United States Environmental Protection Agency

# Analysis of arsenicals and their sulfur analogs using HPLC with collision cell ICP-MS and ESI-MS/MS.

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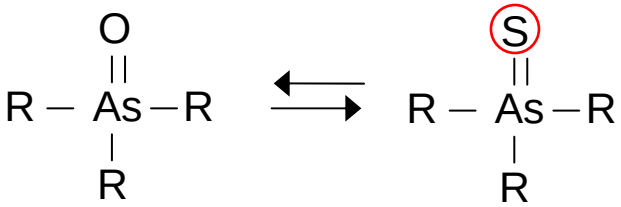
# Introduction - Arsenic

- Because inorganic arsenic is classified as a carcinogen, it has a Maximum Contaminant Level Goal (MCLG) of zero under the Safe Drinking Water Act.
- Potential health risks include:
  - Cancer
    - Lung
    - Bladder
    - Skin
    - Kidney
    - Liver
  - Health Effects
    - Cardiovascular
    - Pulmonary
    - Neurological
    - Endocrine
- Biotransformation of arsenicals may influence how arsenic expresses its toxicity.

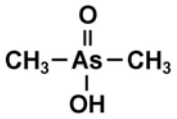
## Basic Terminology of Arsenicals and Thiolated Analogs of Interest



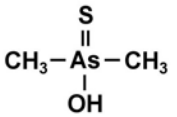

R = $CH_3$, OH, or S

Example:

Dimethylarsinic acid ($DMA^V$)




Dimethylthioarsinic acid ($DMTA^V$)



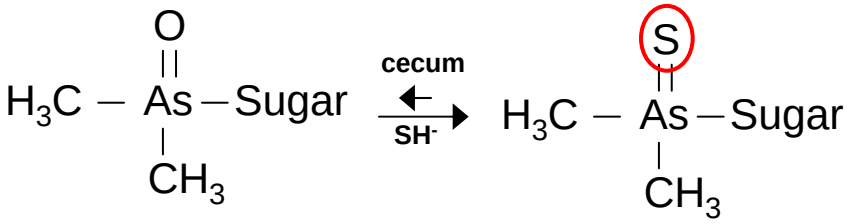
# Introduction – Thioarsenicals

- Arsenic sulfides have been found as metabolites:
  - $DMTA^{III}$ in rat liver[1], $DMTA^{V}$ in sheep wool[2] and human urine[3,4], and $DMTA^{V}$ and TMAS in mouse urine[5].
  - Inorganic As-S and mono- and di-methyl As-S in sulfidic waters[6].
- Specifically, $DMTA^{V}$ has genotoxic[7] and cytotoxic[8] properties greater than $DMA^{V}$ and similar to that of $DMA^{III}$.


1) Suzuki et al., (2004), *Chem. Res. Toxicol*., 17, 914-921.
2) Hansen et al., (2004), *Chem. Res. Toxicol*., 17, 1086-1091.
3) Raml et al., (2005), *Chem. Res. Toxicol.*, 18, 1444-1450.
4) Raml e. al., (2007), *Tox. Ap. Pharm*., 222, 374-380.
5) Hughes et al., (2007), *Tox. Ap. Pharm*., 227, 26-35.
6) Wallschlager et al., (2008), *Env. Sci. Tech.*, 42, 228-234
7) Kuroda et al., (2004), *Tox. Ap. Pharm., 198*, 345-353.
8) Naranmandura et al., (2007) *Chem. Res. Toxicol., 20*, 1120-1125.

# Introduction – Thioarsenicals

- Arsenic oxides can be biotransformed in the cecum of a mouse in anaerobic environment to form arsenic sulfides[1].




- Can this thiolation be generalized to other arsenic oxides?
  - For Example:
    - $AsO_4$ ($As^V$) → $AsO_3S$ → $AsO_2S_2$ → $AsOS_3$ → $AsS_4$
    - MMA → MMTA → MMDTA → MMTTA
    - DMA → DMTA → DMDTA
    - TMAO → TMAS

1) Conklin et al., (2006), *The Analyst*, 131, 648-655

| $TMAO^V$ | $DMA^V$ | $MMA^V$ | $As^V$ |
|---|---|---|---|
| $(CH_3)_3AsO$ | $(CH_3)_2AsO_2^-$ | $CH_3AsO_3^{-2}$ | $AsO_4^{-3}$ |
| $TMAS^V$ | $DMMTA^V$ | $MMMTA^V$ | MTAs |
| $(CH_3)_3AsS$ | $(CH_3)_2AsOS^-$ | $CH_3AsO_2S^{-2}$ | $AsO_3S^{-3}$ |
| | $DMDTA^V$ | $MMDTA^V$ | DTAs |
| | $(CH_3)_2AsS_2^-$ | $CH_3AsOS_2^{-2}$ | $AsO_2S_2^{-3}$ |
| | | $MMTTA^V$ | TTAs |
| | | $CH_3AsS_3^{-2}$ | $AsOS_3^{-3}$ |
| | | | TeTAs |
| | | | $AsS_4^{-3}$ |

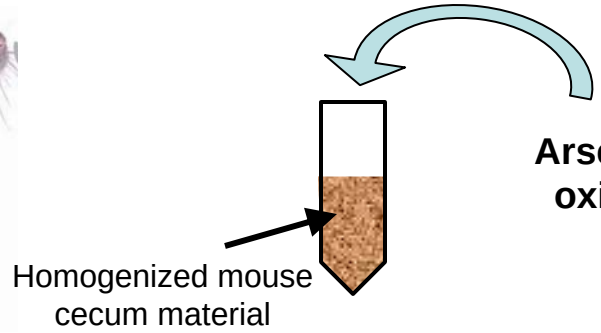
THIOLATION

# Why study mouse cecum?

- Mimics actions of microflora bacteria present in gastrointestinal tract.
- Changes exhibited by cecum material represent those that occur prior to systemic uptake.
- One of first significant areas of biotransformation in the body.
- Easy to collect from mouse – large area (larger than in humans).
- Cost effective.

