



Dose-Response Assessment for Arsenic: A Case Study for Why the LNT Doesn't Work

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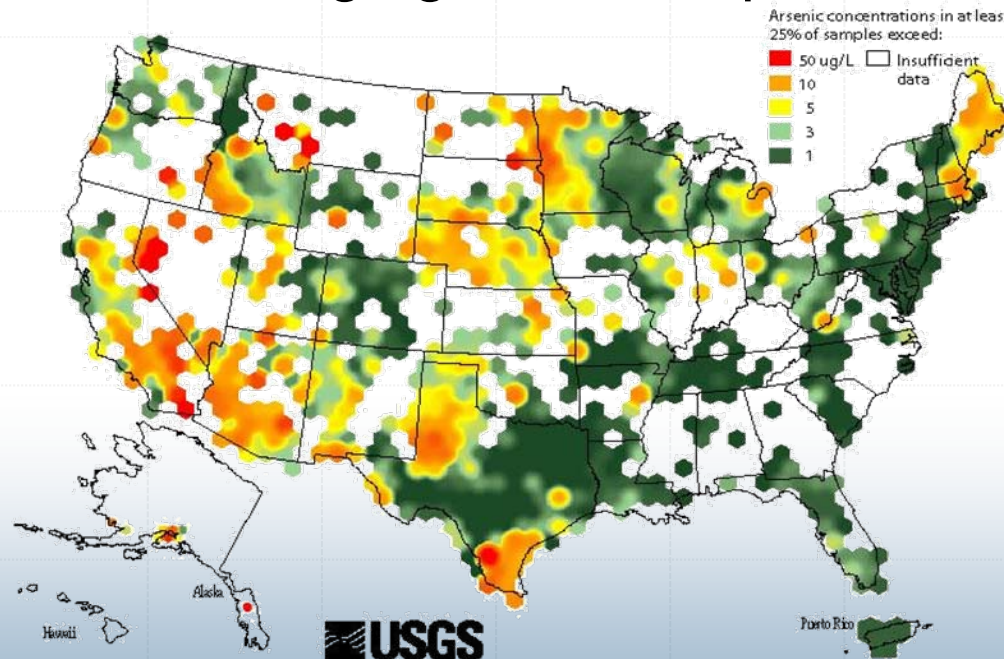
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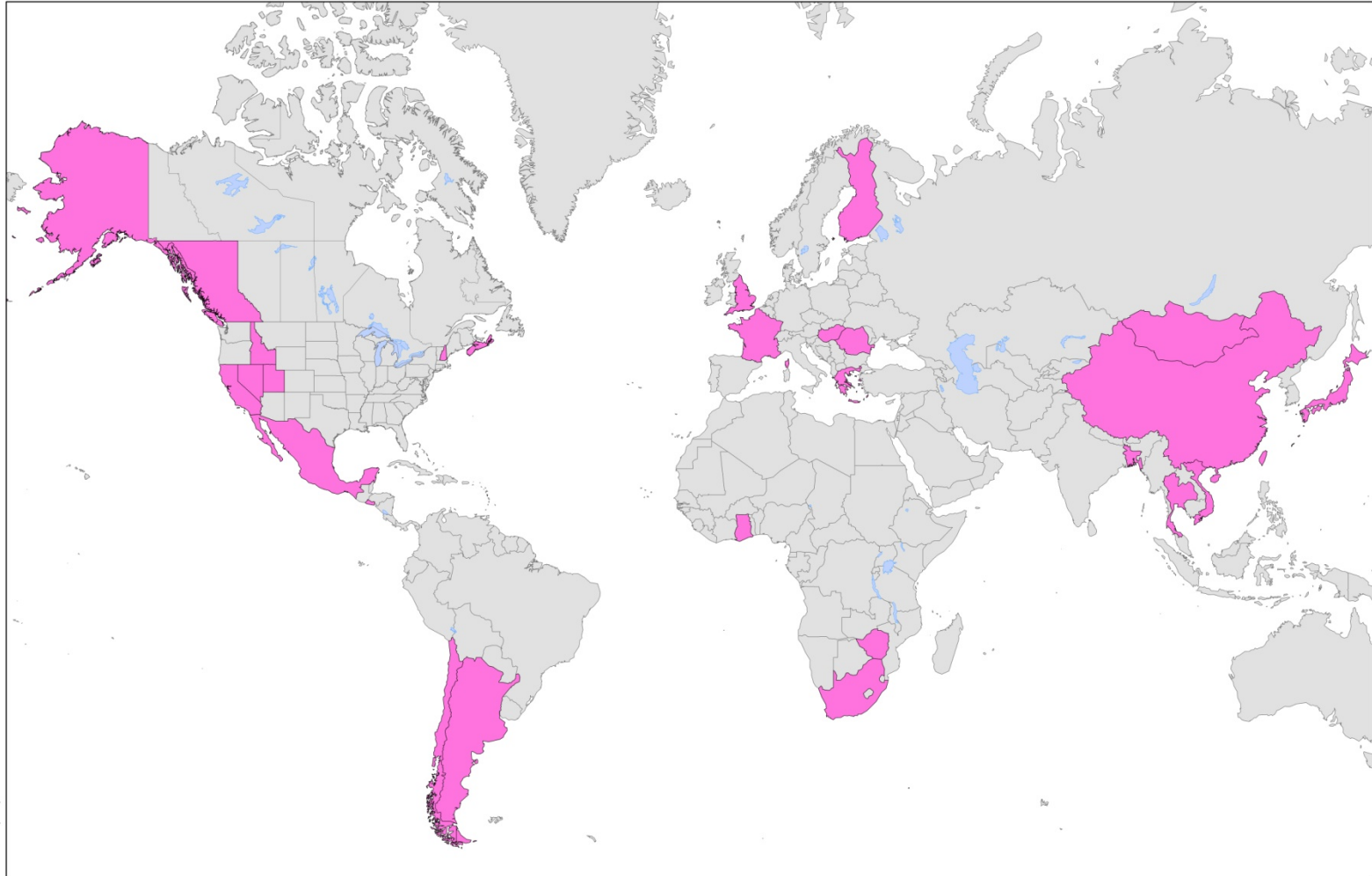
Introduction to Arsenic

