Metal speciation by IC-ICP-MS: Arsenic and Chromium case studies

**Tatyana S. Pinyayev[1], Robert A. Wilson[2], Karen Herbin-Davis[3],
Michael J. Kohan[3], John T. Creed[1] and David J. Thomas[3]**

[1] United States Environmental Protection Agency, National Exposure Research Laboratory,
Microbiological and Chemical Exposure Assessment Research Division, Cincinnati, OH, 45268

[2] Student Services Contractor, NERL, MCEARD, Cincinnati, OH, 45268

[3] United States Environmental Protection Agency, National Health and Environmental Effects Research
Laboratory, Experimental Toxicology Division,
Research Triangle Park, NC, 27711

*Mention of trade names or commercial products does not
constitute endorsement or recommendation for use*

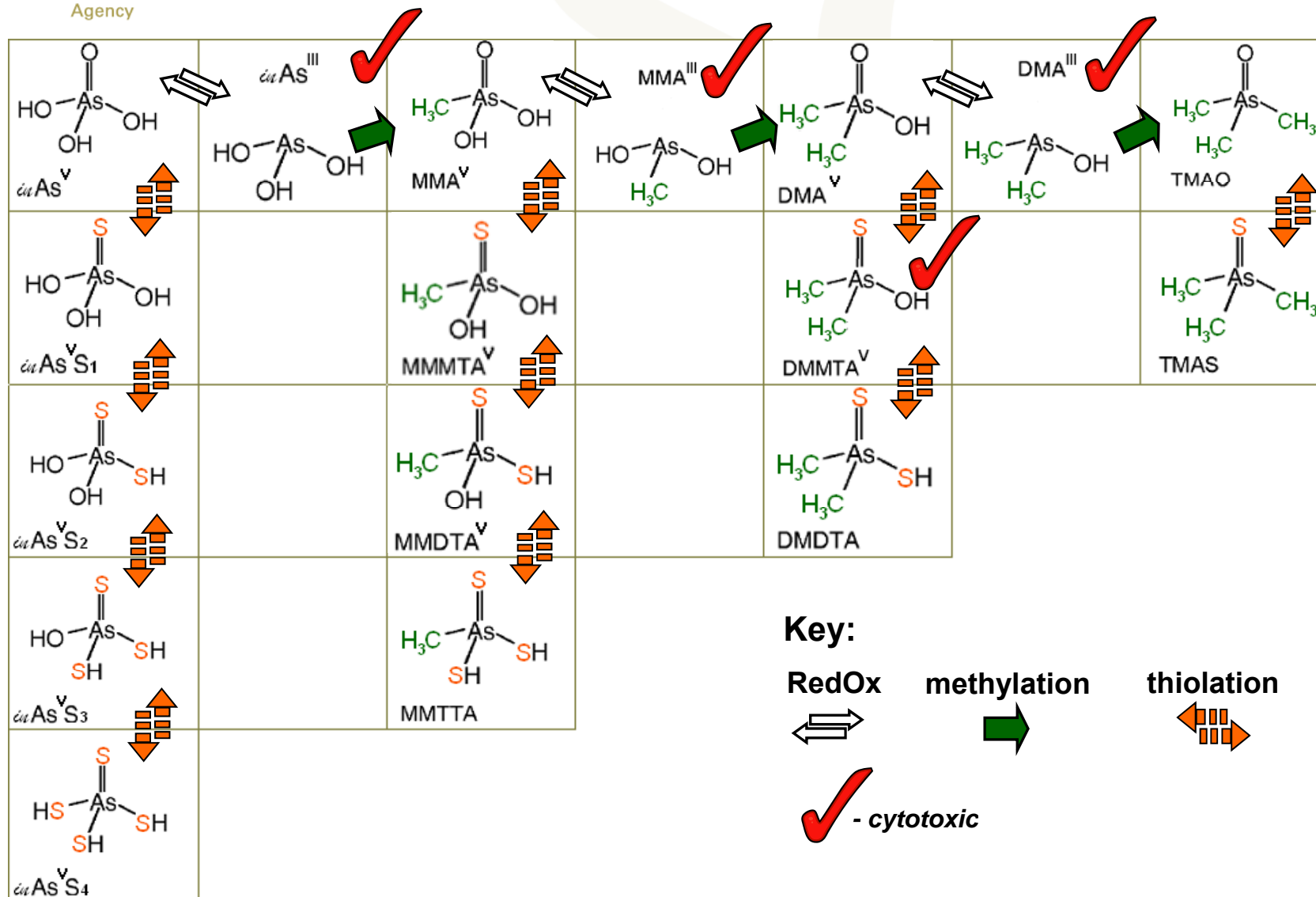**Office of Research and Development**
National Exposure Research Laboratory, Microbiological and Chemical Exposure Assessment Research Division, Chemical Exposure Research Branch

