The Use of ICP-MS and IC-ICP-MS in Environmental and Exposure Assessment Analyses

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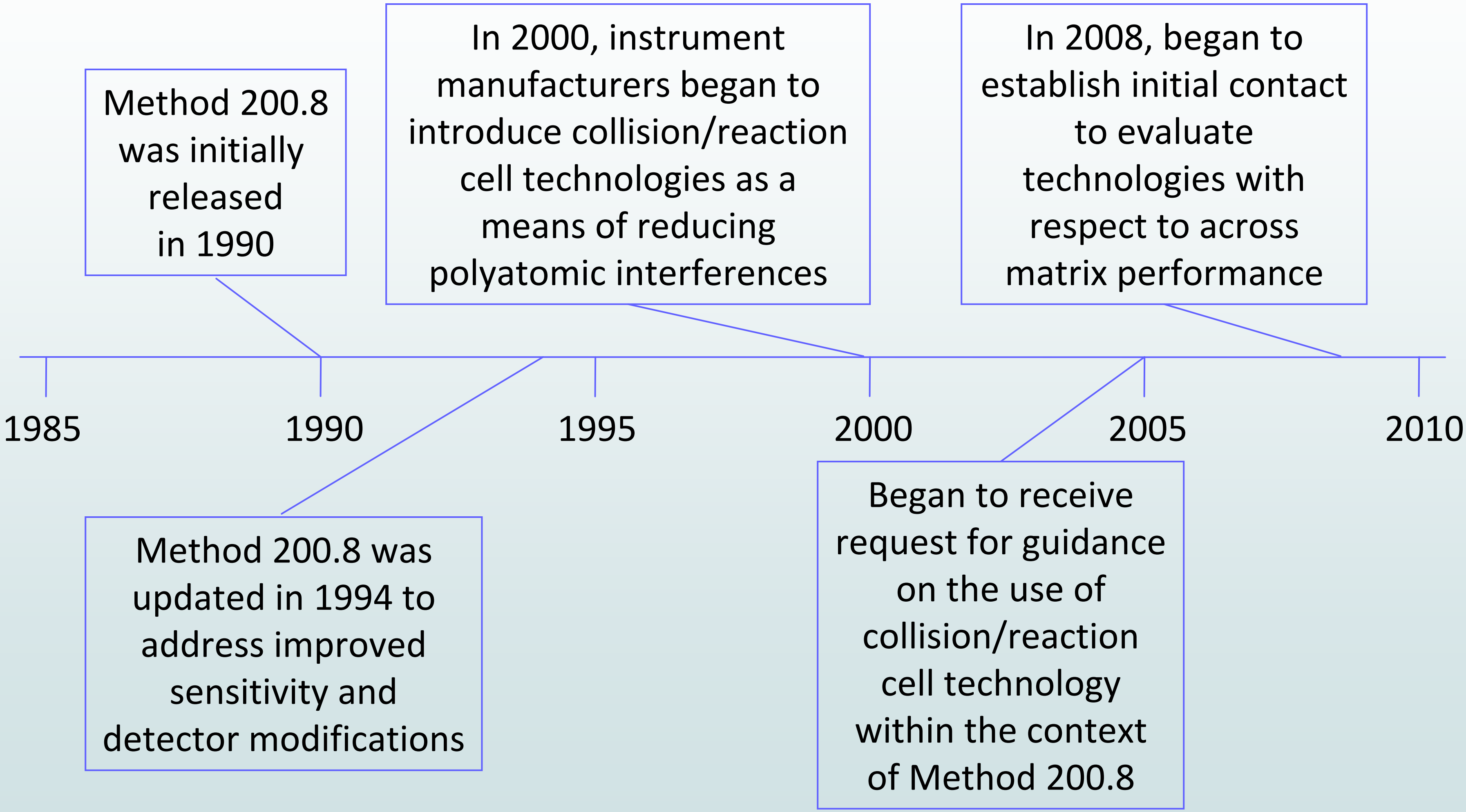
Overview

- Application of collision/reaction cell interference reduction technology for the minimization of polyatomic interferences in environmental matrices.
- Improved risk assessments through the use of arsenic speciation approaches that estimate the bio-accessibility associated with the exposure.
- Presystemic bio-transformation of arsenic oxides and their exposure assessment implications.

History of EPA Method 200.8



Why Update Method 200.8: ICP-MS?

1. Existing method suffers from matrix induced polyatomics.

Matrix Elements

- H
- ^{12}C ^{13}C
- ^{16}O ^{18}O
- ^{23}Na
- ^{28}Si
- ^{32}S ^{34}S
- ^{35}Cl ^{37}Cl
- ^{39}K ^{41}K
- ^{40}Ca ^{42}Ca ^{44}Ca
- ^{36}Ar ^{38}Ar ^{40}Ar

Ca Matrix		Element
56	$^{40}Ca^{16}O$, $^{40}Ar^{16}O$	^{56}Fe
57	$^{40}Ca^{16}OH$	^{57}Fe
58	$^{40}Ca^{18}O$, $^{42}Ca^{16}O$	^{58}Fe, ^{58}Ni
59	$^{40}Ca^{18}OH$, $^{42}Ca^{16}OH$	^{59}Co
60	$^{42}Ca^{18}O$, $^{44}Ca^{16}O$	^{60}Ni
61	$^{42}Ca^{18}OH$, $^{44}Ca^{16}OH$	^{61}Ni
62	$^{44}Ca^{18}O$	^{62}Ni
63	$^{44}Ca^{18}OH$	^{63}Cu

Why Update Method 200.8: ICP-MS?

1. Existing method suffers from matrix induced polyatomics.

2. Vendors have developed two instrumental techniques to minimize polyatomics

    a. Collision Cell: He or H_2 gas

        - Based on Physics: Collision Energy vs. Bond Energy
        - Generally simplifies the spectra

    b. Reaction Cell

        - Based on Chemistry
        - Generally complicates the spectra

Why Update Method 200.8: ICP-MS?

1. Polyatomic problem: Simple example.

Matrix Elements

- H
- ^{12}C ^{13}C
- ^{16}O ^{18}O
- ^{23}Na
- ^{28}Si
- ^{32}S ^{34}S
- ^{35}Cl ^{37}Cl
- ^{79}Br ^{81}Br
- ^{40}Ca ^{42}Ca ^{44}Ca
- ^{36}Ar ^{38}Ar ^{40}Ar

	Br Matrix	Element
80	^{79}BrH	^{80}Se
82	^{81}BrH	^{82}Se

^{82}Se Recommended Analysis Mode ^{81}BrH

Matrix	Vendor 1	Vendor 2	Vendor 3	Vendor 4
Na	0.05	0.47	<LOD	0.04
H_2SO_4	0.16	0.58	0.04	0.16
Mo	-0.03	0.19	<LOD	0.01
C	0.09	0.39	<LOD	0.07
Br	**106.30**	**3795.00**	**1625.00**	**355.50**
Ba	0.03	1.97	<LOD	-0.01
Ca	0.16	<LOD	0.83	-0.05
K	-0.03	3.73	0.22	-0.39
Mg	0.55	0.24	0.05	0.28
Rb	-0.01	0.46	0.66	0.01

MDL (0.5ppb)
MCL (50ppb)

Preliminary Data Set

Why Update Method 200.8: ICP-MS?

1. Existing method suffers from matrix induced polyatomics.

Matrix Elements

- H
- ^{12}C ^{13}C
- ^{16}O ^{18}O
- ^{23}Na
- ^{28}Si
- ^{32}S ^{34}S
- ^{35}Cl ^{37}Cl
- ^{79}Br ^{81}Br
- ^{40}Ca ^{42}Ca ^{44}Ca
- ^{36}Ar ^{38}Ar ^{40}Ar

Calcium Chloride Matrix		Element
51	$^{16}O^{35}Cl$	^{51}V
•		
53	$^{16}O^{37}Cl$	^{53}Cr
•		
•		
•		
75	$^{40}Ar^{35}Cl$, $^{40}Ca^{35}Cl$	^{75}As
76	$^{40}Ar^{36}Ar$	^{76}Se
77	$^{40}Ar^{37}Cl$, $^{40}Ca^{37}Cl$, $^{42}Ca^{35}Cl$	^{77}Se
•		
•		
•		
82	^{81}BrH	^{82}Se

Chloride ratio

Selenium ratio

$$C^{As}_{75} = C_{75} - 3.12 \times [C_{77} - (0.815 \times C^{Se}_{82})]$$

Why Update Method 200.8: ICP-MS?