- Metalloid
- Naturally occurring in environment in multiple forms
- Inorganic form common in some ground waters and soils
 - › Groundwater: mean $> 1 \mu\text{g/L}$, but can be $> 1000 \mu\text{g/L}$
 - › Soil: mean $\sim 5 \text{ mg/kg}$, with 95th percentile $\sim 20 \text{ mg/kg}$



Introduction to Arsenic (cont.)

Also found at high concentrations in many countries outside the US (Bangladesh, Taiwan, Chile, Argentina)



Health Effects of Arsenic in Drinking Water

Many organ systems affected

› Noncancer:

- Nervous system
- Liver
- Lungs
- Skin
- Potentially endocrine (*e.g.*, diabetes)
- Potentially cardiovascular, *etc.*

› Cancer:

- Bladder
- Lung
- Skin

History of Arsenic Cancer Slope Factor (CSF)

Southern Taiwanese population highly studied:

- › Skin cancer study in late 1960s
- › Internal cancer studies in 1980s and '90s
- › Basis for much of quantitative arsenic cancer risk assessment



Map Source: ArcGIS Online data.

History of Arsenic CSF (cont.)

Ongoing concerns regarding Taiwan study

- › Exposure uncertainty
 - Ecological study
 - Max *versus* median arsenic in water concentration for a village
- › Choice of reference population and influence on dose-response model
- › Wide range in dose-response estimates

History of Arsenic CSF (cont.)

Different interpretation of low-dose linearity over time, but same data!

Slope Factor (mg/kg-day) ⁻¹	Report	Agency	Comments
1.5	Integrated Risk Information System (IRIS)	EPA/ORD 1998	Currently listed in IRIS; based on skin cancer incidence in Taiwan; only “blessed” value
0.4- 3.67	Final Rule for arsenic MCL	EPA/OW 2001	Range based on Taiwanese water intake and arsenic in food; also based on bladder and lung cancer
23	NRC Arsenic in Drinking Water Report	NAS 2001	Calculated based on excess lung and bladder cancer risk estimates
3.67	Draft CCA RED, probabilistic CCA risk assessment, and Organic arsenical herbicide RED	EPA/OPP 2003	Based on upper range established in MCL rule
0.41 -23	Petition to ban CCA wood	CPSC 2003	Based on EPA and NRC assessments
9.5	Public health goal for drinking water	CalEPA 2004	Based on bladder and lung cancer in Taiwan, considers others data.
25.7 (female)	Proposed IRIS revision	EPA/ORD 2010	Based on bladder and lung cancer; uses many of NRC's recommendations