August 25, 2011

Arsenic

Chromium

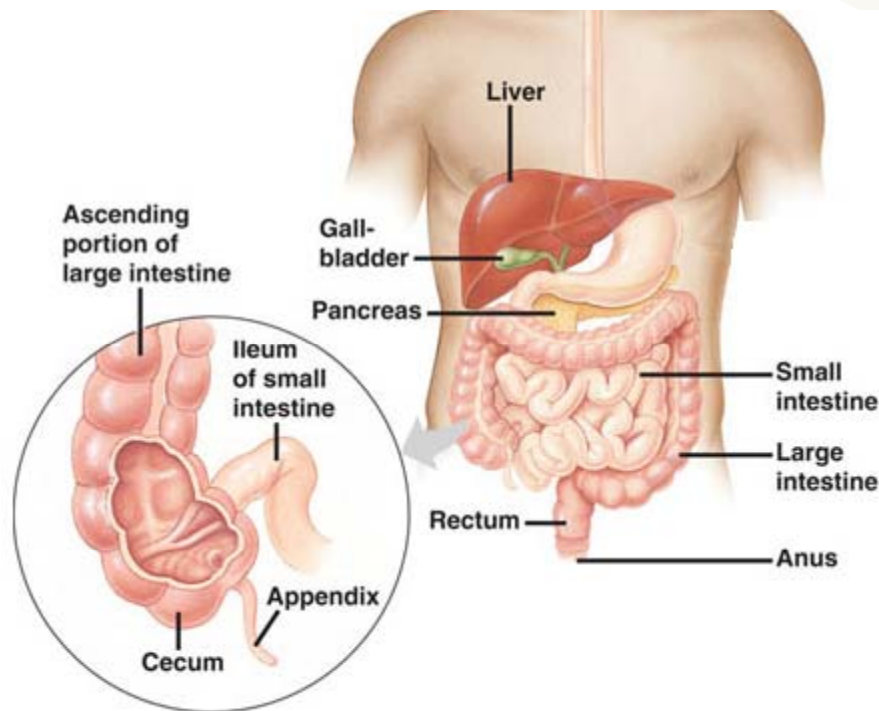| As^V / As^{III} Type 1 carcinogen	Cr^{VI} / Cr^{III} Type 1 carcinogen AND essential nutrient
World Health Organization:	
10 ppb <u>guideline</u> for drinking water	50 ppb <u>guideline</u> for total chromium in drinking water
US EPA:	
<u>enforceable</u> maximum contaminant level (MCL) of 10 ppb - ultimate goal (MCLG) – 0 ppb	<u>enforceable</u> maximum contaminant level (MCL) of 100 ppb -Office of Environmental Health Hazard Assessment recently proposed 20 ppt public health goal (PHG)
Research objective:	
To study metabolism of arsenic oxides by intestinal microbiota and to understand the exposure and toxicity implications of these transformations	*To develop a reliable analytical method for determination of Cr^{III} and Cr^{VI} in drinking water with low ppt detection limit*
Analytical challenges:	
- Speciate multiple (up to 12) unknown metabolites from a complex sample matrix in one chromatographic run - Synthesize and characterize STDs (no SRMs)	- Assure preservation of Cr^{VI} during sample handling - Designing a separation system that has good long term stability and minimizes polyatomic interference due to mobile phase and matrix constituents: $^{40}Ar^{12}C$, $^{34}S^{18}O$, $^{16}O^{37}Cl$

Part 1: Arsenic analysis by IC-ICP-MS

➢ *Multiple metabolites are isolated from a complex matrix (2-phase)*

➢ *Ion-chromatographic separation with elemental detection by ICP-MS*

➢ *Distribution of metabolites within sample phases*

Office of Research and Development
National Exposure Research Laboratory, Microbiological and Chemical Exposure Assessment Research Division, Chemical Exposure Research Branch

2

Arsenic species (metabolic pathway)

Key:

RedOx   methylation   thiolation

✓ - *cytotoxic*

Pre-systemic metabolism of MMA in GI tract by anaerobic bacteria

Arsenical of interest
[$_{in}$As^V , MMA, DMA, etc.]

Homogenized mouse cecum anaerobic incubation at 37°C

As, ppb	Hours at 37°C					
	0	3	6	12	24	48
0	3	—	—	—	3	3
20	3	—	—	—	3	3
200	3	3	3	3	3	3
1000	3	3	3	3	3	3
2000	6	3	3	3	3	6

Snap-freeze @ -70°C → ship

Analyze supernatant by IC-ICP-MS

- Liver
- Gall-bladder
- Pancreas
- Small intestine
- Large intestine
- Rectum
- Anus
- Ascending portion of large intestine
- Ileum of small intestine
- Cecum
- Appendix

➢ Cecum contains H$_2$S-producing bacteria
➢ Potential thiolation site

IC-ICP-MS mass chromatogram of MMA metabolites in supernatant

HPLC conditions: anion-exchange AS-16 Dionex column, mobile phase constituents (A)-DDI and (B)-0.68% TMA(OH). Step gradient: 0-7 min 12%B; 7-17 min 50% B; 17-27 min 100% B; 27- 40 min-12% B.

Office of Research and Development
National Exposure Research Laboratory, Microbiological and Chemical Exposure Assessment Research Division, Chemical Exposure Research Branch

5

Preparation of cecum samples for IC-ICP-MS analysis

Cecum mixture after incubation

↓

Freeze @ -70°C

↓

Thaw @ ambient T

↓

Spin @ 2.5k rpm, 5 min

Supernatant   Solids

↓

Suspend in 0.5 mL VPI   ⎫
                         ⎬ *Repeat 3 times*
Spin @ 2.5k rpm, 5 min   ⎭