2. Vendors have developed two instrumental techniques to minimize polyatomics.

   a. Collision Cell: $^{40}Ar^{35}Cl \xrightarrow{He,\ H} X_1(^{40}Ar + ^{35}Cl) + Y_1(^{40}Ar^{35}Cl)$

   $X_2(^{40}Ar + ^{37}Cl) + Y_2(^{40}Ar^{37}Cl)$

   $X_3(^{81}Br + H) + Y_3(^{81}BrH)$

   b. Reaction Cell: ^{75}As vs. $^{40}Ar^{35}Cl$

Ge is an internal standard in this method: ^{73}Ge, ^{74}Ge

$^{73}Ge \xrightarrow{O_2} {}^{73}Ge^{18}O$ = m/z = 91

$^{74}Ge \xrightarrow{O_2} {}^{74}Ge^{16}OH$ = m/z = 91

$^{74}Se \xrightarrow{O_2} {}^{74}Se^{16}OH$ = m/z = 91

$^{75}As \xrightarrow{O_2} {}^{75}As^{16}O$ = m/z = 91

•
•
•

$^{91}Zr \xrightarrow{O_2} {}^{91}Zr^{16}O$ = m/z = 107 Ag

^{75}As Recommended Analysis Mode Results

Matrix	Vendor 1	Vendor 2	Vendor 3	Vendor 4
Na	0.04	0.04	0.02	0.01
H_2SO_4	0.19	<LOD	0.01	0.00
Mo	-0.03	0.07	0.01	0.01
C	-0.14	0.00	<LOD	-0.01
Br	96.43	0.03	<LOD	-0.03
Ba	0.16	<LOD	<LOD	-0.01
Ca	-0.28	0.11	0.13	0.14
K	-0.06	0.01	<LOD	0.03
Mg	-0.11	0.02	0.00	0.04

MDL (0.1ppb)
MCL (10ppb)

Preliminary Data Set

Why Update Method 200.8: ICP-MS?

2. Vendors have developed two instrumental techniques to minimize polyatomics

   a. Collision Cell: He or H_2 gas

      - Based on Physics: Collision Energy vs. Bond Energy
      - Generally simplifies the spectra

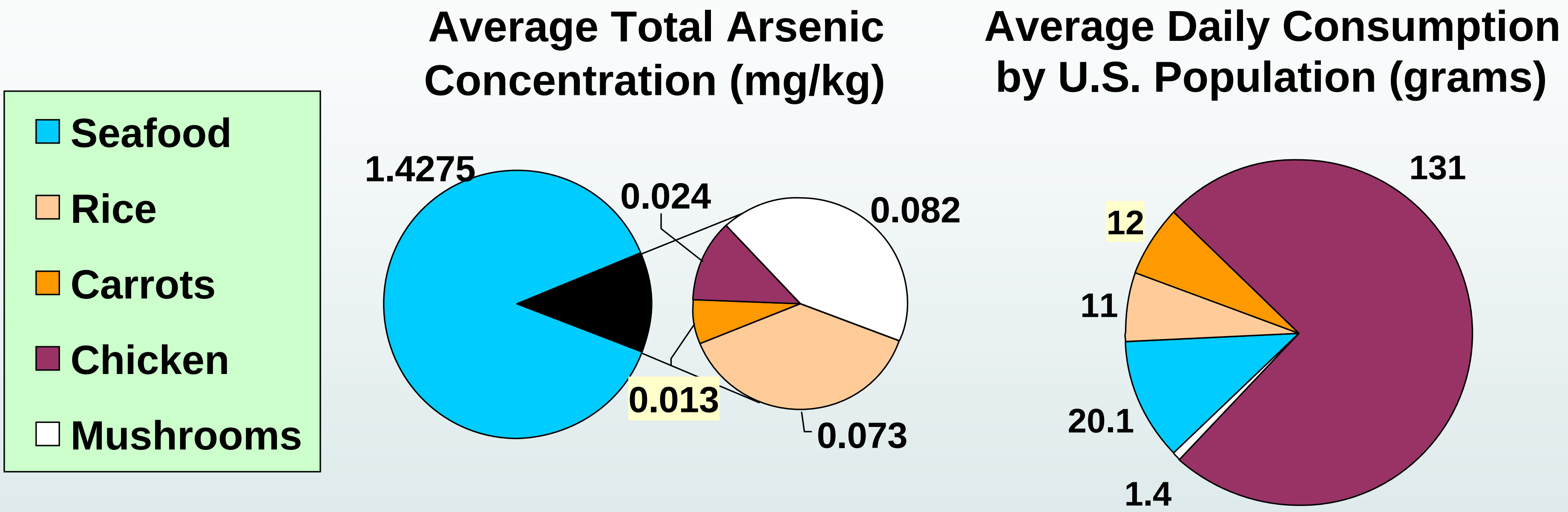
   b. Reaction Cell

      - Based on Chemistry
      - Generally complicates the spectra

Two approaches produce different across matrix performance leading to vendor specific performance.

Dietary Arsenic Exposure Assessment

Dietary Arsenic Exposure Assessment

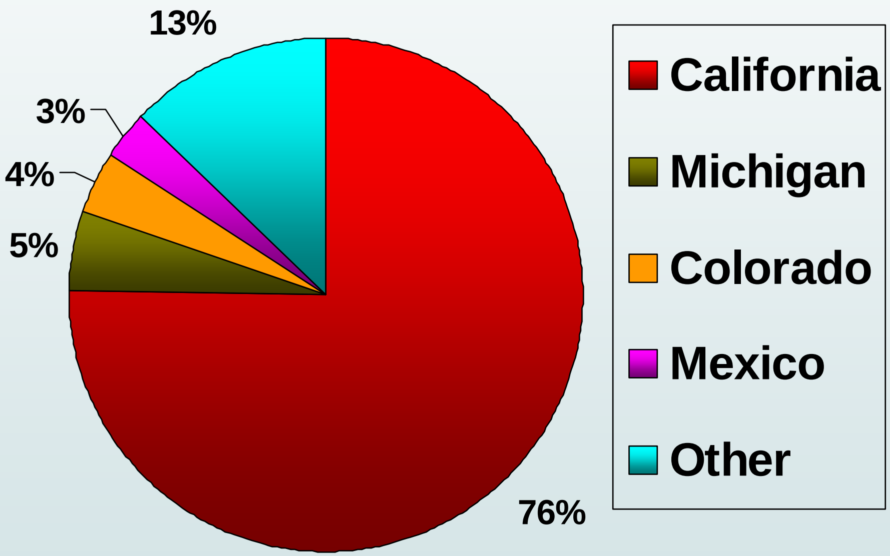


Exposure = "Concentration" x Consumption

Use Carrots as an Example

Exposure = Concentration x Consumption

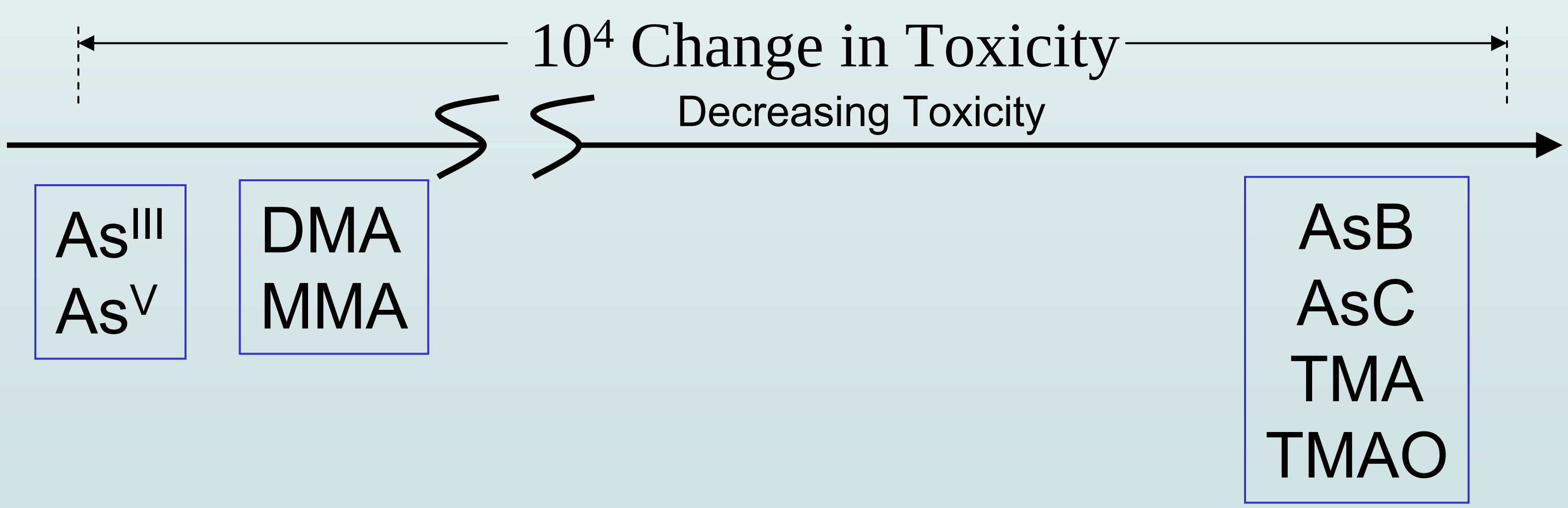
Sampling Based on Harvest Demographics for Carrots