Implications

- LNT approach - cancer risk estimated above 10^{-4} for background exposures to arsenic
- Petito-Boyce *et al.*, 2008 - a probabilistic exposure analysis to determine background exposures to As in diet, water and soil
- High baseline risk from arsenic - risk communication challenge

Lifetime Cancer Risks from Background Exposures to Arsenic

Background Cancer Risk	Endpoint	Mean	5 th percentile	50 th percentile	95 th percentile
1.5 (mg/kg-d) ⁻¹	Skin Cancer	1.4×10^{-4}	4.4×10^{-5}	1.1×10^{-4}	3.4×10^{-4}
6.6 (mg/kg-d) ⁻¹	Female Bladder + Lung- No Comparison Pop	1.4×10^{-4}	1.9×10^{-5}	4.3×10^{-4}	1.5×10^{-3}
25.7 (mg/kg-d) ⁻¹	Female Bladder + Lung- Comparison Pop	2.4×10^{-3}	7.5×10^{-4}	1.8×10^{-3}	5.8×10^{-3}

Biological Basis for Nonlinearity

- All plausible mechanisms are nonlinear
 - › Inhibition of DNA repair
 - › Modulation of signal transduction pathways
 - › Interference with cell cycle
 - › Inhibition of apoptosis
 - › Cytotoxicity followed by regeneration (bladder cancer, possibly lung and skin)
- No evidence that arsenic causes point mutations
- Adaptive low levels followed by cytotoxicity at higher levels (0.01-0.1 μM *in vitro*, < 2 mg/L *in vivo* mice)
- Possible hormetic (beneficial) effects at low doses

Comprehensive Literature Analysis Re: Changes in Gene/Protein Expression Associated with Arsenic

Gentry *et al.* (2010)

- › 800 studies identified
- › 160 studies with relevant information on gene/protein changes associated with arsenic
 - *In vitro* exposures
 - › Primary cell lines
 - › Tumor-derived cell lines

Biological Basis: from Adaptation to Apoptosis

Findings (at tissue or cellular concentrations, and NOT at environmental levels, which are much higher)

- › $< 0.1 \mu\text{M}$ ($7.5 \mu\text{g/L}$)
 - Adaptive state
 - No induction of cell cycle genes
- › $0.1\text{-}10 \mu\text{M}$ ($7.5\text{-}750 \mu\text{g/L}$)
 - Proliferative state
 - Changes in genes associated with cell cycle control and DNA repair
- › $> 10 \mu\text{M}$ ($> 750 \mu\text{g/L}$)
 - Cell cycle stasis
 - Genes characteristic of apoptotic change

Biological Basis: from Adaptation to Apoptosis (cont.)

In Vitro Genomic Changes

	~Stress / adaptation		~Cell cycle control, DNA repair, relevance to carcinogenesis		~Apoptosis
	0.01 uM	0.1 uM	1.0 uM	10 uM	100 uM
Oxidative Stress	Trx Trx Reductase SOD1	AP-1	HO-1 GSR TPX-11		MT-1 MT-2 NRF-2
Inflammation	COX-2			IL-8	
Proteotoxicity	HSP-32		HSP-70		HSP-60 HSP-27
Proliferation	FGFR-4	Fos Jun	VEGF Myc P70 Erk		ERK-1 ERK-2 EGFR
DNA Repair	DDB2	Pol beta Ligase I	PARP-1	Ligase I	GADD153
Cell Cycle Control		P53	CDC25A CDC25B CDC25C	P21	
Apoptosis	P53 EGR-1 P105 P65	NF-kB P53	Casp3 Casp8 Casp9		SRC JNK JNK3