Combined VPI extract   Pellet after extraction

Office of Research and Development
National Exposure Research Laboratory, Microbiological and Chemical Exposure Assessment Research Division, Chemical Exposure Research Branch
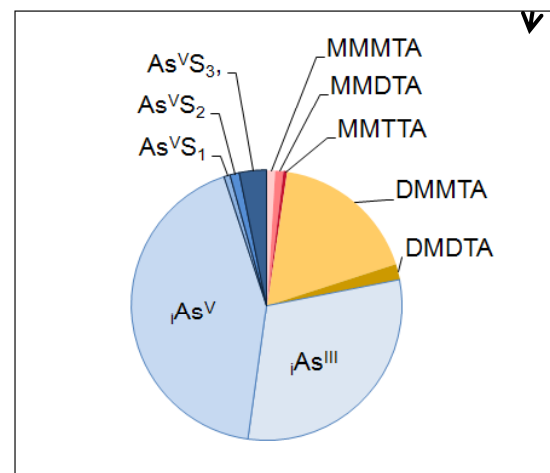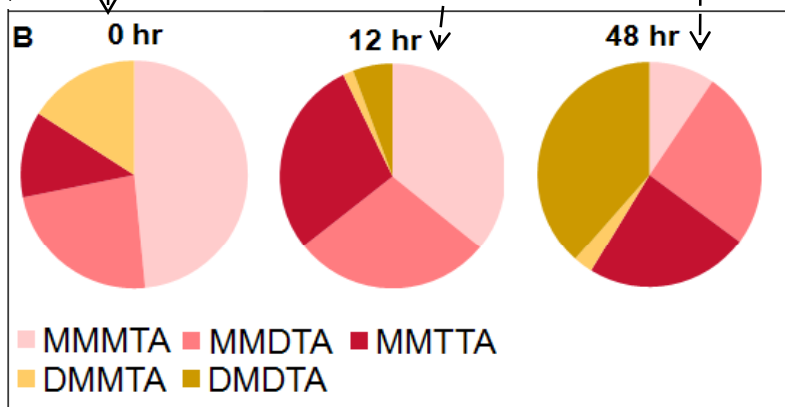
6
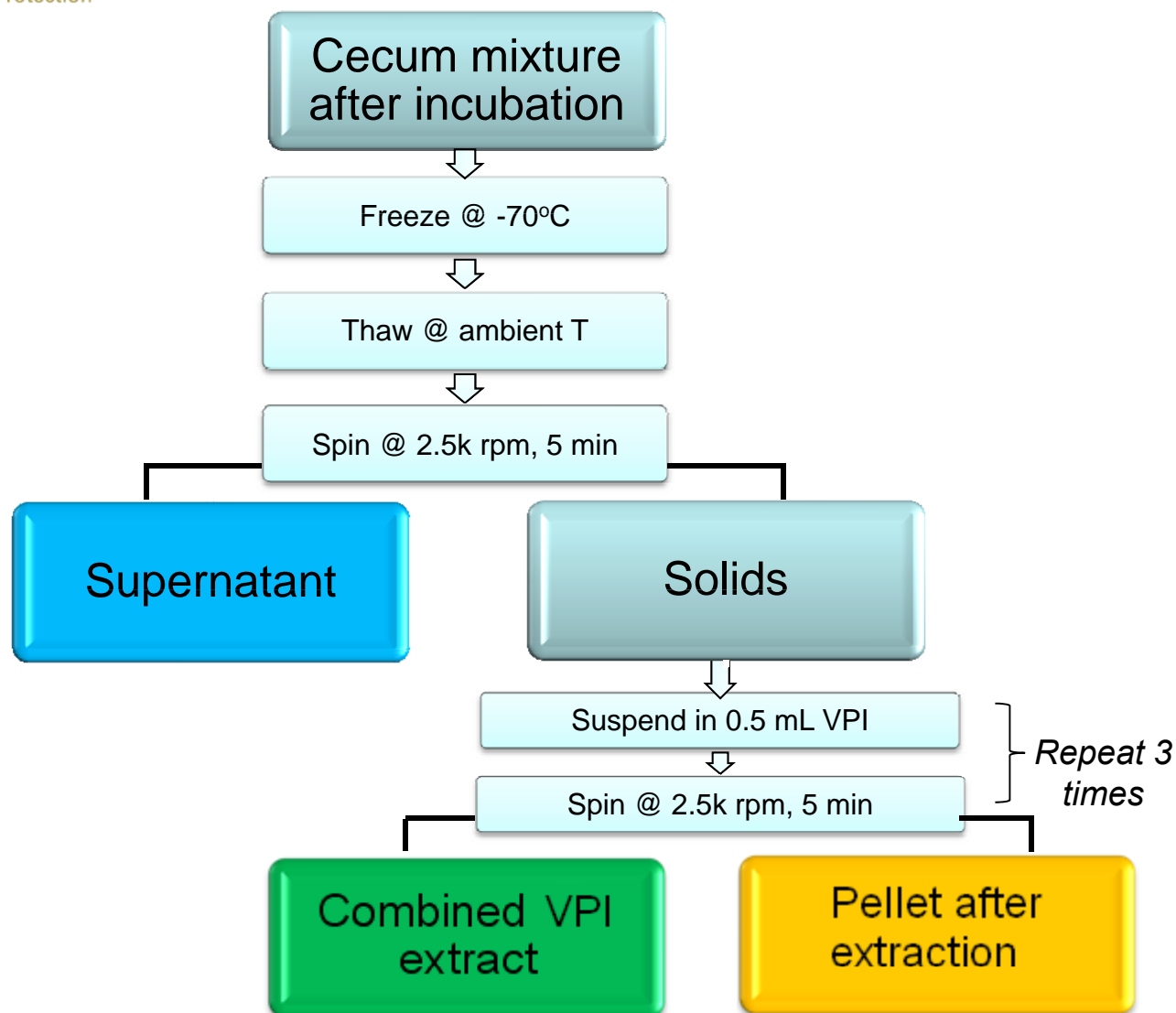
IC-ICP-MS mass chromatogram of MMA metabolites in **extract of cecum solids**
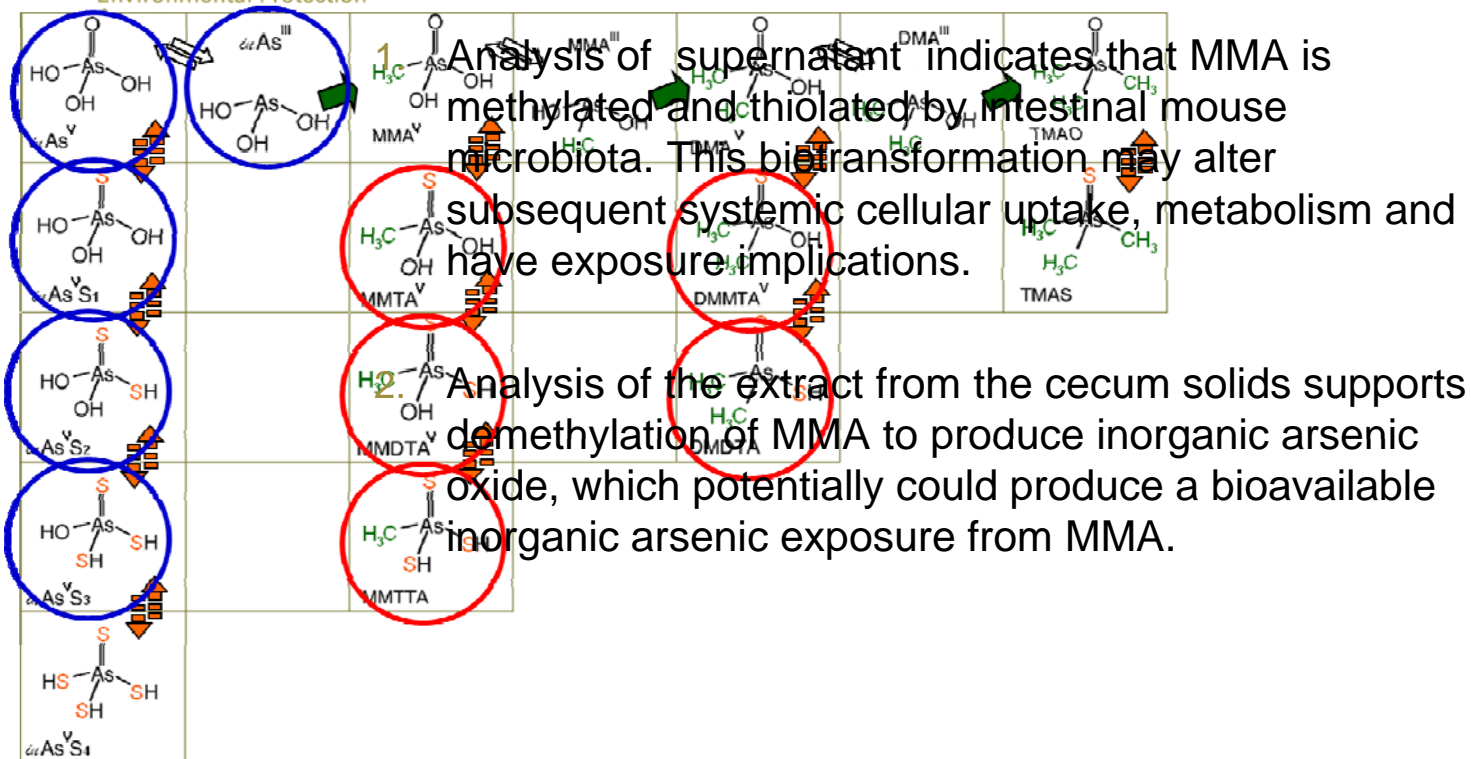
Office of Research and Development
National Exposure Research Laboratory, Microbiological and Chemical Exposure Assessment Research Division, Chemical Exposure Research Branch

