Total and Speciated arsenic in tissues

Effect of arsenic trioxide on the serum activity of alanine aminotransferase (GPT)



Tchounwou, Patlolla, Todorov, and Centeno (in preparation)

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SUMMARY

Significance on Environmental and Military Medicine:

1. Several epidemiological studies have demonstrated marked increased mortality for cancers of: skin, bladder, kidney, liver, and lung for men and women chronically exposed to arsenic from contaminated drinking water.

2. Understanding the relation between arsenic and its chemical forms in environmental and geologic media could help to identify areas where exposure and health problems may exist. This information could be used to systematically map (eg, GIS) arsenic distribution and identify locations of high- and low-As strata.

3. Risk assessment and epidemiological studies of arsenic health effects must consider background exposure, modes of occurrence, chemical/physical speciation, and natural modes of exposure.

Future Directions

- Standardize measurements on “speciation” of arsenic in biological and environmental samples (eg, bioavailability, bioaccessibility studies);

2. To use arsenic “speciation” on the development of epidemiological studies to evaluate chronic low-dose risks (eg, developmental and reproductive toxicology);

3. To study genetic changes and biological markers in various pathological types of major arsenic-induced cancers;

4. To establish the role of arsenic and the occurrence of (non-systemic) clinical outcomes:
   - Diabetes mellitus, hypertension
   - Hematological, neurological and pulmonary manifestations

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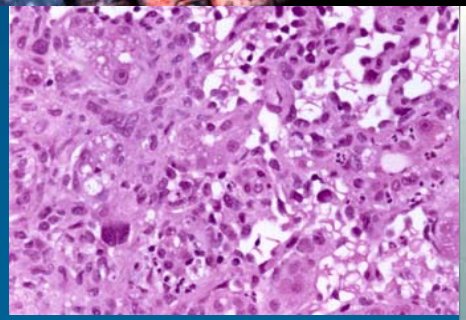
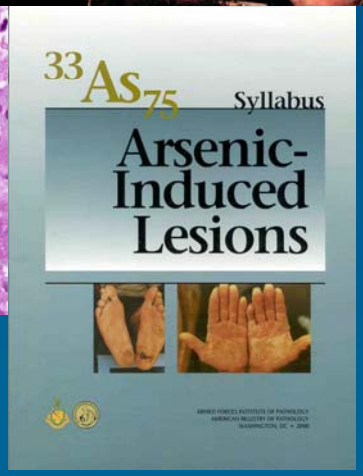
INTERNATIONAL TISSUE REPOSITORY ON CHRONIC ARSENIASIS

Description: **Arsenic is a cancer-inducing agent. A central repository for stored specimens from acute and chronically exposed individuals will assist the military and public health community in improving risk assessment of arsenic-induced health effects.**





Activities:

Archival of tissues and biological specimens;
Consultation on arsenic health effects
Use of archival material on medical *education;*
Research projects: pathology, reproductive toxicology, experimental animal studies; risk management and risk assessment;
Analytical toxicology (speciation studies)