Gene Expression: Increase Decrease

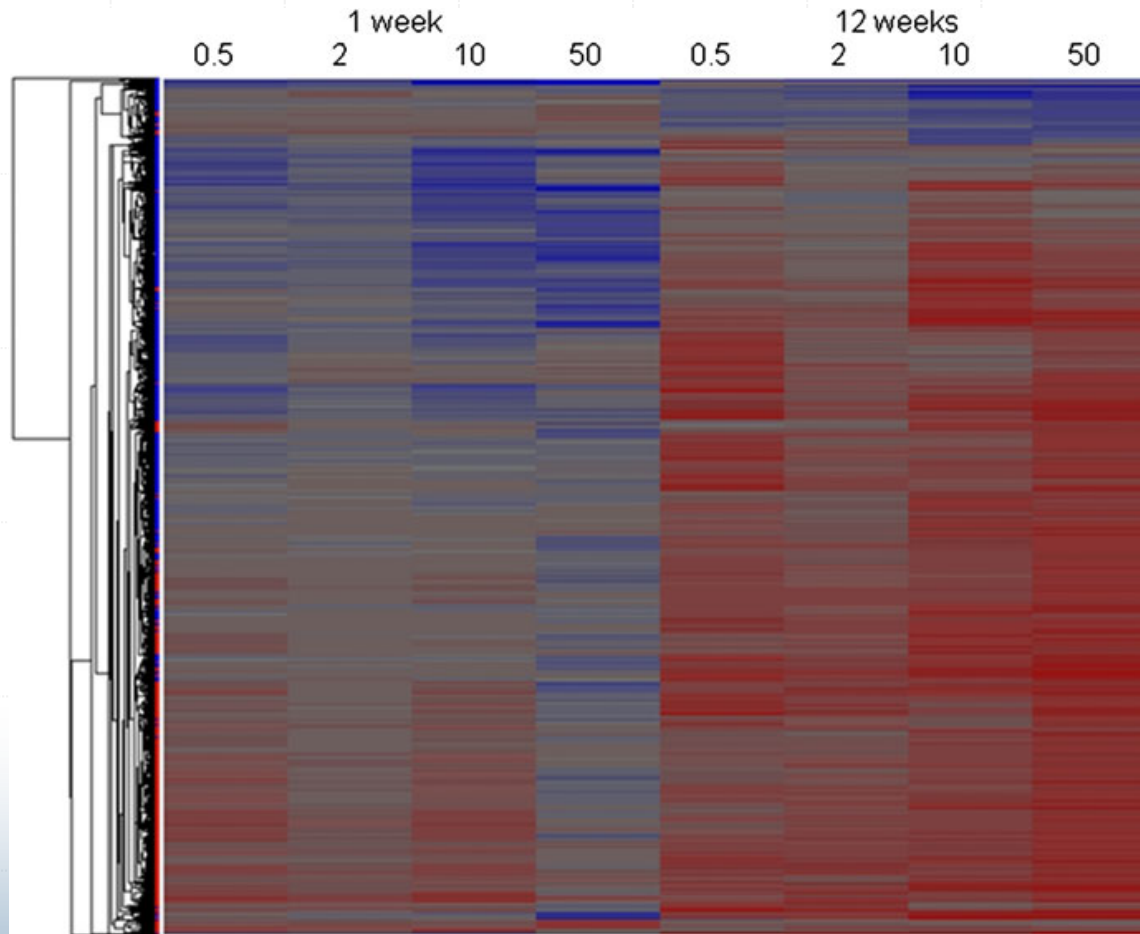
Adapted from Clewell *et al.*, 2007

Biological Basis: Adaptive

- New *in vivo* evidence support *in vitro* results for biphasic dose-response involving adaptive state
 - › Mice administered 0.5, 2, 10 or 50 mg/L arsenate in drinking water for 1 or 12 wks
 - › Gene expression changed measured in bladder
 - Time-dependent changes—week 1 genes downregulated; week 12 genes generally upregulated
 - Bimodal dose-response at both 1 and 12 weeks—inflection point around 2 mg/L
 - › Concentrations above 2 mg/L needed to significantly alter pathways/networks

Biological Basis: Adaptive (cont.)

Gene expression changes at four doses (0.5, 2, 10, and 50 mg As/L) and two time points (1 and 12 weeks)