7
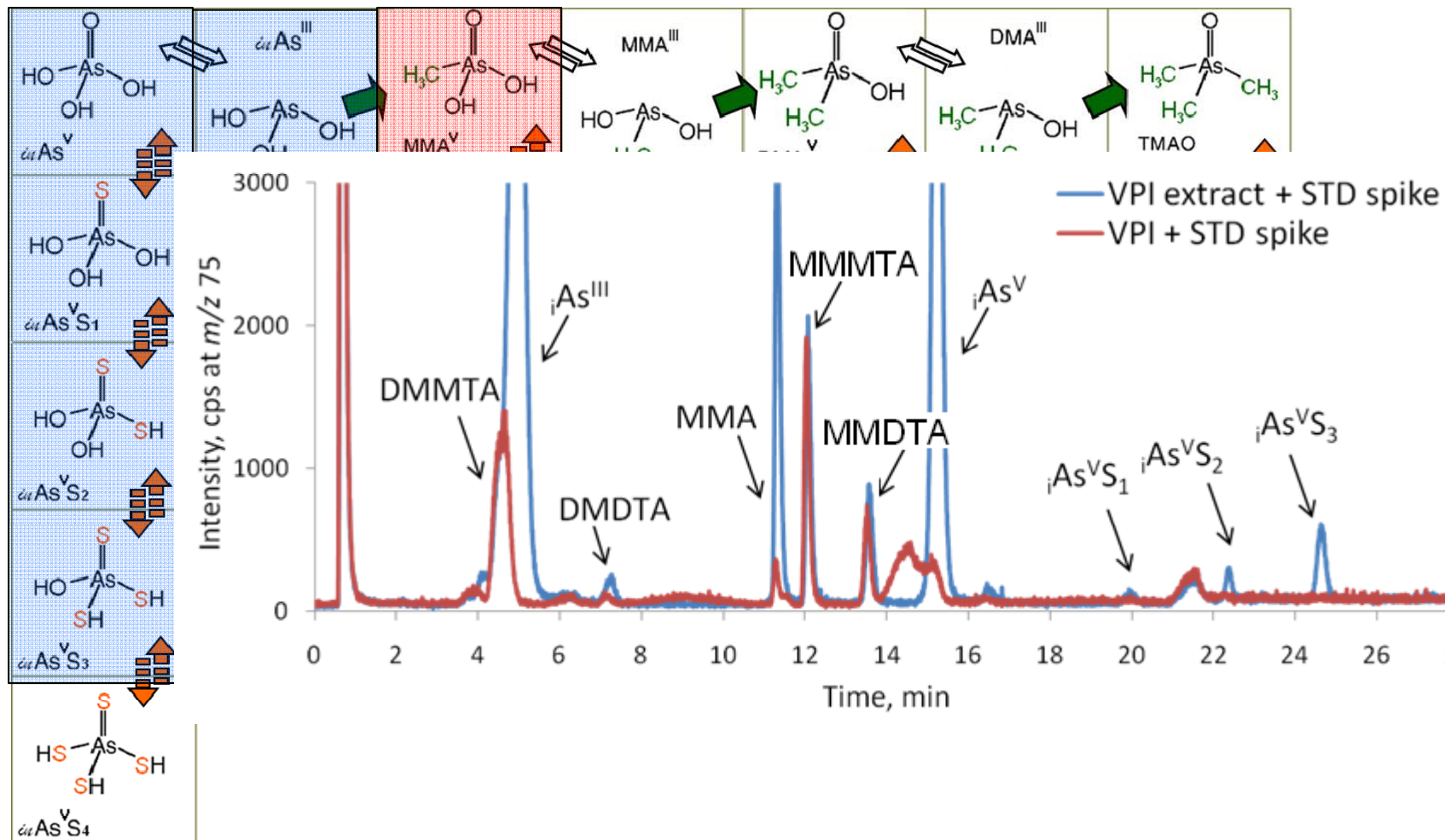
Mass-balance distribution of MMA metabolites in supernatant and cecum solids (at 48 hours)