Exposure = Concentration x Consumption

Sampling Based on Harvest Demographics for Carrots

Speciation reflects chemical form dependent toxicity



Exposure = Concentration x Consumption

Sampling Based on
Harvest Demographics
for Carrots

Speciation α Toxicity

Chemical Extraction

1. Acid / Base
2. MeOH / Water
3. Water

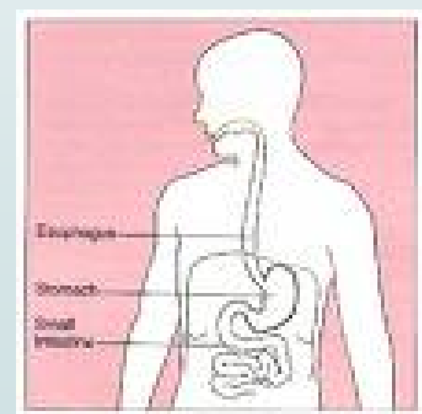
Bioaccessibility

1. *In-vitro* studies that mimic human GI system
2. Better control
3. Estimate of bioaccessible component

Bioavailability

1. Tedious
2. Cross-species correlation?
3. Ethical issues

IDEAL



Exposure = Concentration x Consumption

Sampling Based on Harvest Demographics for Carrots

↓

Speciation α Toxicity

↓

In vitro Assay "Bioaccessibility"

Freeze Dried Carrot Samples

$As_{Total\ Digest}$ (As_{TD})

1. **Rehydrate with DDI**
2. **Add saline solution, 5M HCl and pepsin**
3. **Place in 37°C waterbath with platform agitator for 1 hour**

Carrot Sample after Synthetic Stomach Extraction

1. **pH adjust to 6 with 1M $NaHCO_3$**
2. **Add pancreatin solution & 1M NaOH**
3. **Place in 37°C waterbath with platform agitator for 2 hours**

Carrot Sample after Synthetic Intestine Extraction

Enzymatic Carrot Sample → **LC-ICP-MS**

Mass balance table for species specific bioacce-ssibility based analyses of arsenic in carrots

1	2	3	4	5	6	7	8	9
	As_{Total}				$As_{Speciation}$			
Sample	Wet Weight Moisture Content (%)	Dry Weight Total Digest (ng/g ± 2σ)	Wet Weight Total Digest (ng/g ± 2σ)	Dry Weight Extraction Efficiency (% ± 2σ)	Dry Weight $As_{Inorganic}$ (ng/g ± 2σ)	Wet Weight $As_{Inorganic}$ (ng/g ± 2σ)	Dry Weight Chromatographic Recovery (% ± 2σ)	Dry Weight Overall Recovery (% ± 2σ)
1	91	36 ± 0.8	3.5 ± 0.15	61 ± 11.0	21.4 ± 2.5	2.1 ± 0.2	87 ± 3.7	59 ± 6.9
2	90	37 ± 4.6	4.1 ± 1.03	62 ± 17.5	22.7 ± 2.0	2.5 ± 0.2	101 ± 59.1	61 ± 21.5
3	87	36 ± 4.7	5.4 ± 0.70	82 ± 27.2	28.0 ± 5.6	4.2 ± 0.8	92 ± 22.0	76 ± 15.1
4	90	58 ± 11.2	6.5 ± 1.25	87 ± 21.5	45.8 ± 14.2	5.1 ± 1.6	91 ± 19.6	79 ± 24.5
5	88	48 ± 4.2	6.6 ± 0.57	57 ± 22.0	28.3 ± 9.2	3.9 ± 1.3	103 ± 10.5	59 ± 19.1
6	87	43 ± 3.5	6.5 ± 0.52	69 ± 17.2	27.4 ± 3.8	4.1 ± 0.6	93 ± 16.7	64 ± 8.9
7	88	63 ± 1.7	8.6 ± 0.23	74 ± 18.2	49.1 ± 12.5	6.7 ± 1.7	107 ± 40.4	78 ± 19.9
8	89	74 ± 11.0	9.2 ± 1.35	77 ± 11.0	52.3 ± 8.3	6.5 ± 1.0	93 ± 28.0	71 ± 11.2
9	91	107 ± 1.3	10.5 ± 0.12	53 ± 9.0	72.0 ± 1.1	7.1 ± 0.1	127 ± 22.8	67 ± 1.0
10	90	63 ± 6.0	7.0 ± 0.65	72 ± 2.5	48.7 ± 4.0	5.4 ± 0.4	108 ± 5.6	77 ± 6.37
11	90	8 ± 5.4	0.85 ± 0.60	ND	ND	ND	ND	ND
12	90	79 ± 5.7	8.7 ± 0.63	69 ± 6.4	55.6 ± 4.5	6.2 ± 0.5	102 ± 2.2	70 ± 5.7
13	90	79 ± 5.4	9.0 ± 0.65	71 ± 24.2	62.5 ± 16.7	6.9 ± 1.9	112 ± 40.4	79 ± 21.1
14	90	24 ± 0.6	2.7 ± 0.07	48 ± 15	ND	ND	ND	ND
15	87	57 ± 5.9	8.5 ± 0.88	43 ± 9.0	27.9 ± 6.0	4.2 ± 0.9	114 ± 25.8	49 ± 10.6
16	90	116 ± 14.6	12.8 ± 1.61	29 ± 2.4	32.5 ± 1.5	3.6 ± 0.2	96 ± 8.0	28 ± 1.3
17	89	39 ± 1.3	4.8 ± 0.16	93 ± 56.6	32.4 ± 4.2	4.0 ± 0.5	94 ± 46.1	83 ± 10.9
18	90	43 ± 3.6	4.7 ± 0.39	43 ± 16.2	18.0 ± 7.1	2.0 ± 0.8	72 ± 14.0	42 ± 16.5
Across Matrix Avg ± 2σ	89 ± 2.6	56 ± 55	6.6 ± 5.9	56 ± 15	40 ± 31	4.8 ± 3.1	101 ± 21	65 ± 31

Exposure = Concentration x Consumption

Sampling Based on
Harvest Demographics
for Carrots

↓

Speciation α Toxicity

↓

In vitro Assay
“Bioaccessibility”

↓

4.8 ng/g ± 3.1

Exposure = Concentration x Consumption

Sampling Based on Harvest Demographics for Carrots

↓

Speciation α Toxicity

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4.8 ng/g ± 3.1

- What We Eat In America (WWEIA), NHANES 2005-2006, considers 13,000 commonly eaten foods in US.
- 52,653 participants in the survey provided information on their food consumption.

Exposure = Concentration x Consumption

Sampling Based on Harvest Demographics for Carrots

Speciation α Toxicity

In vitro Assay "Bioaccessibility"

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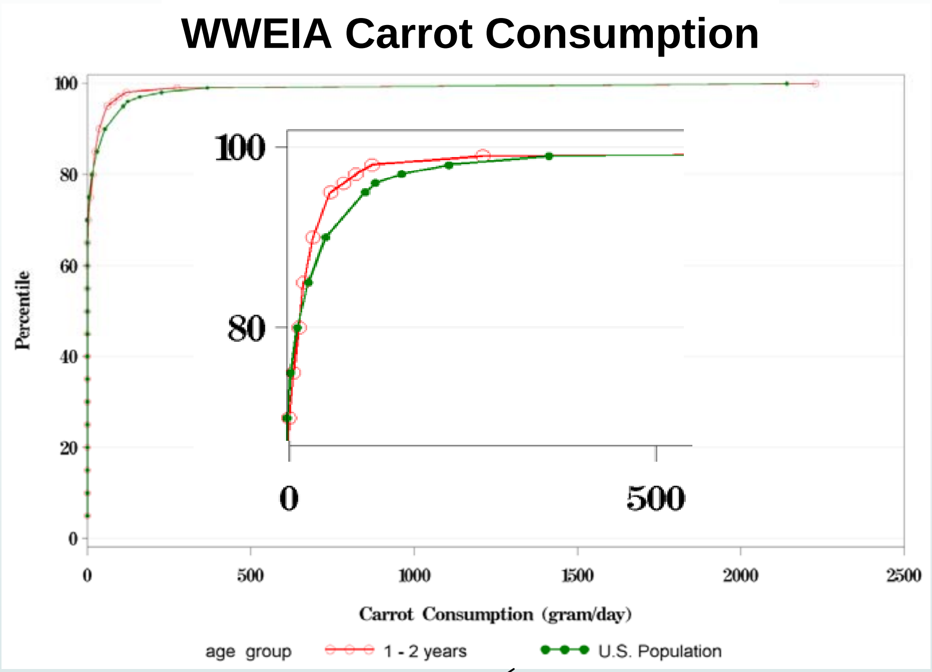
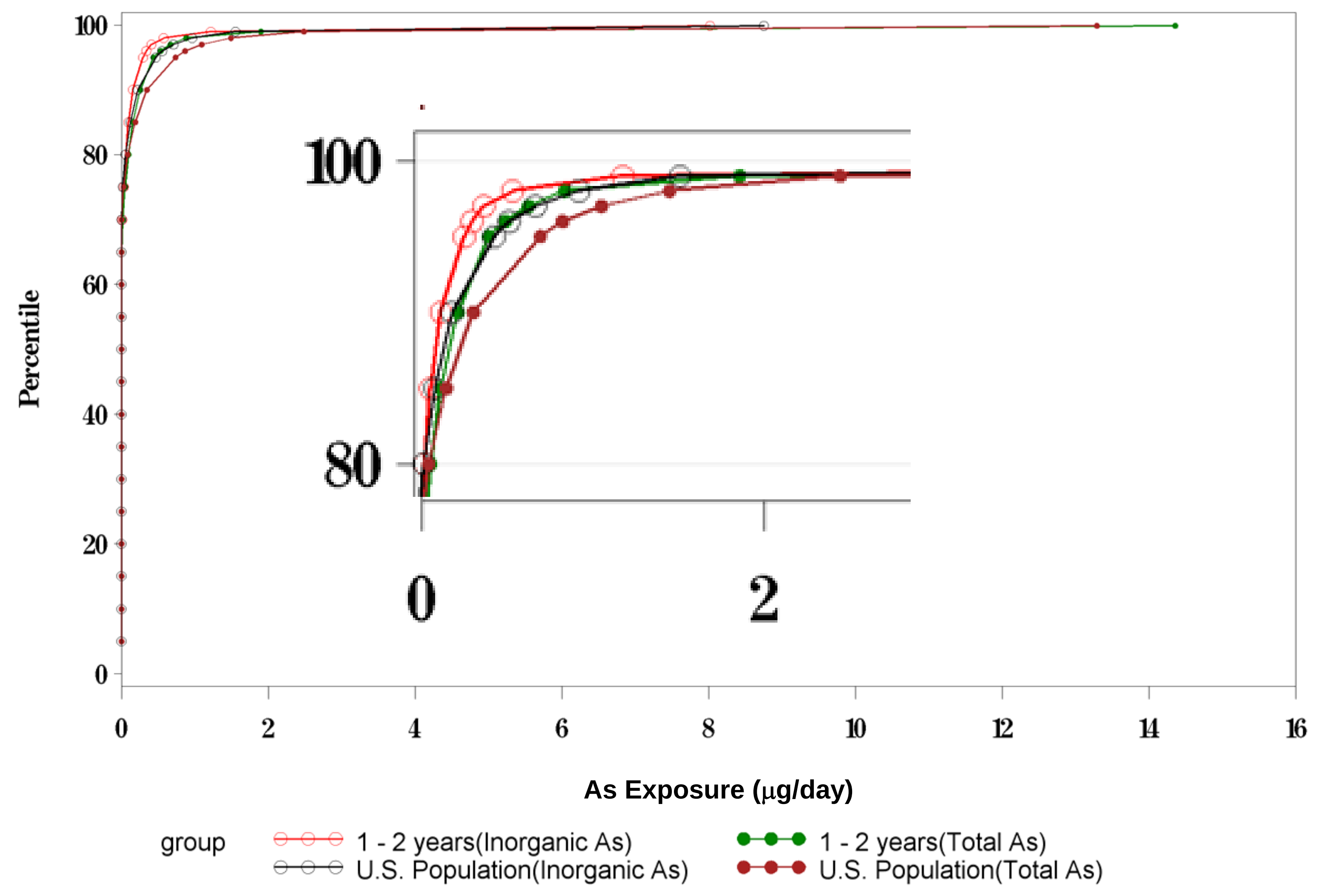