Note relative lack of gene induction at 2 mg/L at both time points

From Clewell *et al.*, 2011

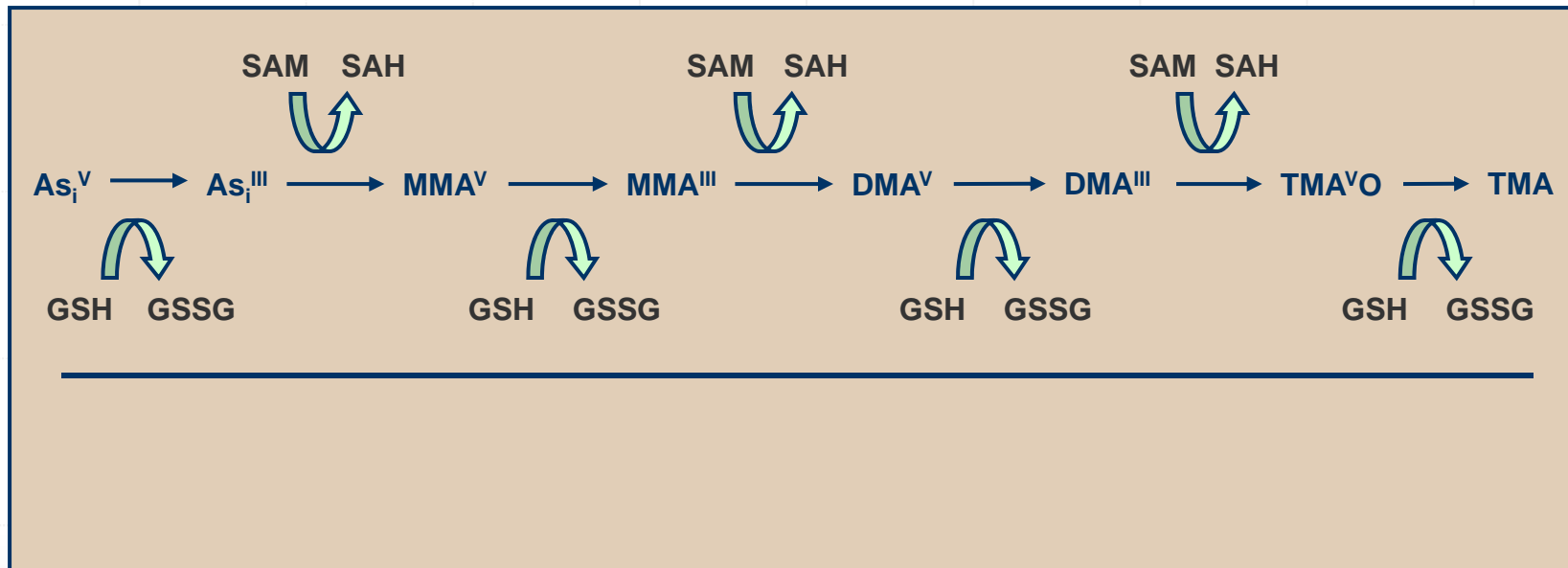
Biological Basis: Adaptive (cont.)

- Other examples of biphasic dose-response/hormesis
- Sykora and Snow, 2008
 - › At low doses, DNA repair activity was increased, whereas at higher doses (above 1 μM) there was significant downregulation
- Yang *et al.*, 2007
 - › In lung fibroblasts, significant increase in cell viability at low concentration (0.5 μM) with inhibition at 5 and 10 μM .

Biological Basis: Metabolism

Research over past decade has suggested role for trivalent metabolites in toxicity and carcinogenicity

Arsenic metabolism: sequential reduction and methylation



Biological Basis: Cytotoxicity

Effects of As^{III} on the Bladder Epithelium in a Dose Response Study (Rat)

Treatment in diet	Histopathology ^[a]		Labeling Index (%)	SEM Classification				
	Normal	Hyperplasia	Mean ± S.E. (n)	1	2	3	4	5
Control	10	--	0.08 ± 0.02(9)	5	4	1	-	-
1 ppm As ^{III}	10	--	0.06 ± 0.01(10)	2	5	3	-	-
10 ppm As ^{III}	8	2	0.06 ± 0.01(10)	2	4	4	-	-
25 ppm As ^{III}	7	3	0.19 ± 0.05(9)	3	1	3	2	1
50 ppm As ^{III} ^[b]	5	5 ^[c]	0.45 ± 0.12(10) ^[c]	-	-	2	2	6
100 ppm As ^{III} ^[b]	3	7 ^[c]	0.17 ± 0.03(9)	-	-	3	4	3

^an = 10 for all groups

Adapted from Suzuki et al., 2010

^bSEM classification significantly different from control group, p<0.05

^cSignificantly different from control group, p<0.05

Similar findings with DMA^{III}

Biological Basis: Cytotoxicity (cont.)