Part 1: conclusions

1. Analysis of supernatant indicates that MMA is methylated and thiolated by intestinal mouse microbiota. This biotransformation may alter subsequent systemic cellular uptake, metabolism and have exposure implications.

2. Analysis of the extract from the cecum solids supports demethylation of MMA to produce inorganic arsenic oxide, which potentially could produce a bioavailable inorganic arsenic exposure from MMA.

Office of Research and Development
National Exposure Research Laboratory, Microbiological and Chemical Exposure Assessment Research Division, Chemical Exposure Research Branch

9

Part 2: Chromium analysis by IC-ICP-MS

➢ *Analytical capabilities of ICP-MS for Cr speciation studies*

  – *Polyatomic signal interference (mobile phase, matrix)*

  – *Separation/Sensitivity*

➢ *Preservation of native Cr^{III} and Cr^{VI} during shipping and analysis*

Office of Research and Development
National Exposure Research Laboratory, Microbiological and Chemical Exposure Assessment Research Division, Chemical Exposure Research Branch

10

# Potential polyatomic interferences associated with detection of Cr by ICP-MS at *m/z* 52 and *m/z* 53

- Chromium isotopes: 50 (4.3%), **52** (83.8%), **53** (9.5%) and 54 (2.4%)

- Any diatomic species at these masses can contribute to background signal and chromatographic peaks

Origin of the diatomic **background interference:**	Possible interferences at m/z 52:	Possible interferences at m/z 53:	Polyatomic induced **chromatographic peaks** from matrix
plasma gas	$^{36}Ar^{16}O$	$^{36}Ar^{17}O$	
	$^{38}Ar^{14}N$	$^{38}Ar^{15}N$	
mobile phase (sulfates)	$^{36}S^{16}O$; $^{34}S^{18}O$	$^{36}S^{17}O$	Sulfate (SO_4^{2-})
mobile phase (carboxylates)	$^{40}Ar^{12}C$	$^{40}Ar^{13}C$	Carbonate (CO_3^{2-})
		$^{37}Cl^{16}O$	Chloride (Cl^-)

Office of Research and Development
National Exposure Research Laboratory, Microbiological and Chemical Exposure Assessment Research Division, Chemical Exposure Research Branch

11

Effect of mobile phase composition on polyatomic background signal

Considered mobile phases:

1. *Ammonium oxalate (AO)*
2. *Ammonium sulfate (SO4)*
3. *Ammonium nitrate (NO3)*

1) Oxalate contributes to high background signal; also reacts with chromium (complexation)

2) Sulfate also reacts with chromium to produce unidentified chromium compound (elutes under 4 minutes); provides less background at *m/z* 52 compared to oxalate