Stochastic Human Exposure and Dose Simulation

Population Based Exposure Assessment for Carrots

Population Based Exposure Assessment for Arsenic in Carrots



Risk Perspective on Population Based Exposure Assessment for Carrots

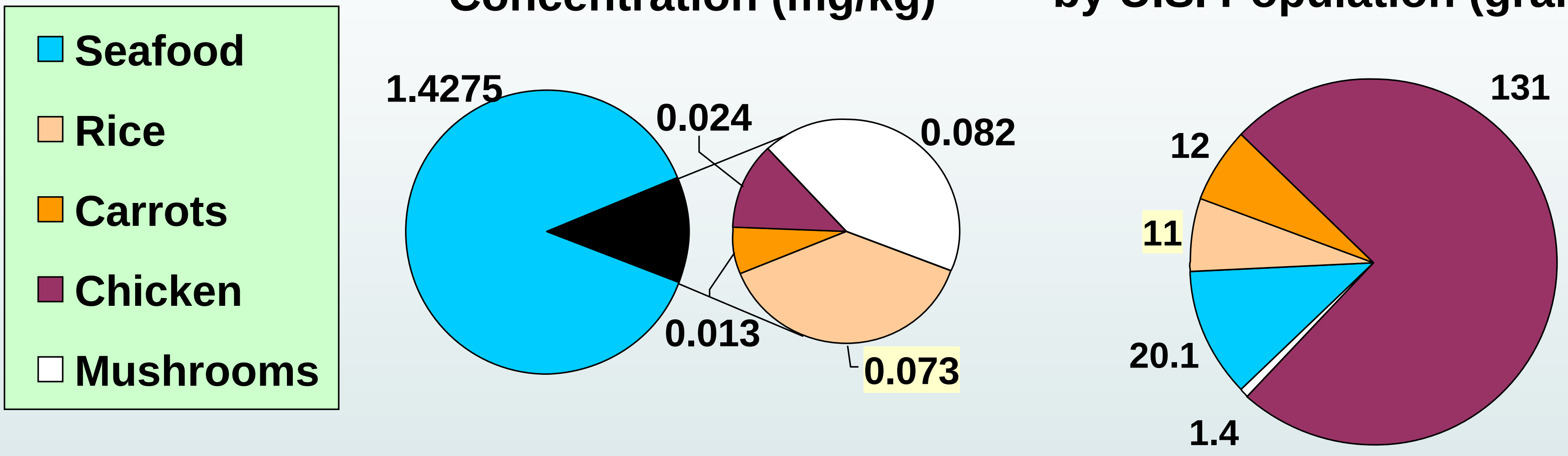
- Using the following estimates:
  - Oral slope factor of 1.5 per mg per kg bodyweight per day
  - 70 kg bodyweight
  - Mean consumption habit of 25 g per day
  - Mean inorganic As exposure of 0.11 μg per day from carrots

  The risk is estimated at 2.3 people in a million will develop cancer from ingesting carrots over an **entire** lifetime.
- Problems:
  1. Infant risk is difficult to assess given that the consumption habit per kg bodyweight is not held constant over a lifetime
  2. Difficulty in extending estimates for 75^{th} and 90^{th} percentiles because consumption habits vary from day to day. Bear in mind the WWEIA is a two day survey

Dietary Arsenic Exposure Assessment

Average Total Arsenic Concentration (mg/kg)

Average Daily Consumption by U.S. Population (grams)



Exposure = Concentration x Consumption

Use Rice as an Example

Exposure = Concentration x Consumption

Sampling Based on Harvest Demographics for Rice

Speciation α Toxicity

In vitro Assay "Bioaccessibility"

Freeze Dried Rice Samples

$As_{Total\ Digest}$ (As_{TD})

1. **Rehydrate with DDI**
2. **Add saline solution, 5M HCl and pepsin**
3. **Place in 37°C waterbath with platform agitator for 1 hour**

Rice Sample after Synthetic Stomach Extraction

1. **pH adjust to 6 with 1M $NaHCO_3$**
2. **Add pancreatin solution & 1M NaOH**
3. **Place in 37°C waterbath with platform agitator for 2 hours**