Urothelial Cytotoxicity and Proliferation Induced by Inorganic Arsenic

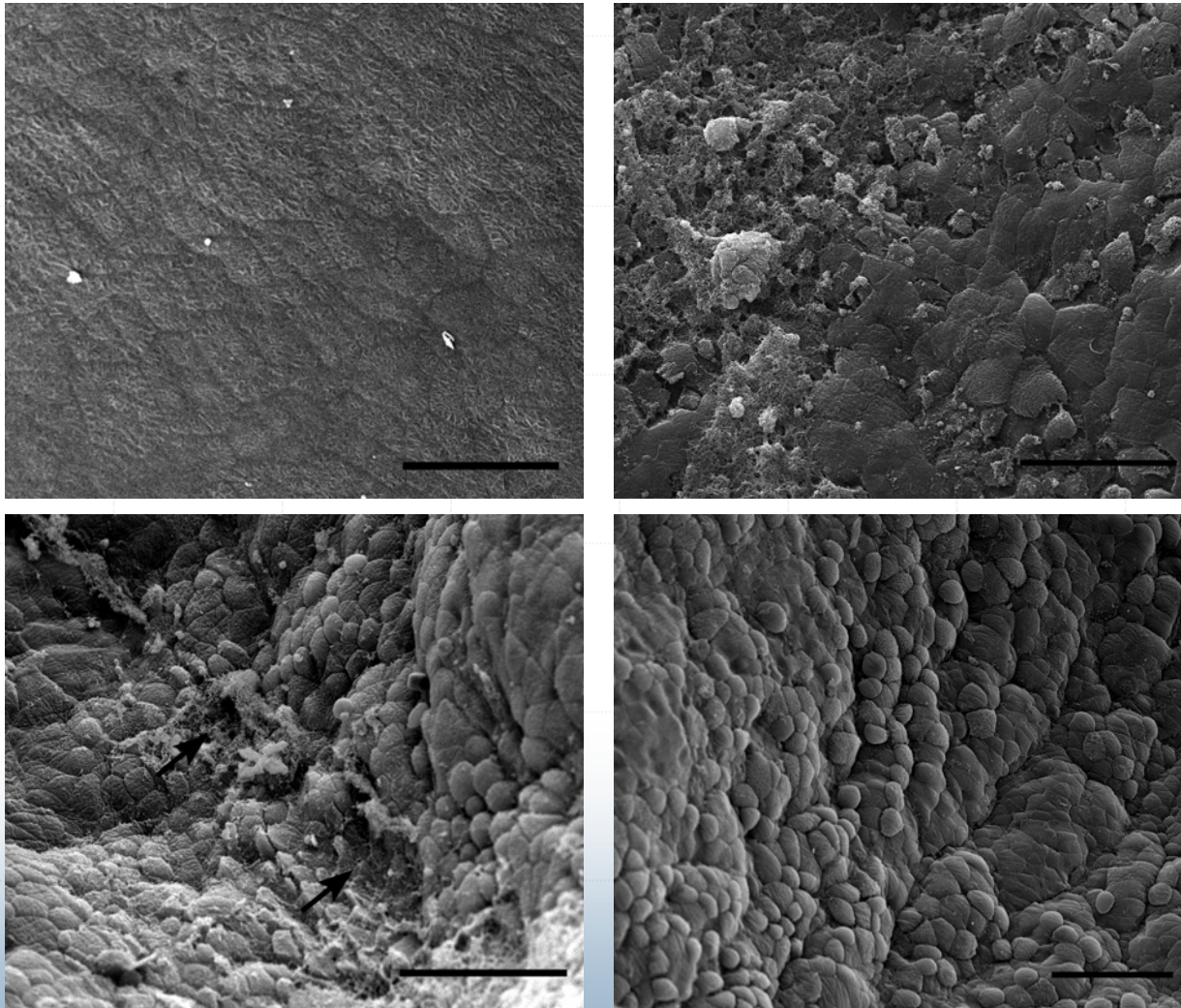


Photo credit: Dr. Sam Cohen, University of Nebraska Medical School

Biological Basis: Cytotoxicity (cont.)