3) Ammonium nitrate allows separation of Cr^{III} and Cr^V under 14 minutes and produces least background signal
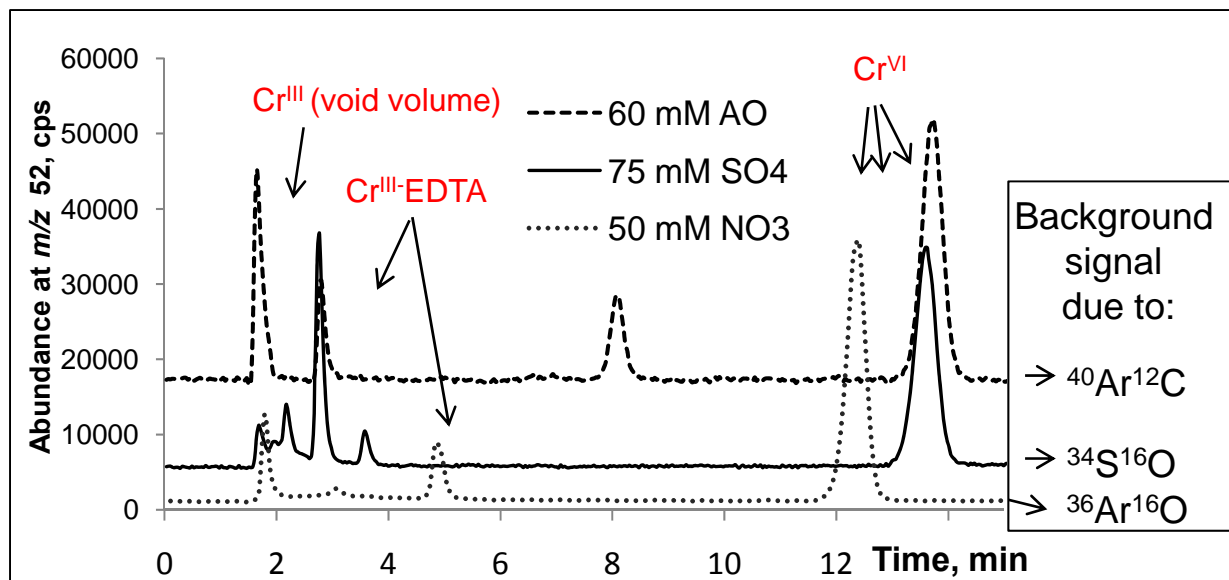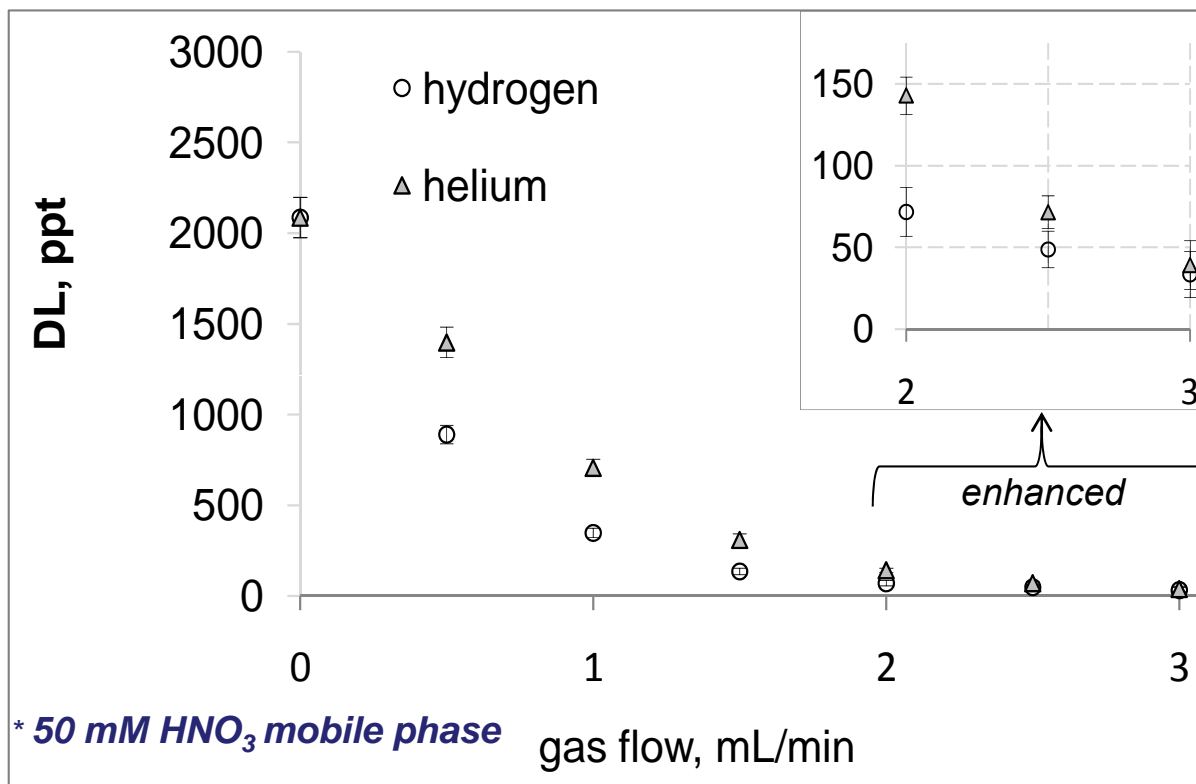

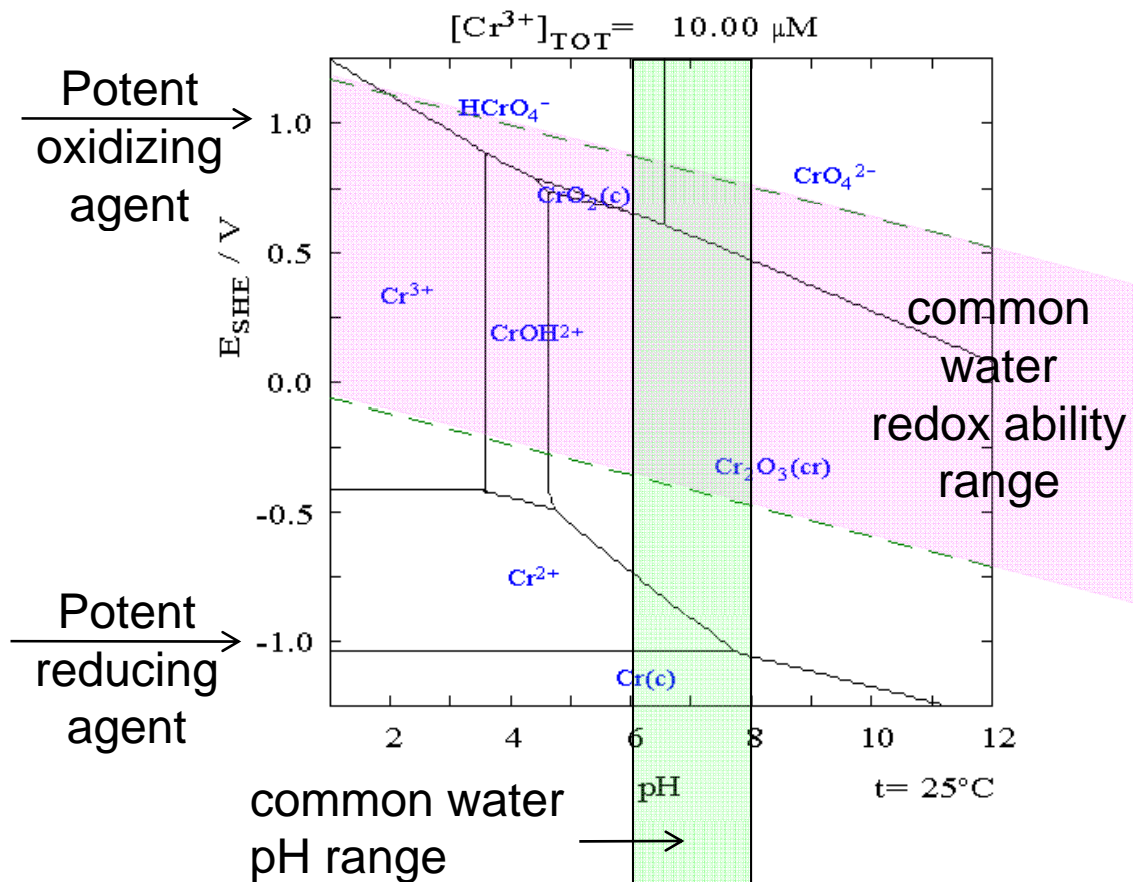*All chromium compounds were spiked at 1 ppb level
* Naturally occurring matrix ions contributing to diatomics such as $^{40}Ar^{12}C$, $^{34}S^{18}O$, $^{16}O^{37}Cl$ are base-line resolved at these conditions (not shown)
** Dionex AS7 ion-exchange column, 1mL/min flow rate, ambient temperature