Rice Sample after Synthetic Intestine Extraction

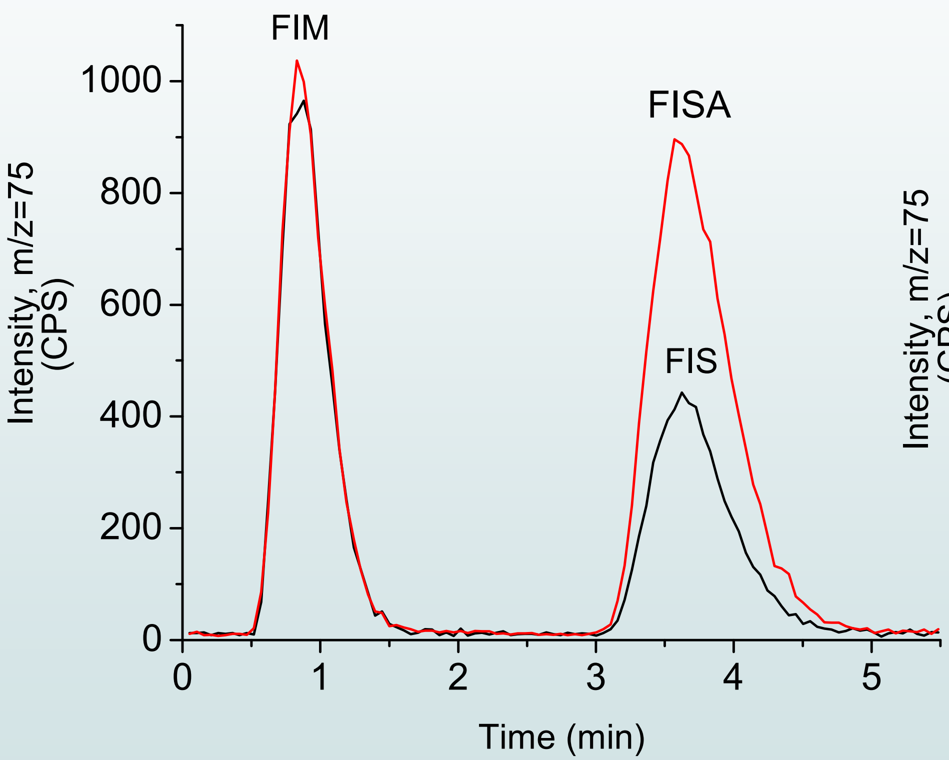
Enzymatic Rice Sample

LC-ICP-MS

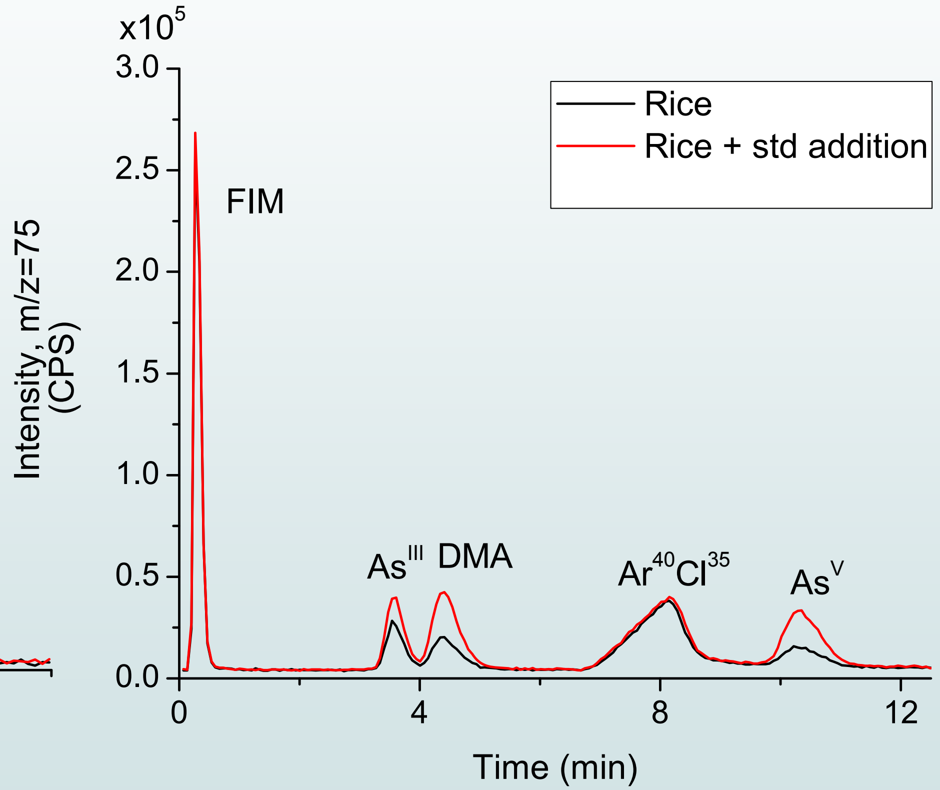
Collision Cell ICP-MS

Enzymatic Extraction of a Rice Sample (High Chloride)

Collision Cell ICP-MS



LC-ICP-MS



Estimating the species specific arsenic concentrations present in U.S. consumed rice with an emphasis on determining the bio-accessible component of the exposure

Rice	Rice Type	Total Digest (As_{TD}) (ng/g ± 2σ)	As Extraction Totals (As_{TE}) in an Enzymatically Extracted Rice Sample		Extraction Efficiency (% Rec ± 2σ) (Bioaccessibility Estimate)	As Speciation (As_{Chrom}) in an Enzymatically Extracted Rice Sample					Chromatographic Recovery (% Rec ± 2σ)	Overall Recovery (% Rec ± 2σ)
						Inorganic As (As_{Ing})		DMA		Sum of Speciation ($As_{\Sigma Chrom}$)		
			Conc (ng/g ± 2σ)	LFM (% Rec)		Conc (ng/g ± 2σ)	LFM (% Rec)	Conc (ng/g ± 2σ)	LFM (% Rec)	Conc (ng/g)		
1	W-LG	230 ± 14.7	126 ± 15.4	83	55 ± 6.7	72.1 ± 15.4	85	36.8 ± 11.1	92	109	92 ± 10.8	47 ± 2.6
2	B-MG	131 ± 56.0	76.2 ± 3.2	91	58 ± 2.5	71.0 ± 8.1	66	11.1 ± 2.0	99	82.1	102 ± 20.1	63 ± 13.9
3	B-MG	237 ± 24.3	152 ± 14.4	116	64 ± 6.1	119 ± 18.5	91	36.1 ± 15.0	93	155	96 ± 7.6	65 ± 12.7
4	W	81.0 ± 19.4	48.2 ± 10.7	94	59 ± 13	37.2 ± 7.8	90	15.7 ± 3.7	94	52.8	102 ± 9.0	65 ± 12.2
5	B-LG	279 ± 17.9	165 ± 23.4	75	59 ± 8.4	117 ± 13.9	78	39.8 ± 8.3	99	157	87 ± 2.4	56 ± 7.9
6	B-LG	282 ± 38.6	165 ± 10.1	90	59 ± 8.4	128 ± 5.1	83	26.4 ± 2.1	93	154	88 ± 7.3	55 ± 2.3
7	W	103 ± 31.7	46.5 ± 2.9	101	45 ± 3.6	44.1 ± 7.0	89	9.8 ± 1.3	96	53.9	95 ± 10.5	53 ± 7.1
8	B-LG	264 ± 29.6	162 ± 12.4	73	61 ± 4.7	132 ± 21.2	91	29.2 ± 2.0	102	161	89 ± 11.5	61 ± 8.6
9	W-LG	313 ± 3.6	180 ± 26.6	82	58 ± 8.5	56.3 ± 4.8	88	116 ± 12.3	81	172	85 ± 10.0	55 ± 2.6
10	W-LG	144 ± 30.7	74.1 ± 17.0	80	51 ± 12	60.2 ± 1.0	76	24.8 ± 10.4	91	85.0	99 ± 14.6	59 ± 7.8
11	B-LG	107 ± 21.1	64.0 ± 17.9	93	60 ± 17	48.0 ± 7.4	91	10.8 ± 5.5	97	58.7	80 ± 23.0	55 ± 12.0
12	B	120 ± 29.6	103 ± 34.4	81	86 ± 29	42.1 ± 4.7	82	26.9 ± 1.6	96	69.0	73 ± 18.8	57 ± 5.3
13	W	248 ± 2.8	133 ± 5.9	98	54 ± 2.4	66.8 ± 5.3	75	53.1 ± 3.0	101	120	80 ± 7.9	48 ± 2.6
14	W	213 ± 25.2	136 ± 31.3	81	64 ± 15	81.3 ± 10.7	86	45.7 ± 9.2	98	127	85 ± 23.6	60 ± 6.7
15	W-MG	259 ± 54.9	172 ± 7.5	123	66 ± 2.9	63.4 ± 12.8	102	59.8 ± 34.8	90	123	79 ± 18.4	48 ± 15.3
16	W	294 ± 98.0	233 ± 28.9	101	76 ± 9.8	131 ± 15.0	86	83.8 ± 16.7	102	215	91 ± 12.1	73 ± 7.6
17	W	242 ± 22.0	159 ± 43.8	100	66 ± 18	106 ± 18.9	67	76.4 ± 7.6	93	183	104 ± 28.0	75 ± 6.7
Across Matrix Avg ± 2σ		209 ± 153	129 ± 106	92 ± 27.7	62 ± 19.3	80.9 ± 67.7	84 ± 18.5	41.3 ± 58.2	95 ± 10.4	122 ± 99.0	90 ± 18.0	59 ± 16.1

Exposure = Concentration x Consumption

Sampling Based on Harvest Demographics for Rice

↓

Speciation α Toxicity

↓

In vitro Assay "Bioaccessibility"

↓

80.9 ng/g ± 67.7

- What We Eat In America (WWEIA), NHANES 2005-2006, considers 13,000 commonly eaten foods in US.
- 52,653 participants in the survey provided information on their food consumption.