# Experimental Design

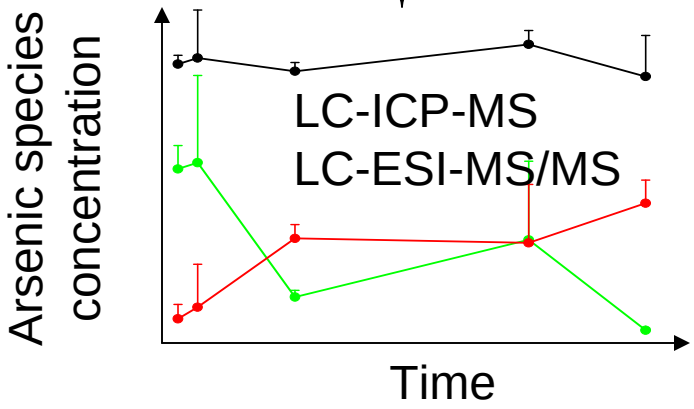
Harvest cecal contents




**Arsenic oxide**

Study arsenic species formation as a function of time

**Anaerobic environment**



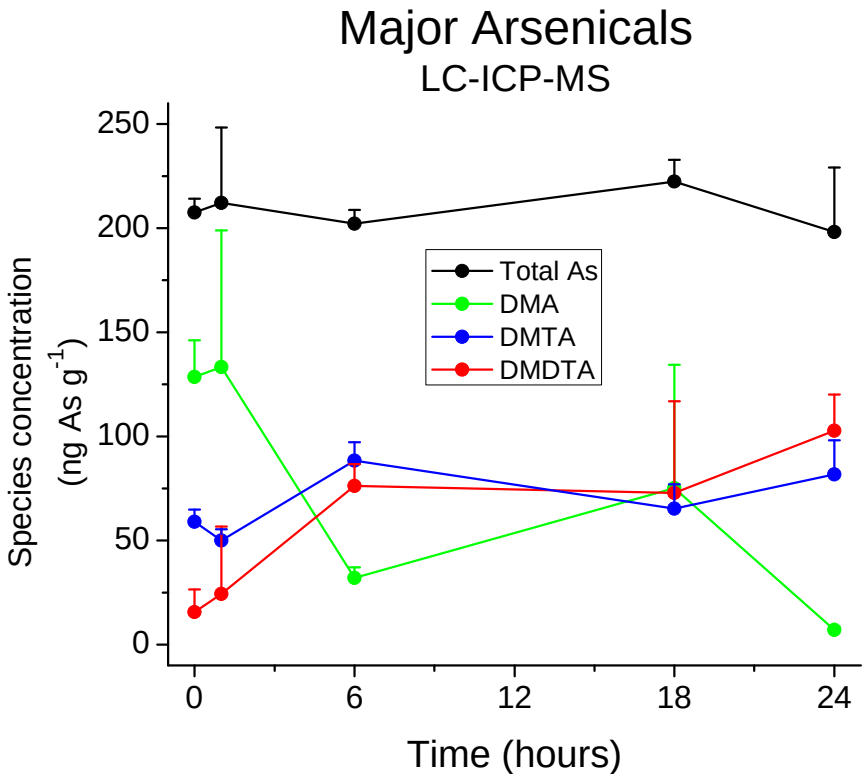

$DMA^V$

$DMTA^V$

DMDTA

# Time Dependent Arsenic Species Distribution

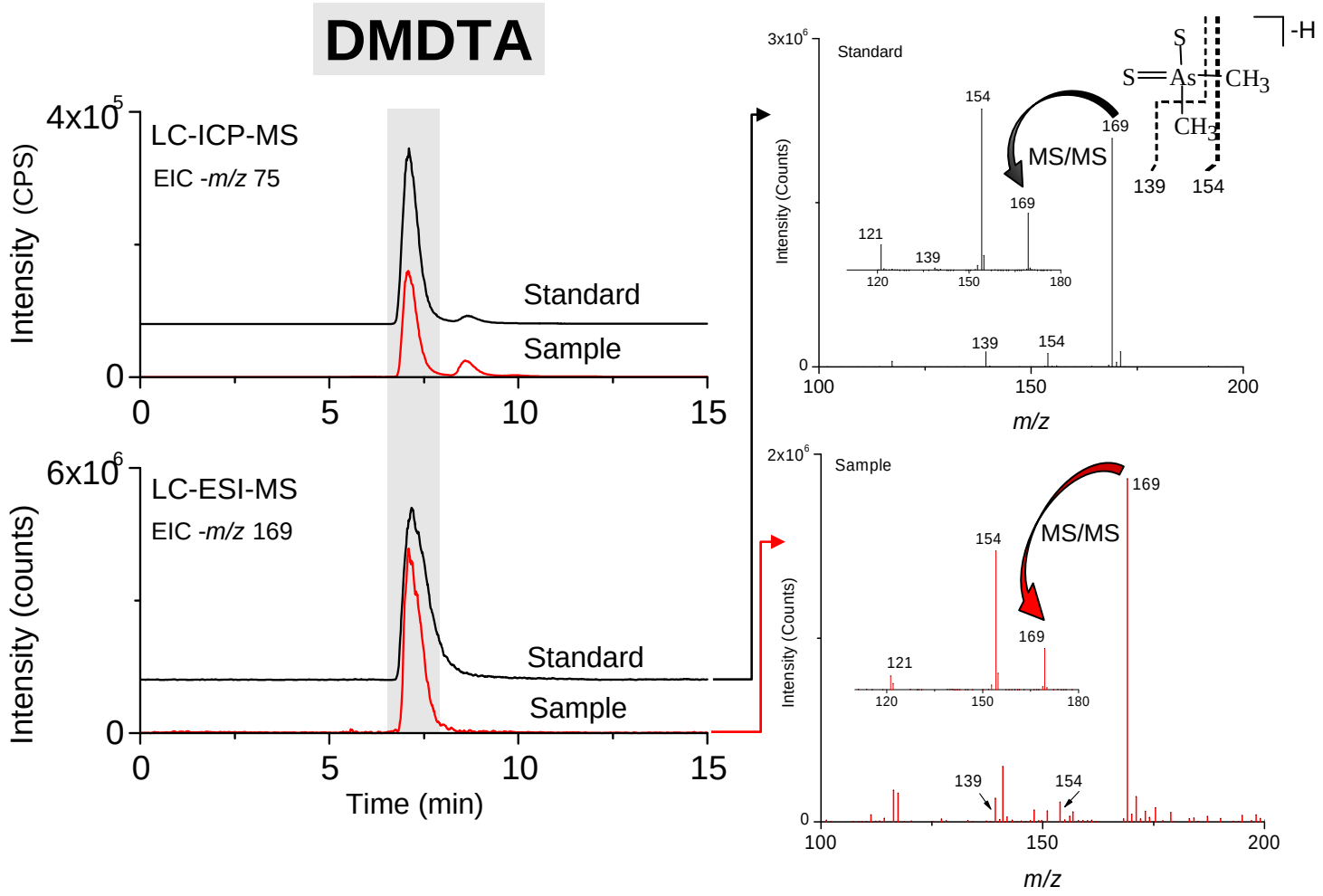
Cecum supplemented with $DMA^V$ (200 ng As $g^{-1}$)