Office of Research and Development
National Exposure Research Laboratory, Microbiological and Chemical Exposure Assessment Research Division, Chemical Exposure Research Branch

12

Effect of collision gas flow rate on Cr^{VI} detection limit (3σ)

* 50 mM HNO$_3$ mobile phase

Office of Research and Development
National Exposure Research Laboratory, Microbiological and Chemical Exposure Assessment Research Division, Chemical Exposure Research Branch

13

Potent oxidizing agent →

Potent reducing agent →

common water redox ability range
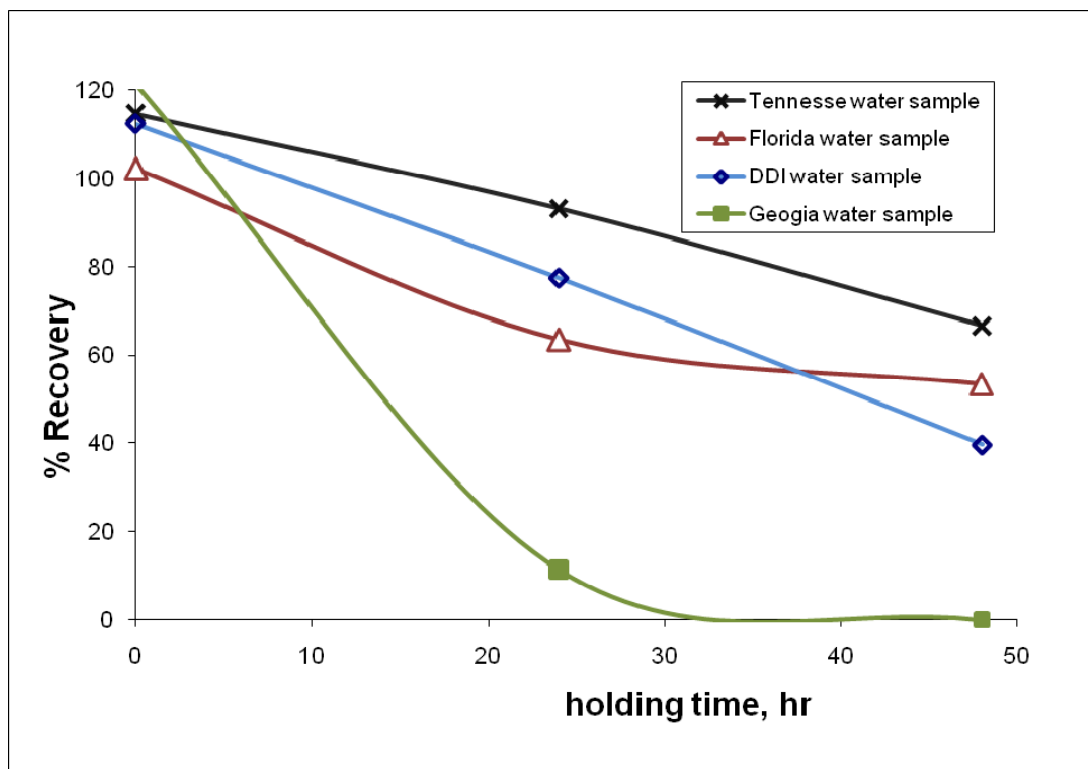
common water pH range →

[Cr³⁺]ₜₒₜ = 10.00 μM

1. Cr^{VI} is only predominant in oxidative waters

2. Disinfectants added to portable water are oxidizing in nature, favoring formation of Cr^{VI} from Cr^{III}

3. Analytical challenge is to develop a method that preserves native Cr^{III} and Cr^{VI} from the time of sample collection until detection

Pourbaix diagram for chromium adapted from Kotaś, J.; Stasicka, Z Environmental Pollution (2000) 107 (3): 263–283

Office of Research and Development
National Exposure Research Laboratory, Microbiological and Chemical Exposure Assessment Research Division, Chemical Exposure Research Branch