Stochastic Human Exposure and Dose Simulation

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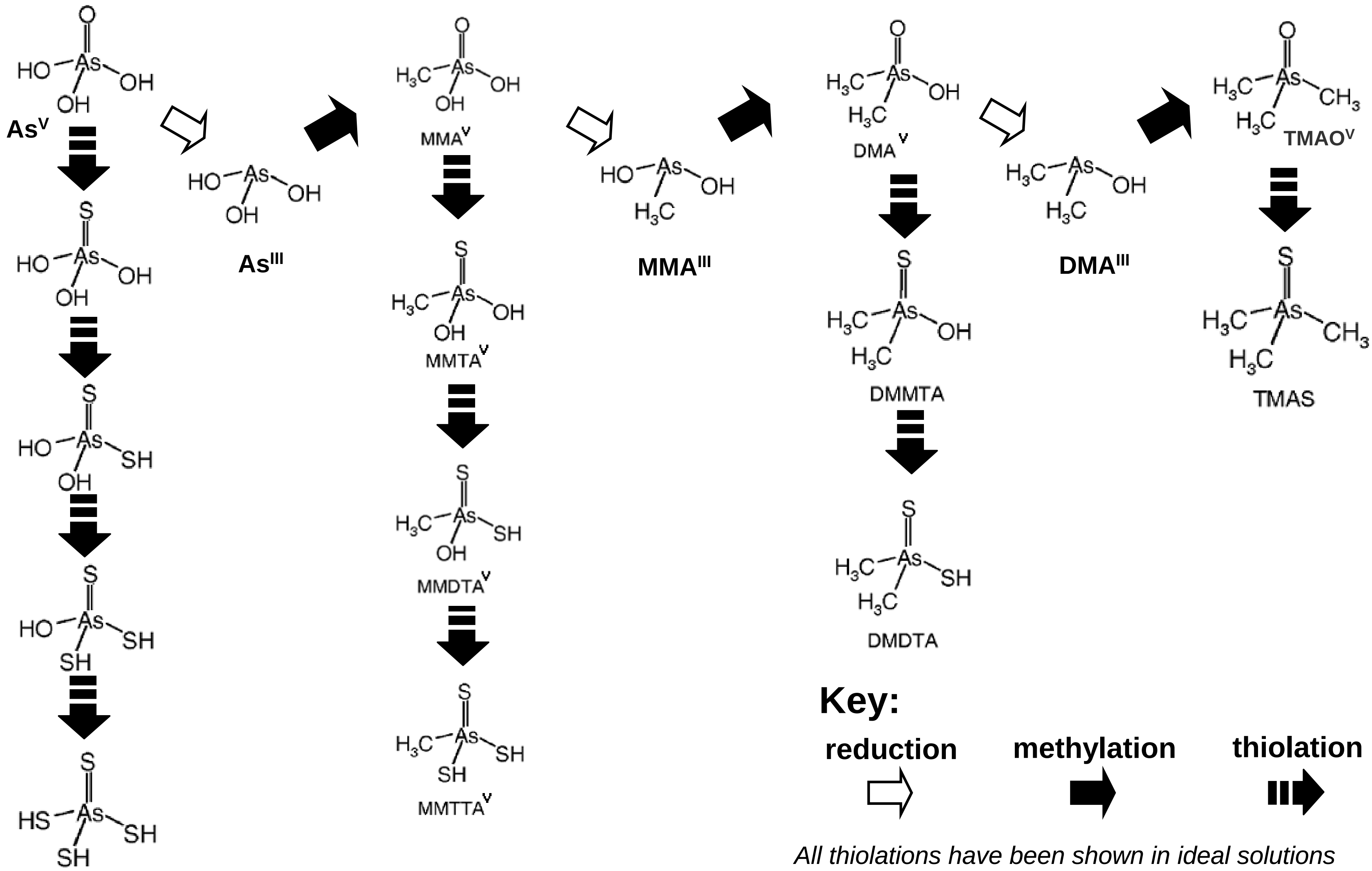
Population Based Exposure Assessment for Rice

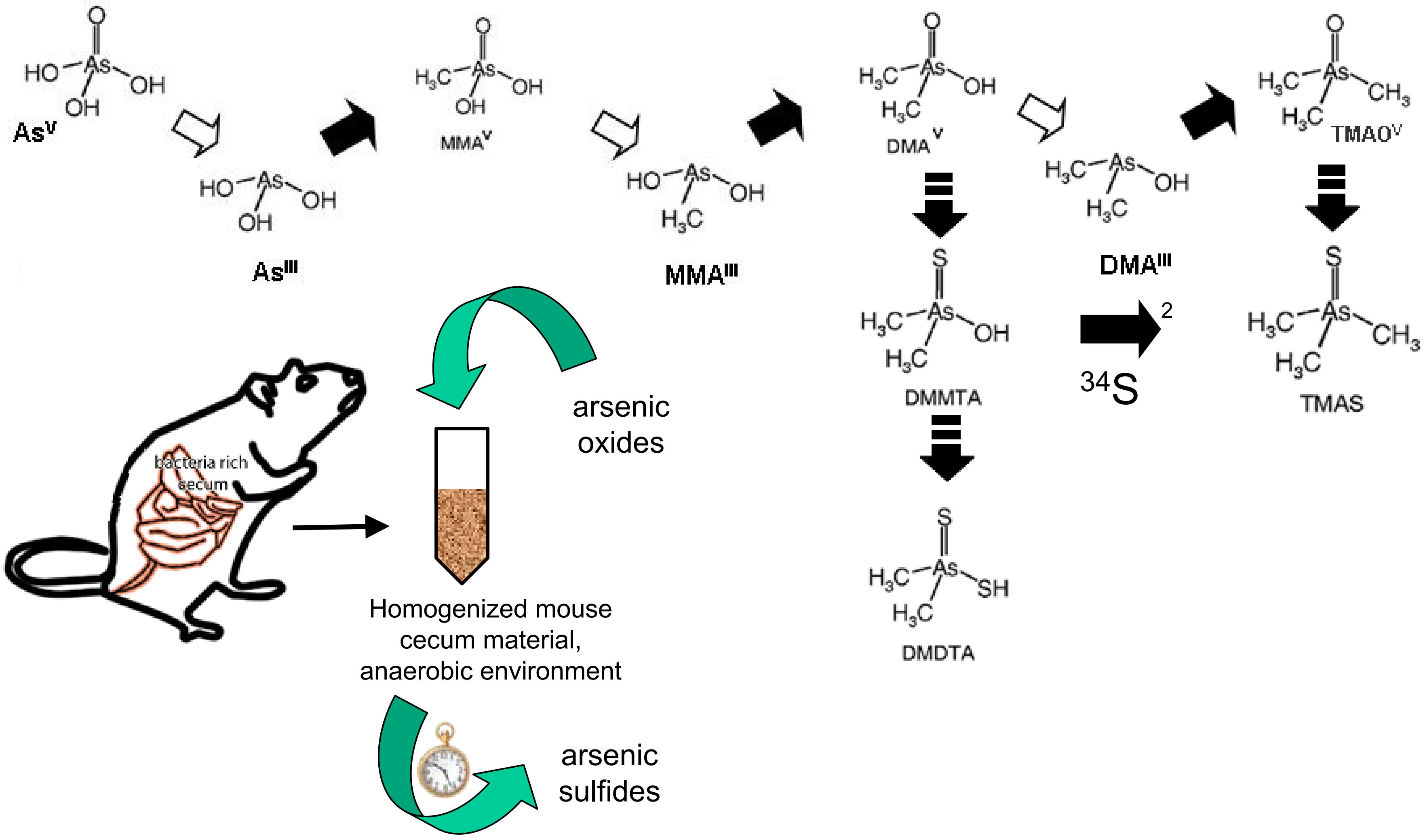
Exposure Assessment and Presystemic Biotransformation of Arsenic Oxides

Introduction: Challenger Pathway



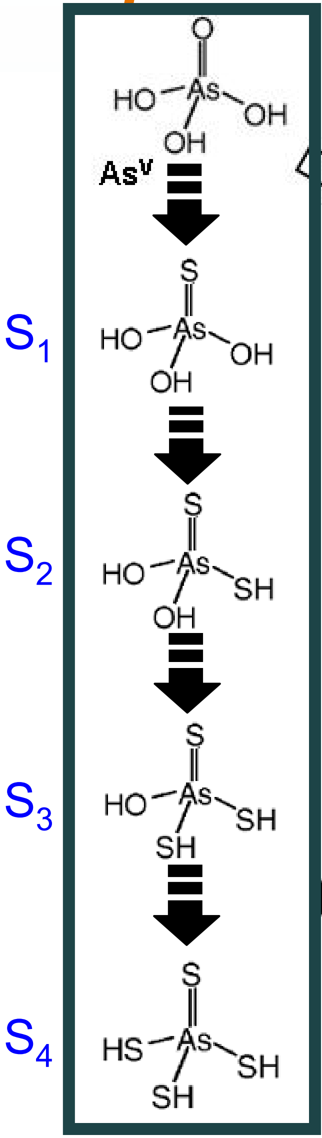
All thiolations have been shown in ideal solutions

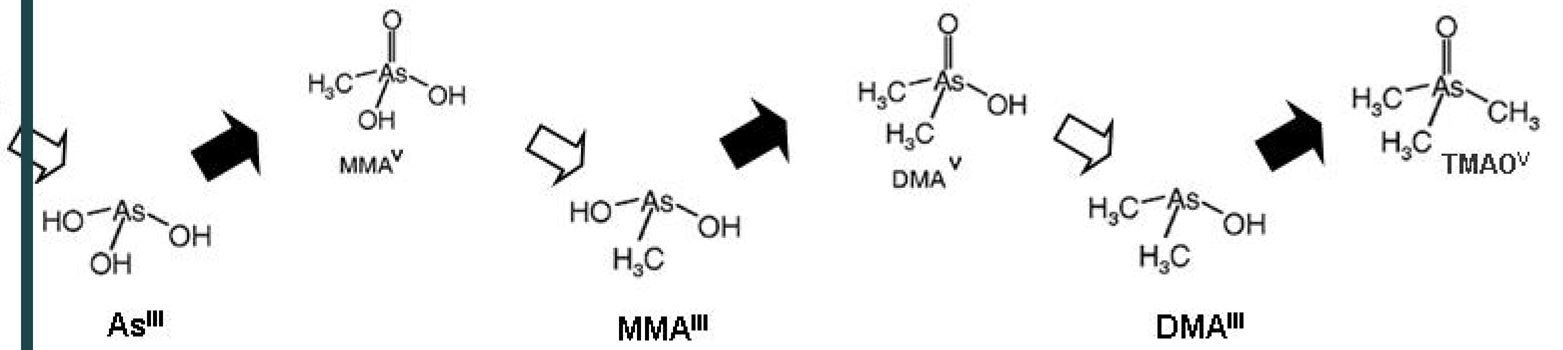
In Vitro Biotransformation of Arsenic Oxides