DMA → DMTA → DMDTA

DMTA and DMDTA were confirmed with ESI-MS and MS/MS

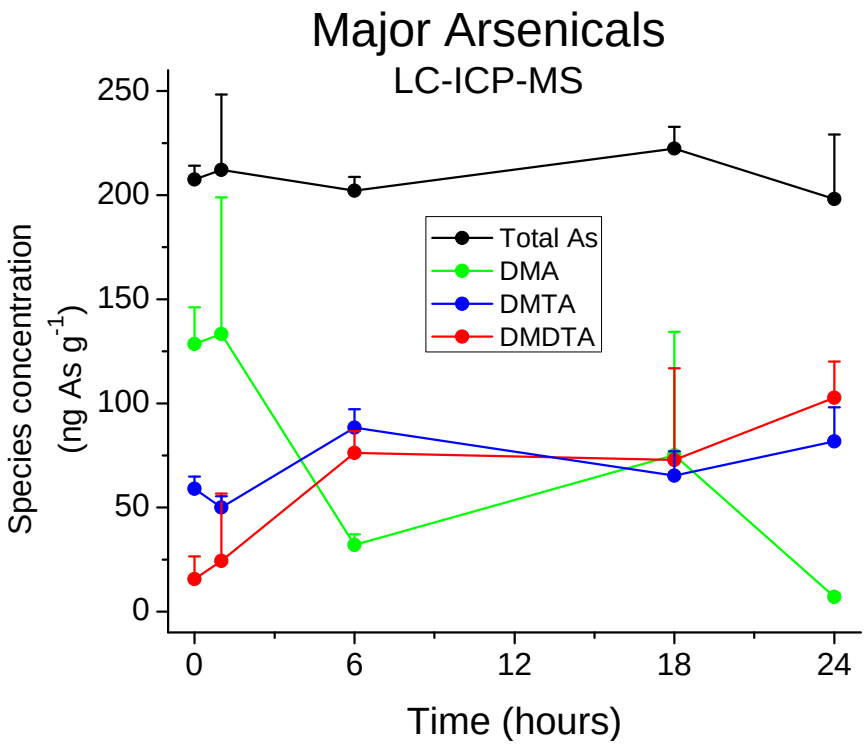
# Confirmation of DMDTA using ESI-MS/MS

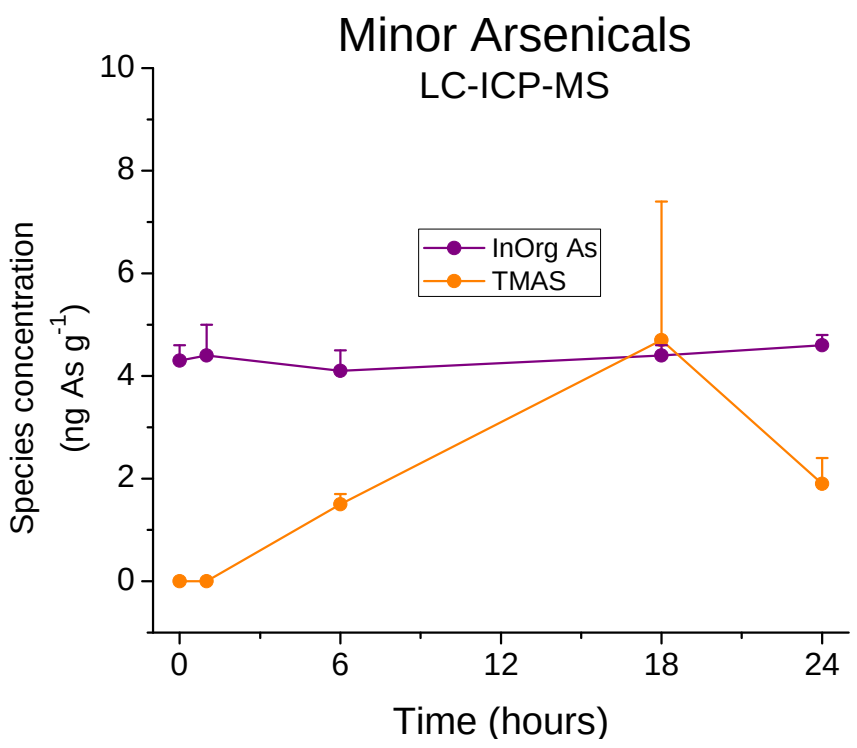



**DMTA was confirmed with ESI-MS and MS/MS previously by our group (not shown here)**

# Time Dependent Arsenic Species Distribution

Cecum supplemented with $DMA^V$ (200 ng As $g^{-1}$)