14

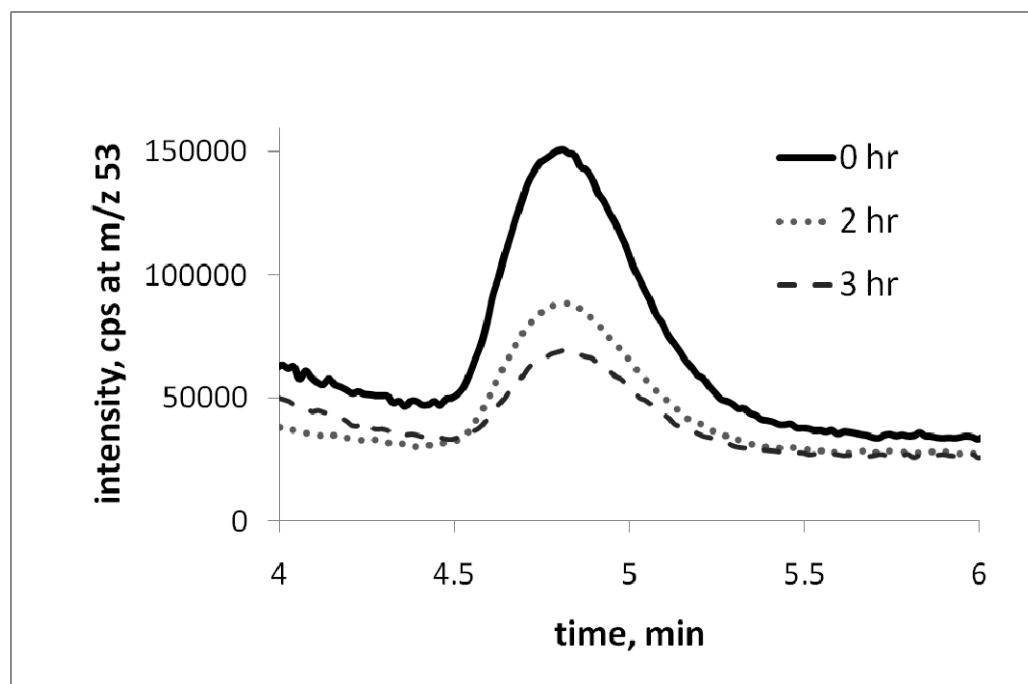# Stability of Cr^{III} in drinking water as a function of time

1. Water samples were collected in different states and transported to lab

2. Cr^{III} standard spike was added to waters in lab and held at room temperature

3. Aliquots of each water were taken at time intervals, heated with EDTA and a formed Cr^{III}-EDTA complex was detected

4. Cr^{III} recoveries were 0% for all waters (except Tennessee, 50%) after 8 days

1 ppb standard spikes, 0.8 mL/min He

Office of Research and Development
National Exposure Research Laboratory, Microbiological and Chemical Exposure Assessment Research Division, Chemical Exposure Research Branch

15

Stability of Cr^{III} in EDTA-spiked drinking water at room temperature

1. Water samples were collected in different states and transported to lab

2. EDTA followed by Cr^{III} standard spike was added to waters, held at room temperature

3. Aliquots of each water were taken at time intervals, heated for 1 hour and a formed Cr^{III}-EDTA complex was detected

* 6 ppb Cr^{III} standard spike, 0.8 mL/min He

Office of Research and Development
National Exposure Research Laboratory, Microbiological and Chemical Exposure Assessment Research Division, Chemical Exposure Research Branch

16

Conclusions

– Ion-exchange chromatography is suitable for separation of Cr^{III} and Cr^{VI} in drinking water

– Preservation of Cr^{III} spike in water samples is challenging unless immediately heated after addition of EDTA

Office of Research and Development
National Exposure Research Laboratory, Microbiological and Chemical Exposure Assessment Research Division, Chemical Exposure Research Branch

17

Acknowledgement

Jack Creed   David Thomas   Carol Brockhoff   Madhavi Mantha   Patty Creed   Tony Wilson

Office of Research and Development
National Exposure Research Laboratory, Microbiological and Chemical Exposure Assessment Research Division, Chemical Exposure Research Branch

18
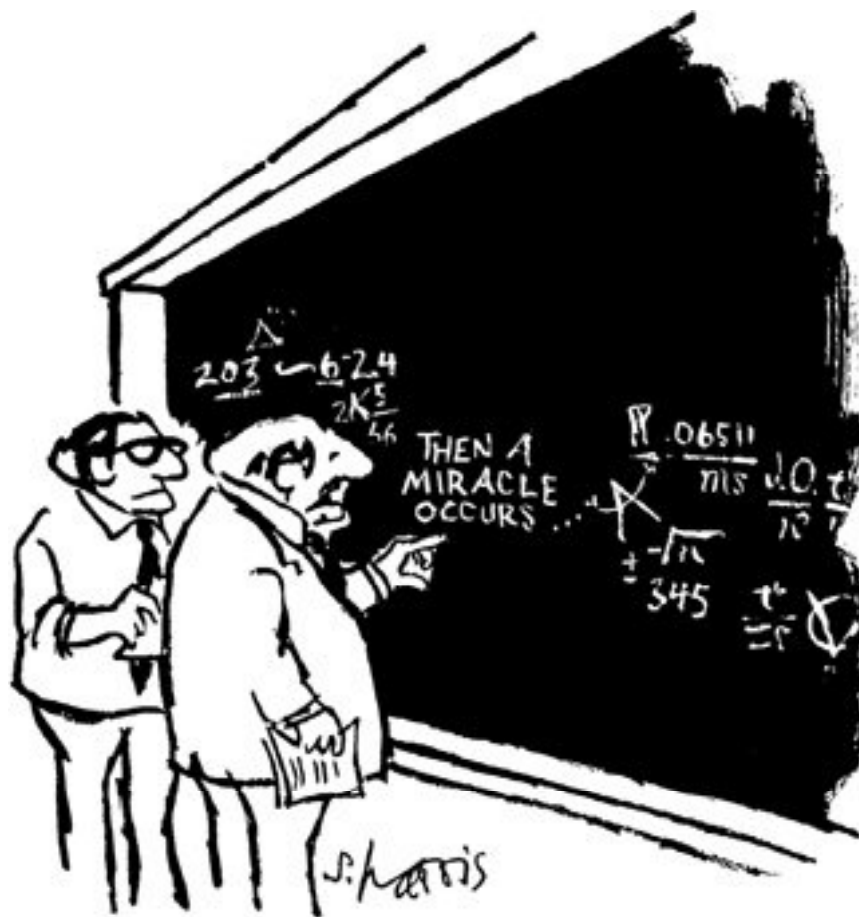
"I think you should be more explicit here in step two."

Office of Research and Development
National Exposure Research Laboratory, Microbiological and Chemical Exposure Assessment Research Division, Chemical Exposure Research Branch

19