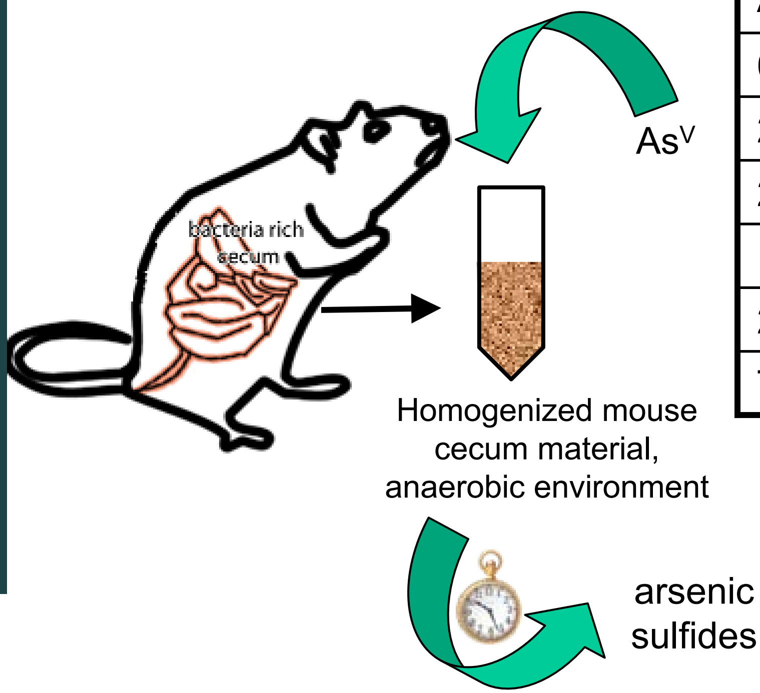


1) Kubachka et al., *J. Anal. At. Spectrom.*, **2009**, *24*, 1062-1068
2). Kubachka et.al., *Toxic Appl Pharm*, **2009**, 239,137-143

Expanding arsenic oxides to include inorganic arsenic







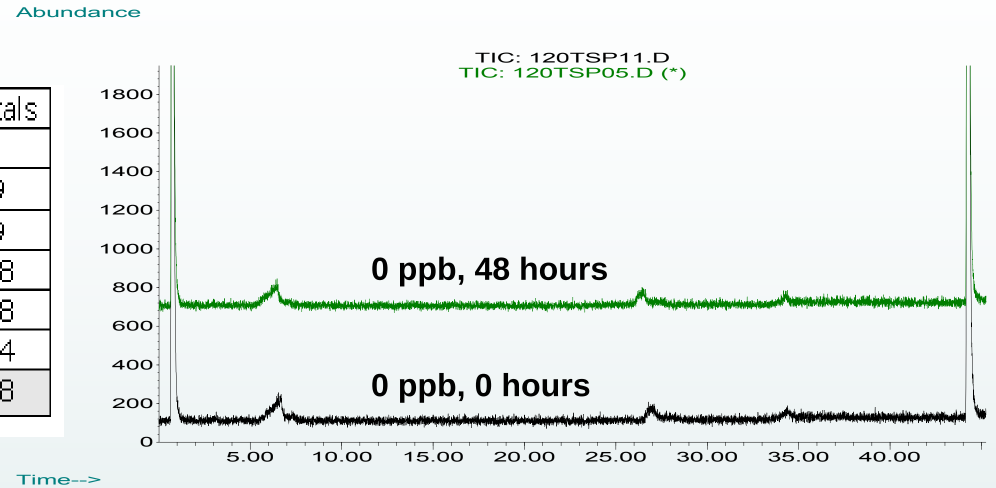
	Hours at 37°C						Totals
As, ppb	0	3	6	12	24	48	
0	3	-	-	-	3	3	9
20	3	-	-	-	3	3	9
200	3	3	3	3	3	3	18
1000	3	3	3	3	3	3	18
2000	6	3	3	3	3	6	24
Totals	18	9	9	9	15	18	78

Key:

reduction ⇨ **methylation** ➡ **thiolation** ▮▮➡

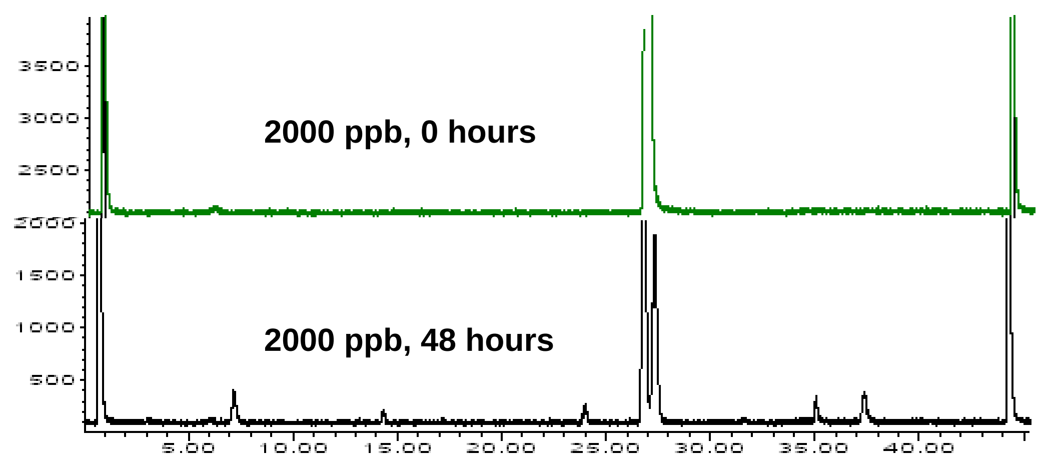
Incubated Bacterial Cultures

	Hours at 37°C						Totals
As, ppb	0	3	6	12	24	48	
0	3	—	—	—	3	3	9
20	3	—	—	—	3	3	9
200	3	3	3	3	3	3	18
1000	3	3	3	3	3	3	18
2000	6	3	3	3	3	6	24
Totals	18	9	9	9	15	18	78

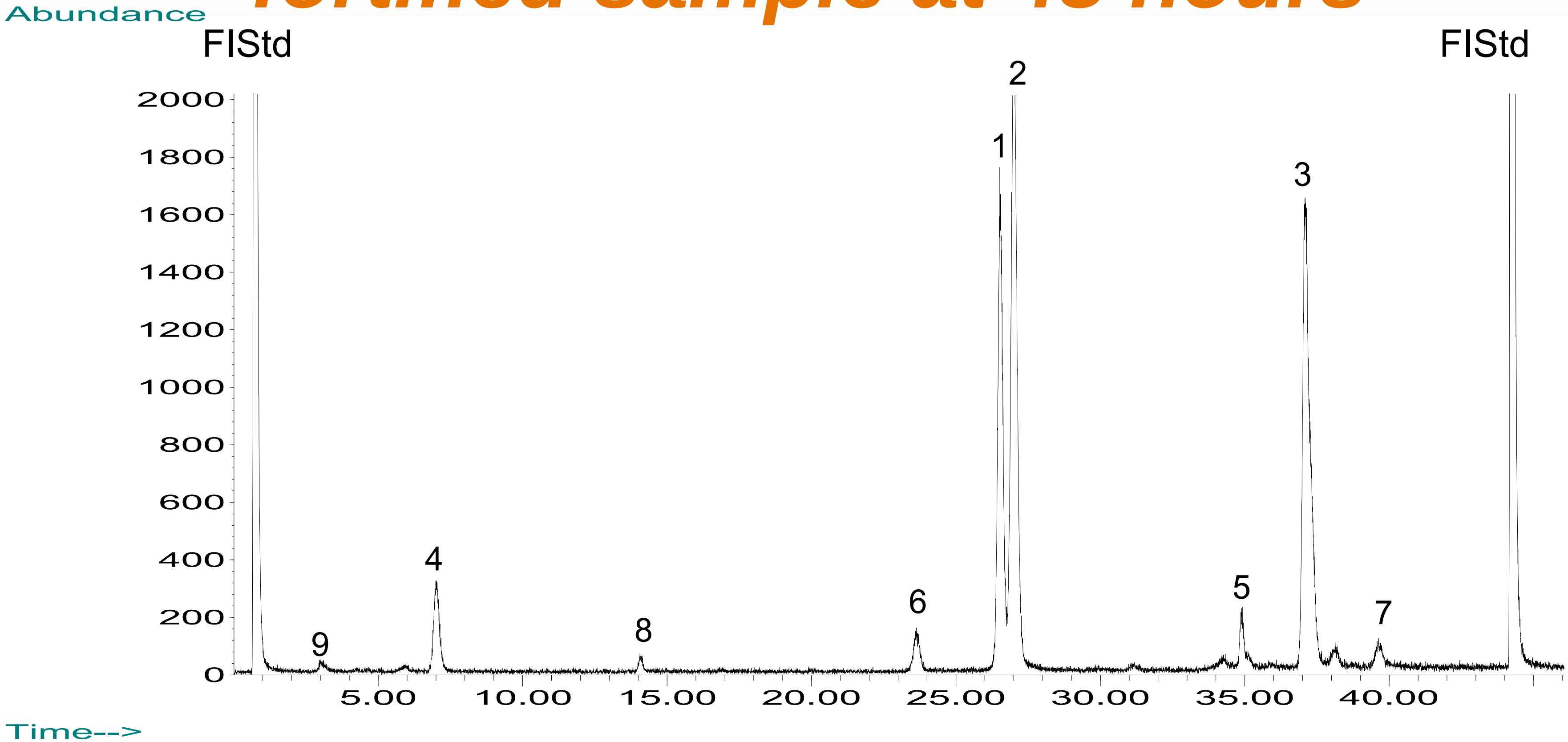


- Insignificant amount of As^{V} in cecal contents

	Hours at 37°C						Totals
As, ppb	0	3	6	12	24	48	
0	3	—	—	—	3	3	9
20	3	—	—	—	3	3	9
200	3	3	3	3	3	3	18
1000	3	3	3	3	3	3	18
2000	6	3	3	3	3	6	24
Totals	18	9	9	9	15	18	78