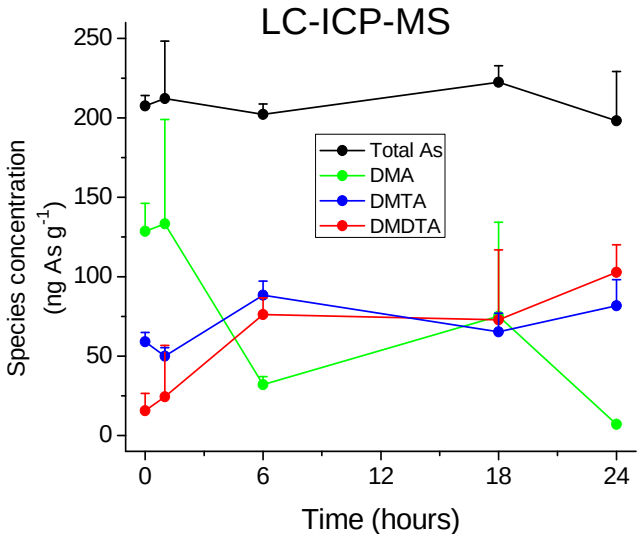



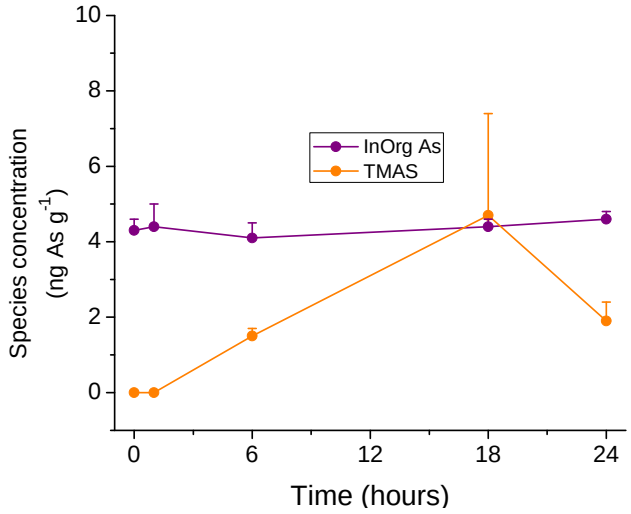

DMA → DMTA → DMDTA

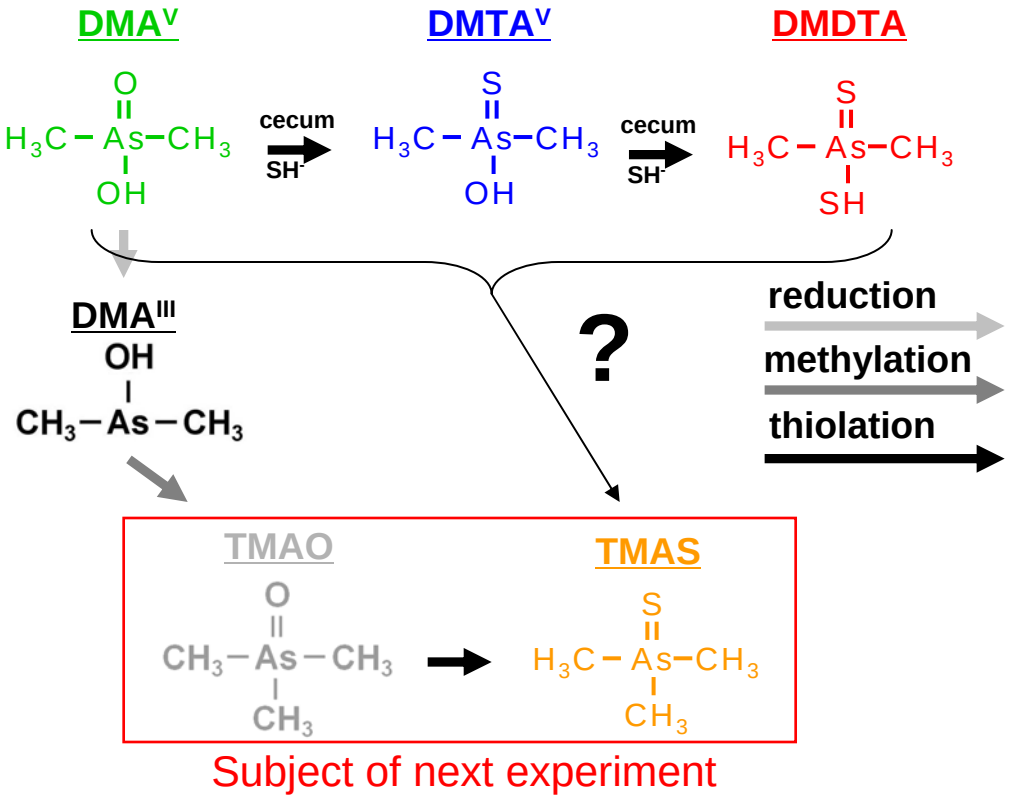
DMA $\xrightarrow{?}$ TMAS

DMTA, DMDTA, and TMAS were confirmed with ESI-MS and MS/MS

# Mechanistic implications of $DMA^V$ supplementation

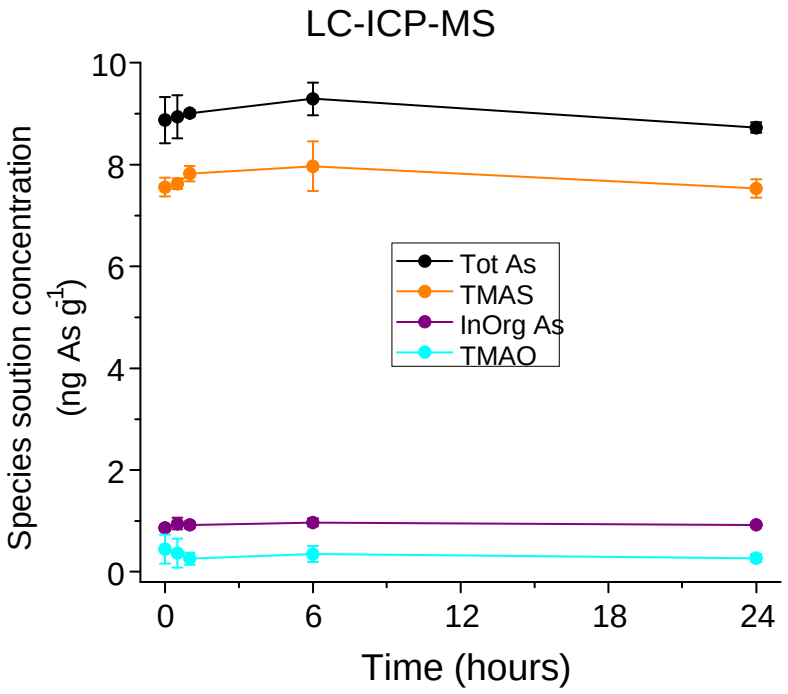


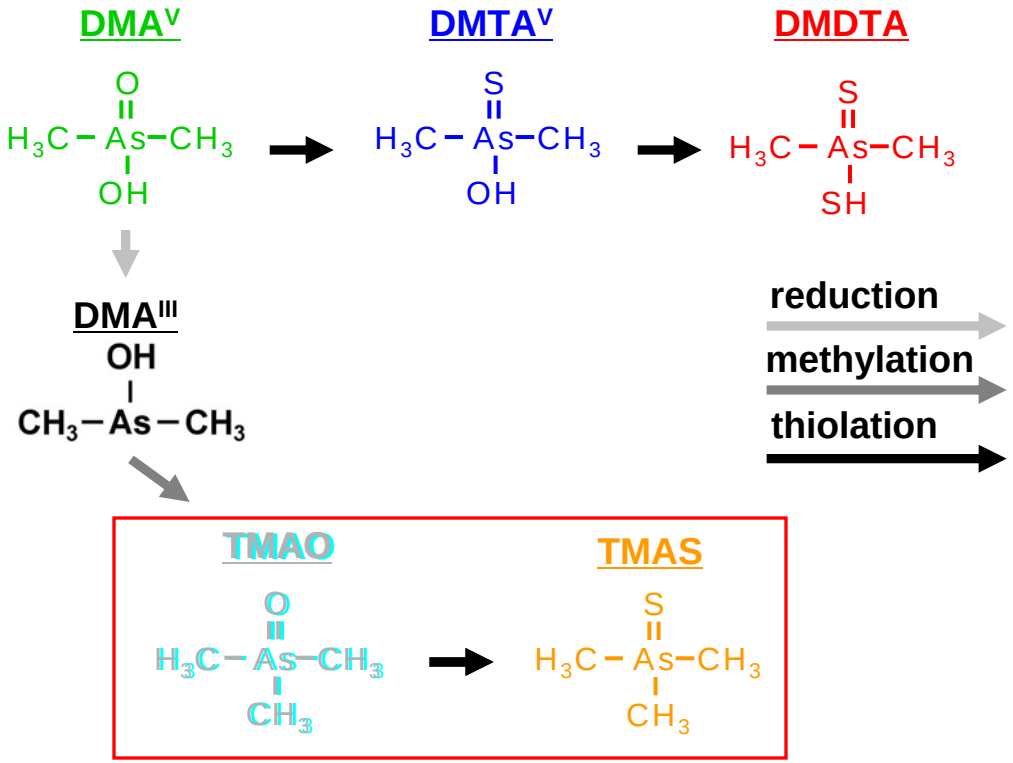



# Time Dependent Arsenic Species Distribution

Cecum supplemented with TMAO (20 ng As $g^{-1}$)