- Accumulating evidence that lung and skin carcinogenesis involves cytotoxicity followed by regeneration
- In both cases noncancerous effects precede cancer effects
 - › Skin
 - Arsenic accumulates in skin because of presence of sulfhydryl groups
 - Initial skin changes are superficial (similar to urothelium)
 - Inflammatory response and regenerative hyperplasia leading to cancer
 - › Lung
 - Bronchial epithelial cells in culture are susceptible to cytotoxic effects, similar to urothelial cells

Biological Basis: Metabolism + Cytotoxicity

- Overall animal and *in vitro* studies observe cytotoxic changes to bladder cell when exposed to concentration of about 0.1-0.2 μM in urine
- Supported with human data
 - › Water concentration < 100 $\mu\text{g/L}$, non-carcinogenic exposure
 - Urinary trivalent below 0.1-0.2 μM (non-cytotoxic range)
 - › Water concentration > 100 $\mu\text{g/L}$, carcinogenic exposure
 - Urinary trivalent above 0.1-0.2 μM (cytotoxic range)

Epidemiological Evidence: Outside the US

- Epidemiological evidence consistent with a nonlinear dose-response for arsenic
- Overall, studies outside US show increased risk above several hundred $\mu\text{g/L}$
 - › Taiwan, Chile, Argentina, Bangladesh

Recent Studies

Study	Location	Study Type	Endpoint	Finding
Chen <i>et al.</i> , 2010a	Northeastern Taiwan	Cohort	Lung cancer	No significant increase in risk <100 $\mu\text{g/L}$
Chen <i>et al.</i> , 2010b	Northeastern Taiwan	Cohort	Urinary tract cancer	No significant increase in risk < 100 $\mu\text{g/L}$
Pou <i>et al.</i> , 2011	Argentina	Ecological	Bladder cancer	No significant increase in risk <320 $\mu\text{g/L}$ (F)

Epidemiological Evidence: US Studies

US case-control & cohort studies do not show an increased cancer risk in populations exposed to arsenic in drinking water at mean concentrations up to 190 µg/L

- NH: Bladder cancer study (Karagas *et al.*, 2001)
- NV: Multi-site cancer study (Soto-Pena *et al.*, 2006)
- NV and CA: Bladder cancer study (Steinmaus *et al.*, 2003)
- NV: Childhood multi-site cancer study (Moore *et al.*, 2002)
- UT: Multi-site cancer study (Lewis *et al.*, 1999)
- UT: Bladder cancer study (Bates *et al.*, 1995)

Epidemiological Evidence: Meta-Analysis

Meta-analysis by Mink *et al.* (2008): "Low-level arsenic exposure in drinking water and bladder cancer: A review and meta-analysis"

- › 8 studies
- › Study locations
 - 1 Argentina
 - 2 Finland
 - 1 Northeastern Taiwan
 - 4 US

Epidemiological Evidence: Meta-Analysis (cont.)

- Among never smokers, “low level” (100-200 $\mu\text{g/L}$) summary relative risk estimate:
 - › 0.81 (0.60-1.08)
 - › All central tendency estimates < 1.0
- Among ever smokers, 1.12 (0.88-1.66)
- Prediction based on southwest Taiwan
 - › 1.2- to 2.5-fold increase in risk at these concentrations
 - › Based on NRC (2001) modeling
- Updated analysis (2010) with more recent studies
 - › 0.83 (0.65-1.06)

Conclusions

- LNT Model based on direct acting mutagenicity
 - › One hit sufficient to cause cancer
- In case of arsenic, LNT fails to consider
 - › Not all forms of genotoxicity are alike
 - Different test needed to distinguish among different types
 - › Adaption leading to biphasic dose response apparent from *in vitro* and *in vivo* experiments
 - In particular role of DNA repair
 - › Role of generation of reactive metabolites in dose-response
 - › Cytotoxicity followed by regeneration as a plausible MOA

Conclusions (cont.)

- LNT fails to consider
 - › Role of increased cell proliferation
 - Critical step in carcinogenesis for many chemicals (and even for direct DNA-reactive chemicals, *e.g.*, 2-acetylaminofluorene in bladder)
- Epidemiology studies
 - › Provide reality check on likelihood of and association at different exposure levels
 - › Meta-analysis- no stat. sig. increase in risk; inconsistent with NRC (2001) prediction
- All evidence supports existence of a threshold