SELECTED REFERENCES

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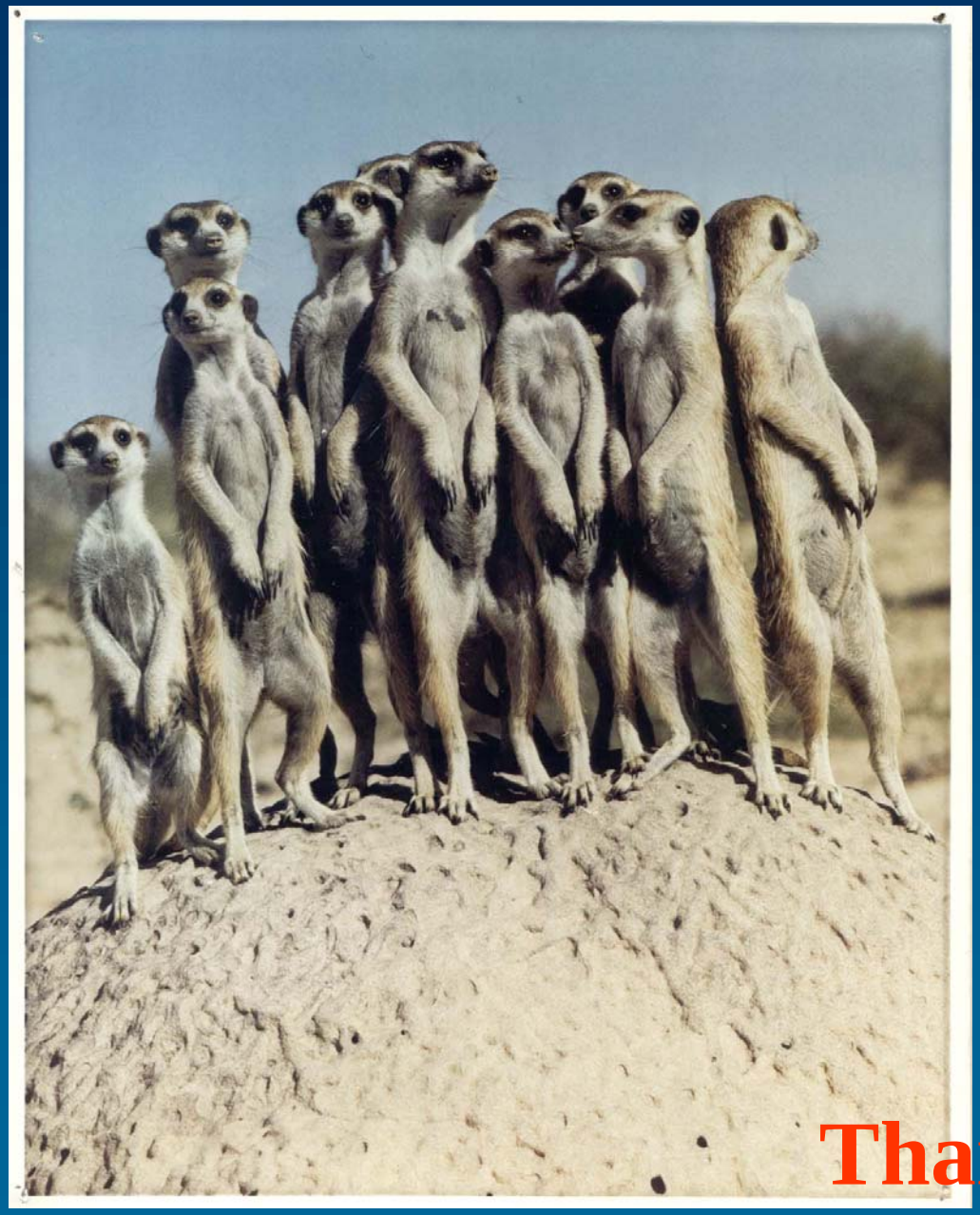
2. Tchounwou PB, Patlolla AK, Centeno JA. Carcinogenic and Systemic Health Effects Associated with Arsenic Exposure – A Critical Review. *Toxicologic Pathology* 2003;31:575-588.

3. Centeno JA, Mullick FG, Martinez L, et al. Chronic Arsenic Toxicity: An Introduction and Overview. *Histopathology* 2002;41:324-326.

4. Guha Mazumder DN, De BK, Santra A, Dasgupta J, Ghosh N, Roy BK, Ghoshal UC, Saha A,Chatterjee A, Dutta S, Haque R, Smith AH, Chakraborty D, Angle CR, Centeno JA. Chronic arsenic toxicity: Epidemiology, natural history and treatment. In *Arsenic Exposure and HealthEffects* 1999 (Chappell WR, Abernathy CO, Calderon RL, editors).

5. Guha Mazumder DN, Ghospal UC, Saha J, Santra A, De BK, Chatterjee A, Dutta S, Angle CR and Centeno JA. Randomized placebo-controlled trial of 2,3-dimercaptosuccinic acid in therapy of chronic arseniasis due to drinking arsenic-contaminated subsoil water. *Clin Toxicol* 1998;36(7): 683-690.

QUESTIONS?



Thank you!