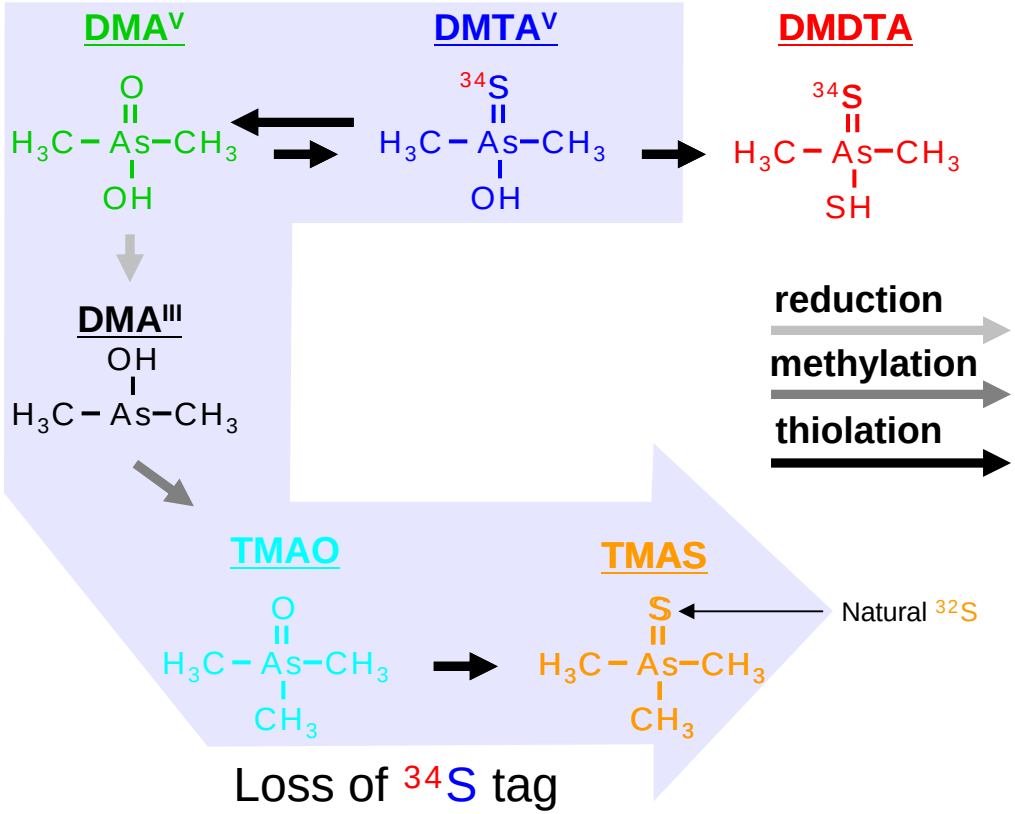



*Similar trend for 200 and 1000 ng As $g^{-1}$ supplementation

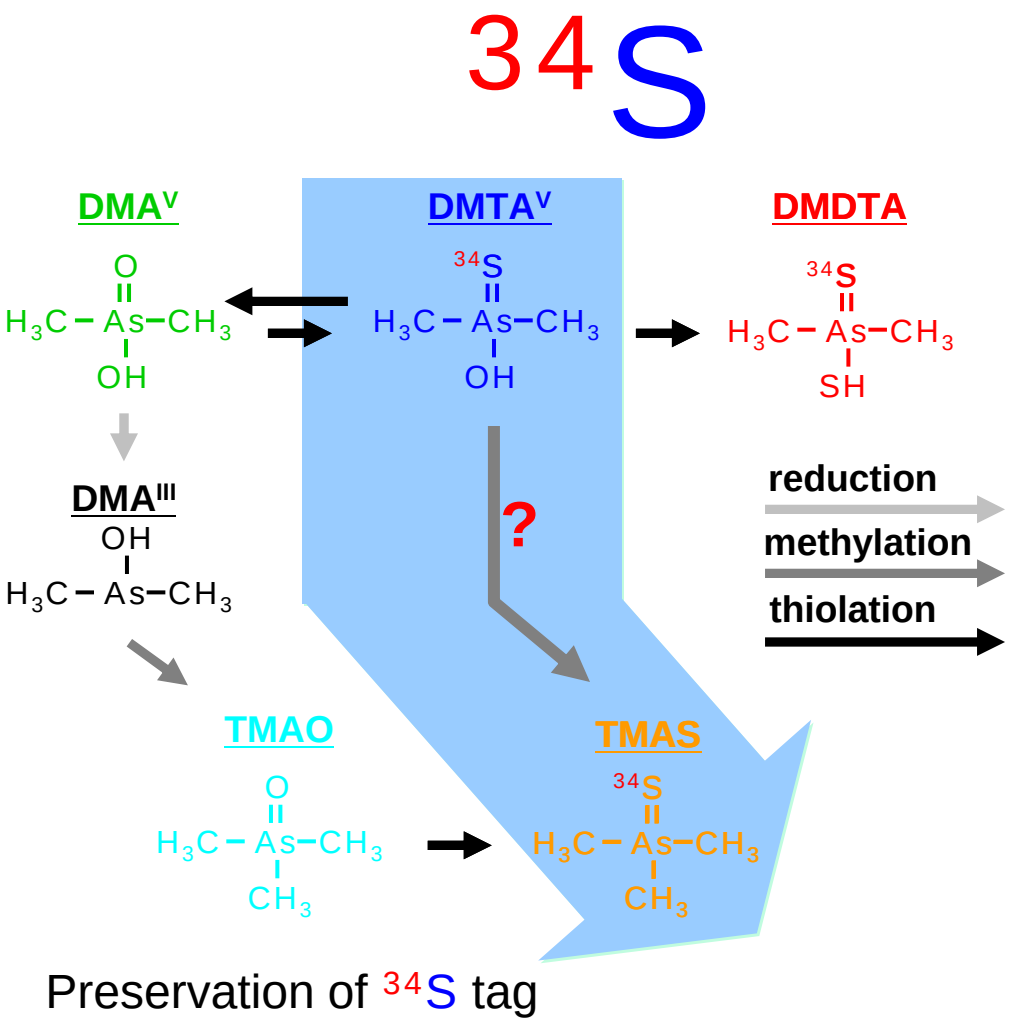


# How is TMAS formed?

$^{34}S$