References

- Bates, MN; Smith, AH; Cantor, KP. 1995. "Case-control study of bladder cancer and arsenic in drinking water." *Am. J. Epidemiol.* 141:523-530.
- Chen, CL; Chiou, HY; Hsu, LI; Hsueh, YM; Wu, MM; Chen, CJ. 2010a. "Ingested arsenic, characteristics of well water consumption and risk of different histological types of lung cancer in northeastern Taiwan." *Environ. Res.* 110(5):455-462.
- Chen, CL; Chiou, HY; Hsu, LI; Hsueh, YM; Wu, MM; Wang, YH; Chen, CJ. 2010b. "Arsenic in drinking water and risk of urinary tract cancer: A follow-up study from northeastern Taiwan." *Cancer Epidemiol. Biomarkers Prev.* 19(1):101-110.
- Clewell, HJ; Thomas, RS; Gentry, PR; Crump, KS; Kenyon, EM; El-Masri, HA; Yager, JW. 2007. "Research toward the development of a biologically based dose response assessment for inorganic arsenic carcinogenicity: A progress report." *Toxicol. Appl. Pharmacol.* 222:388-398.
- Clewell, HJ; Thomas, RS; Kenyon, EM; Hughes, MF; Adair, BM; Gentry, PR; Yager, JW. 2011. "Concentration- and time-dependent genomic changes in the mouse urinary bladder following exposure to arsenate in drinking water for up to 12 weeks." *Toxicol. Sci.* 123(2):421-432.
- Gentry, PR; McDonald, TB; Sullivan, DE; Shipp, AM; Yager, JW; Clewell, HJ III. 2010. "Analysis of genomic dose-response information on arsenic to inform key events in a mode of action for carcinogenicity." *Environ. Mol. Mutagen.* 51(1):1-14.

References (cont.)

- Karagas, MR; Stukel, TA; Morris, JS; Tosteson, TD; Weiss, JE; Spencer, SK; Greenberg, ER. 2001. "Skin cancer risk in relation to toenail arsenic concentrations in a US population-based case-control study." *Am. J. Epidemiol.* 153 :559-565.
- Lewis, DR; Southwick, JW; Ouellet-Hellstrom, R; Rench, J; Calderon, RL. 1999. "Drinking water arsenic in Utah: A cohort mortality study." *Environ. Health Perspect.* 107(5):359-365.
- Mink, PJ; Alexander, DD; Barraj, LM; Kelsh, MA; Tsuji, JS. 2008. "Low-level arsenic exposure in drinking water and bladder cancer: A review and meta-analysis." *Regul. Toxicol. Pharmacol.* 52:299-310.
- Moore, LE; Lu, M; Smith, AH. 2002. "Childhood cancer incidence and arsenic exposure in drinking water in Nevada." *Arch. Environ. Health* 57:201-206.
- Petito Boyce, C; Lewis, AS; Sax, SN; Eldan, M; Cohen, SM; Beck, BD. 2008. "Probabilistic analysis of human health risks associated with background concentrations of inorganic arsenic: Use of a margin of exposure approach." *Hum. Ecol. Risk Assess.* 14:1159-1201.
- Pou, SA; Osella, AR; Diaz Mdel, P. 2011. "Bladder cancer mortality trends and patterns in Córdoba, Argentina (1986-2006)." *Cancer Causes Control* 22(3):407-415.
- Soto-Pena, GA; Luna, AL; Acosta-Saavedra, L; Conde-Moo, P; Lopez-Carillo, L; Cebrian, ME; Bastida, M; Calderon-Aranda, ES; Vega, L. 2006. "Assessment of lymphocyte subpopulations and cytokine secretion in children exposed to arsenic." *FASEB J.* 20(6):779-781.

References (cont.)

Steinmaus, C; Yuan, Y; Bates, MN; Smith, AH. 2003. "Case-control study of bladder cancer and drinking water arsenic in the western United States." *Am. J. Epidemiol.* 158:1193-2001.

Suzuki, S; Arnold, LL; Pennington, KL; Chen, B; Naranmandura, H; Le, XC; Cohen, SM. 2010. "Dietary administration of sodium arsenite to rats: Relations between dose and urinary concentrations of methylated and thio-metabolites and effects on the rat urinary bladder epithelium." *Toxicol. Appl. Pharmacol.* 244(2):99-105.

Sykora, P; Snow, ET. 2008. "Modulation of DNA polymerase beta-dependent base excision repair in cultured human cells after low dose exposure to arsenite." *Toxicol. Appl. Pharmacol.* 228:385-394.

Yang, P; He, XQ; Peng, L; Li, AP; Wang, XR; Zhou, JW; Liu, QZ. 2007. "The role of oxidative stress in hormesis induced by sodium arsenite in human embryo lung fibroblast (HELFL) cellular proliferation model." *J. Toxicol. Environ. Health A* 70:976-983.