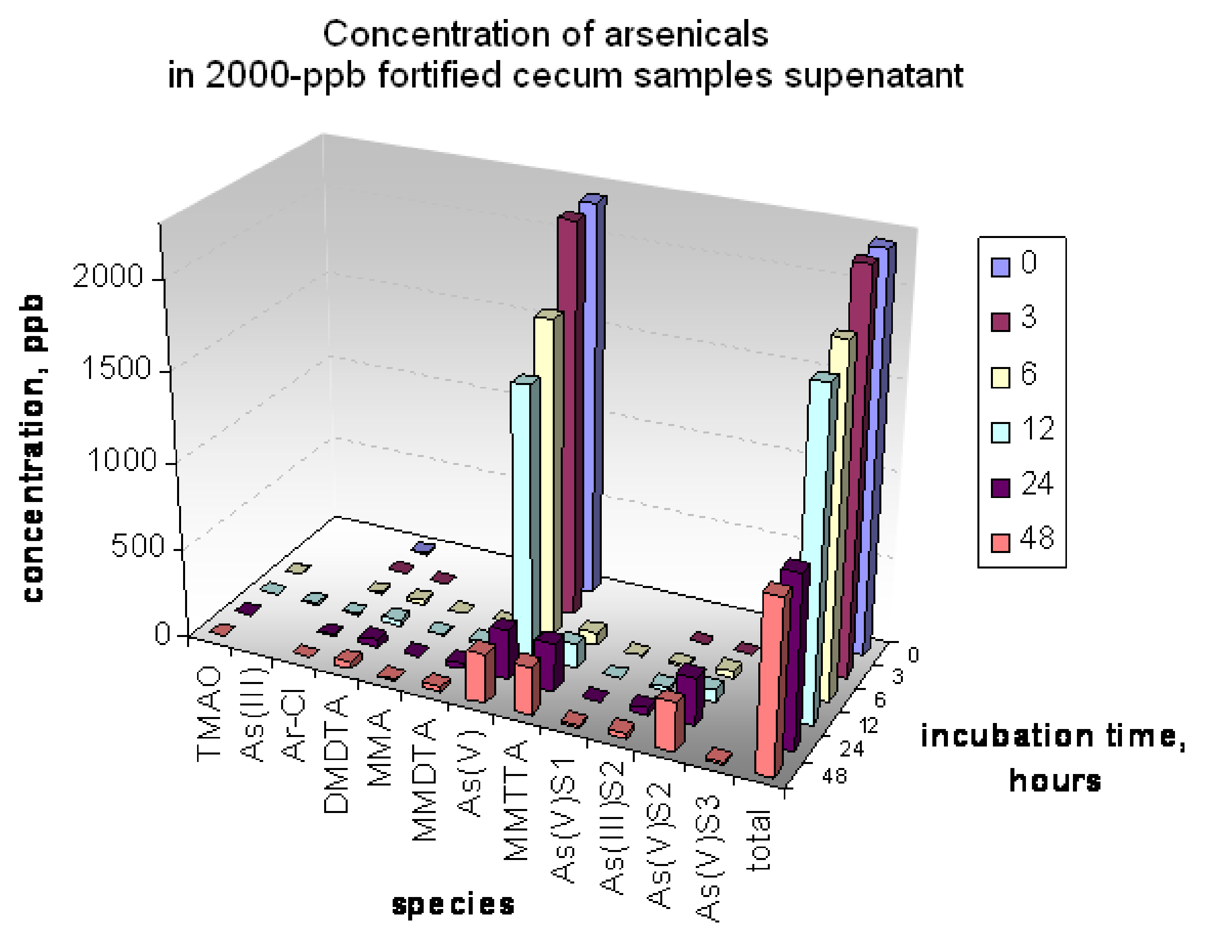
As^{V} metabolites found in 2000 ppb fortified sample at 48 hours



1. Unchanged As^{V}
2. MMTTA
3. $As^{V}O_2S_2H_3$
4. DMDTA
5. $As^{III}OS_2H_3$
6. MMDTA
7. $As^{V}OS_3H_3$
8. MMA
9. TMAO (or small proteins)

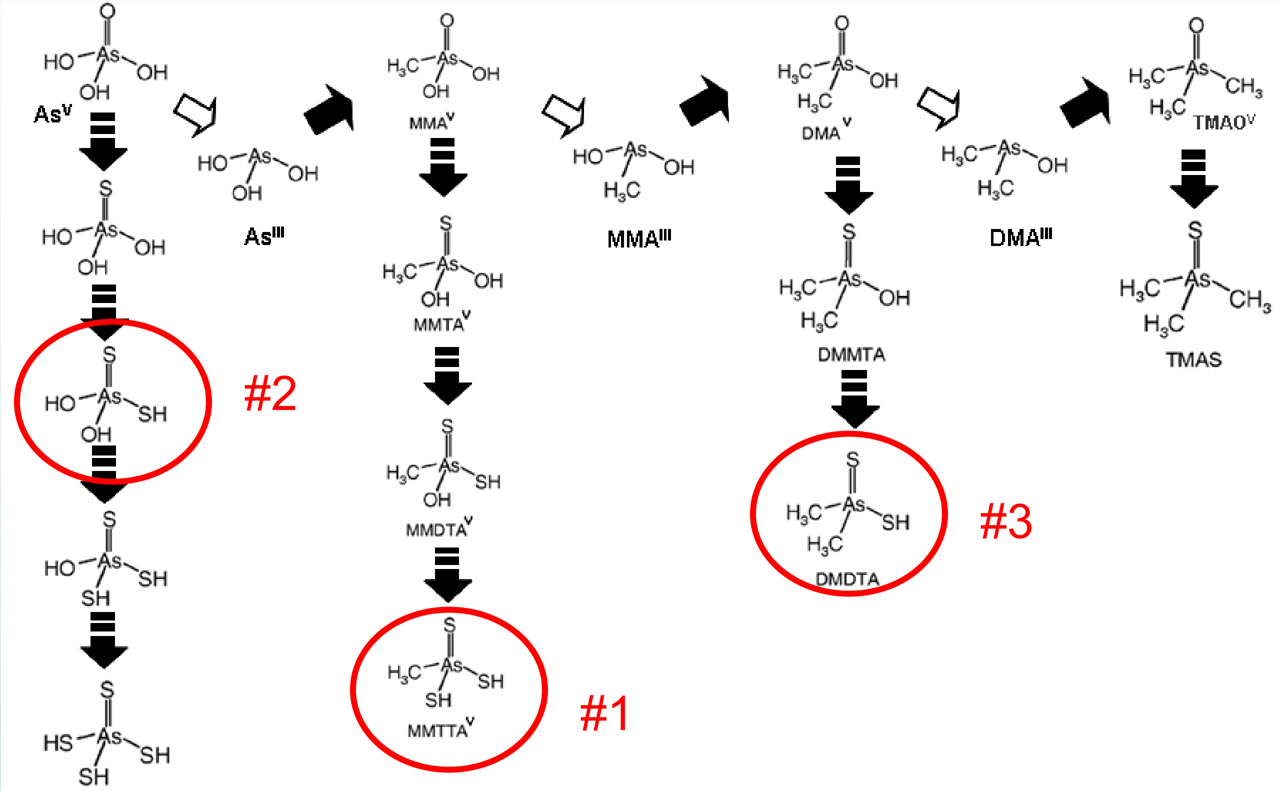
Arsenic oxide to sulfide conversion as a function of incubation time

	Hours at 37°C						Totals
As, ppb	0	3	6	12	24	48	
0	3	-	-	-	3	3	9
20	3	-	-	-	3	3	9
200	3	3	3	3	3	3	18
1000	3	3	3	3	3	3	18
2000	6	3	3	3	3	6	24
Totals	18	9	9	9	15	18	78



Conclusions

- Major fraction of chromatographable arsenicals in the cecum samples is comprised of unchanged As(V)
- Most abundant metabolites at 48 hours:



- The bacteria in the cecum are capable of thiolating inorganic arsenic