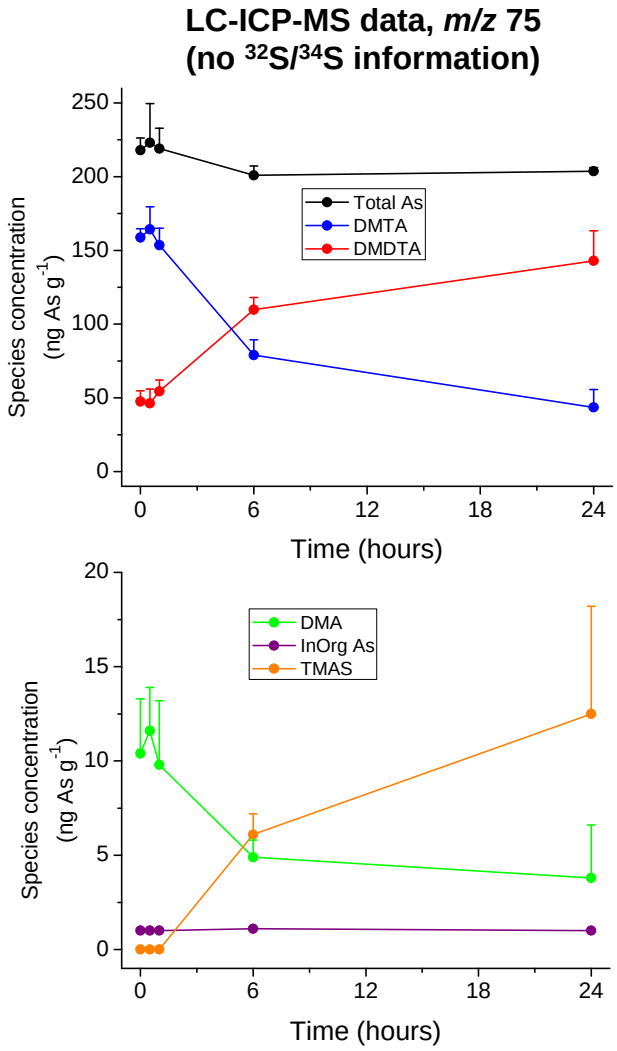
# How is TMAS formed?

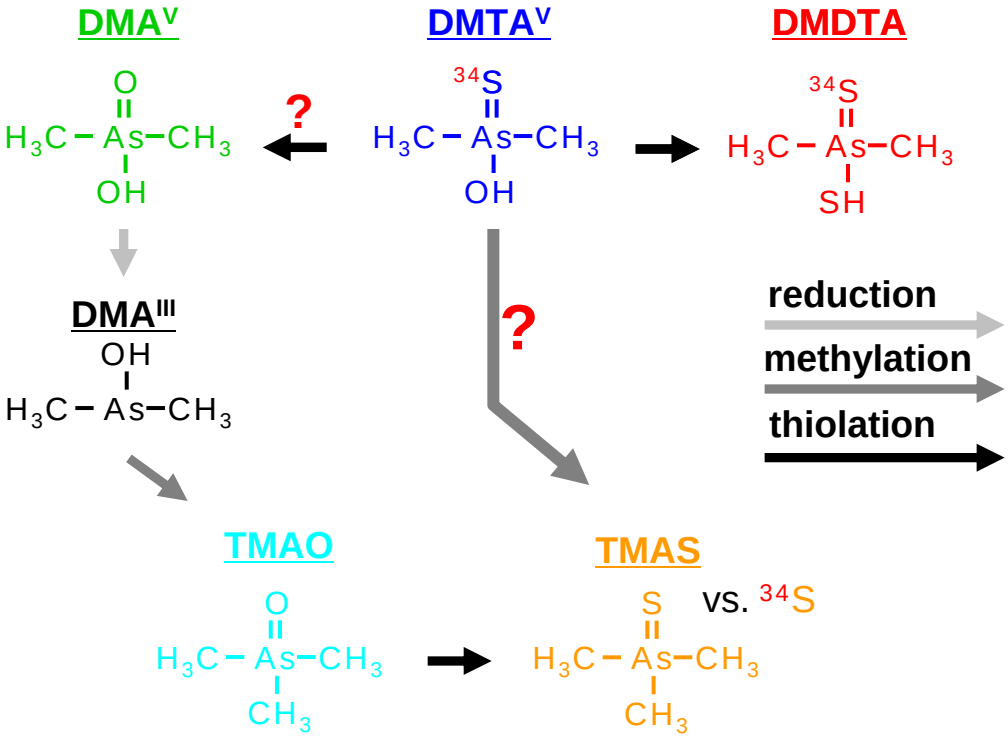



Preservation of $^{34}S$ tag

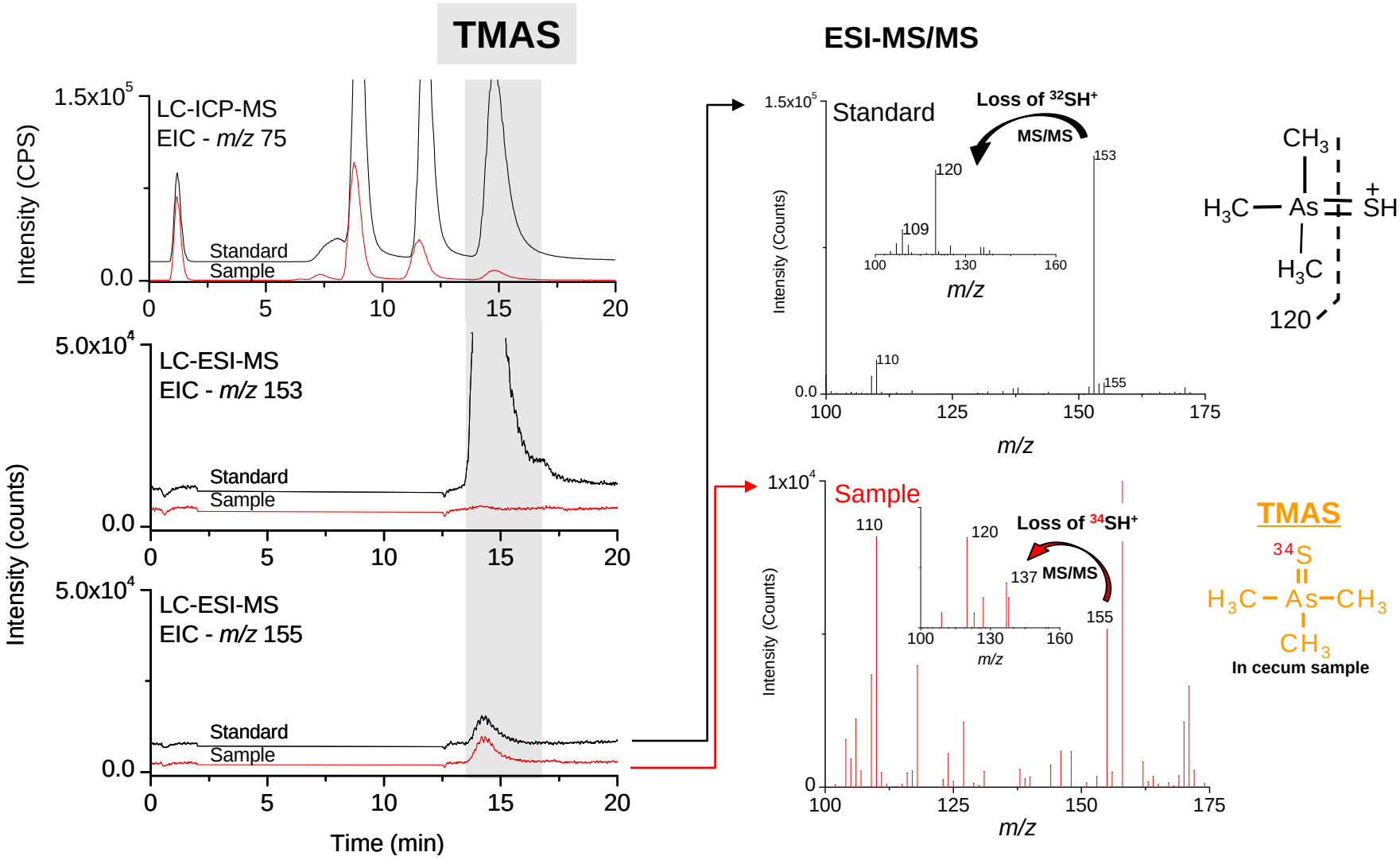
# Time Dependent Arsenic Species Distribution

Cecum supplemented with $DM^{34}TA^{V}$ (200 ng As $g^{-1}$)

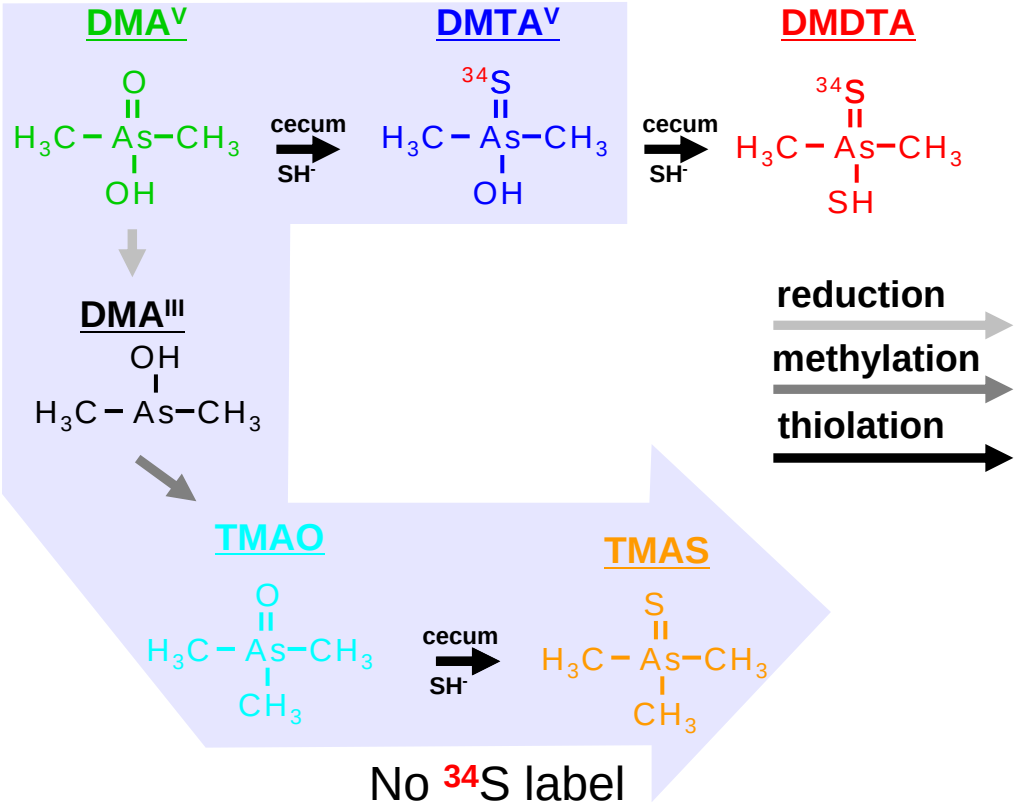


# Confirmation of TMAS by ESI-MS/MS
# TMAS vs TMA$^{34}$S

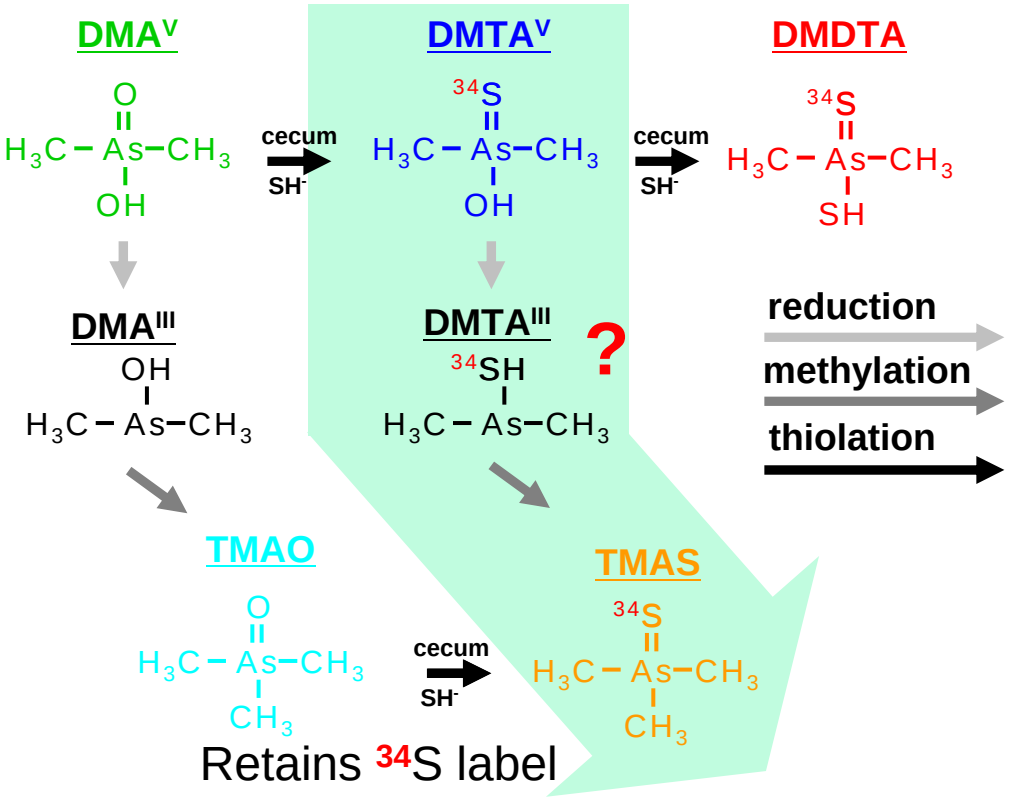


# Summary of Proposed Mechanistic Pathway




Kubachka et al., (2009) *Tox App Pharm*, In Press

# Summary of Proposed Mechanistic Pathway

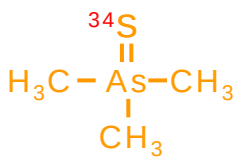


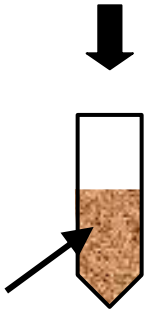

Kubachka et al., (2009) *Tox App Pharm*, In Press

# Time Dependent Arsenic Species Distribution

Cecum supplemented with TMAS (200 ng As $g^{-1}$)

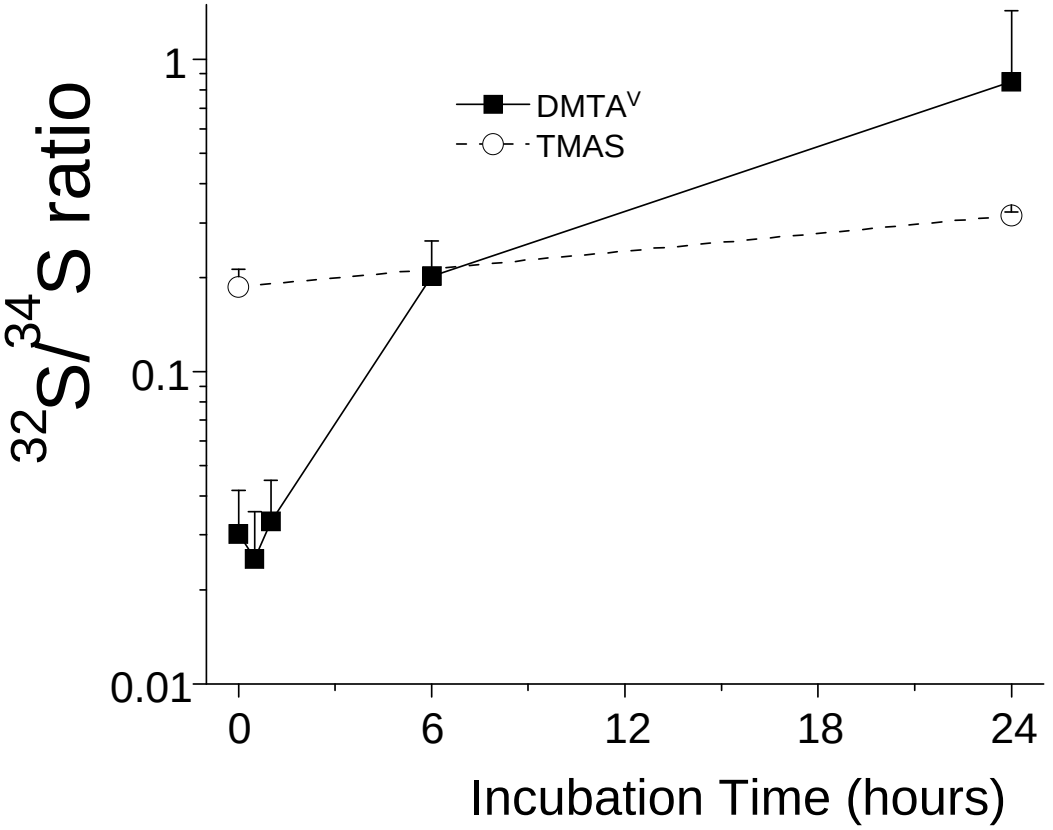
**TMAS**





Homogenized mouse cecum material

**Anaerobic environment**

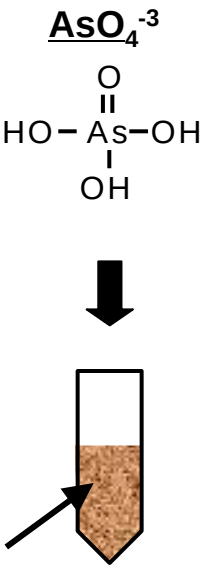



TMA$^{34}$S does not undergo S exchange with the cecum at the same rate as DM$^{34}$TA$^{V}$

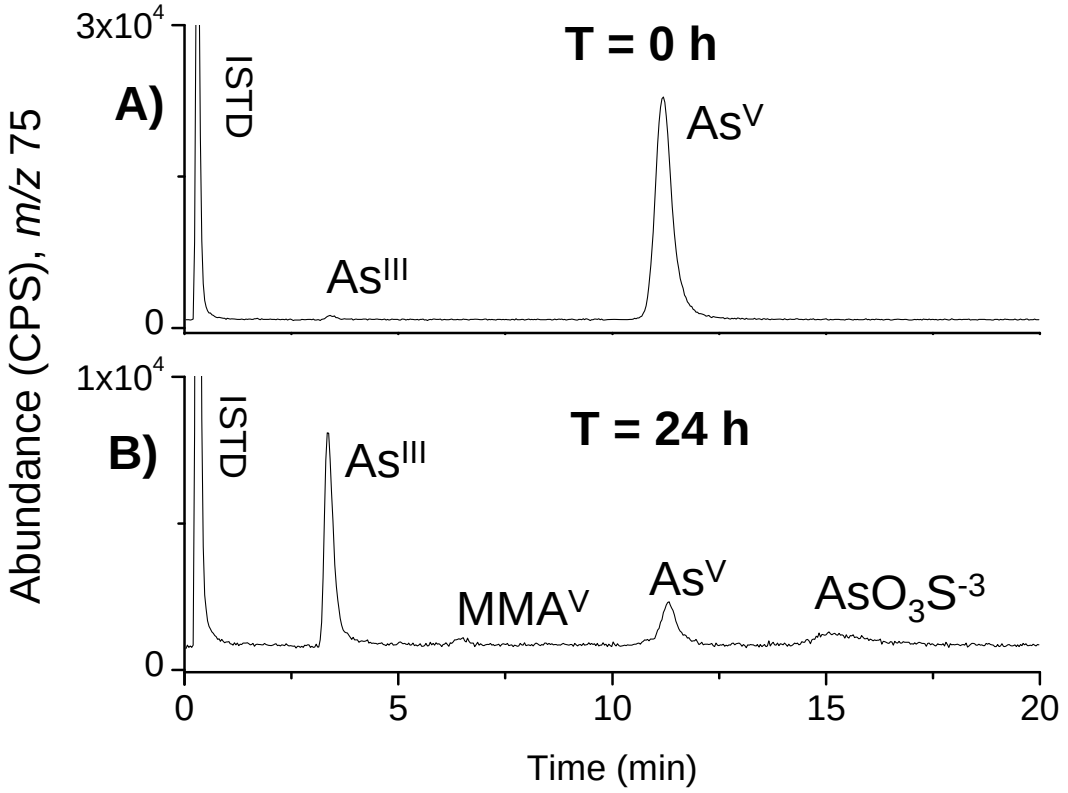
# Time Dependent Arsenic Species Distribution

Cecum supplemented with $AsO_4$ (200 ng As $g^{-1}$)

## Preliminary Results




**Anaerobic environment**




~100% Column Recovery

~50% Column Recovery

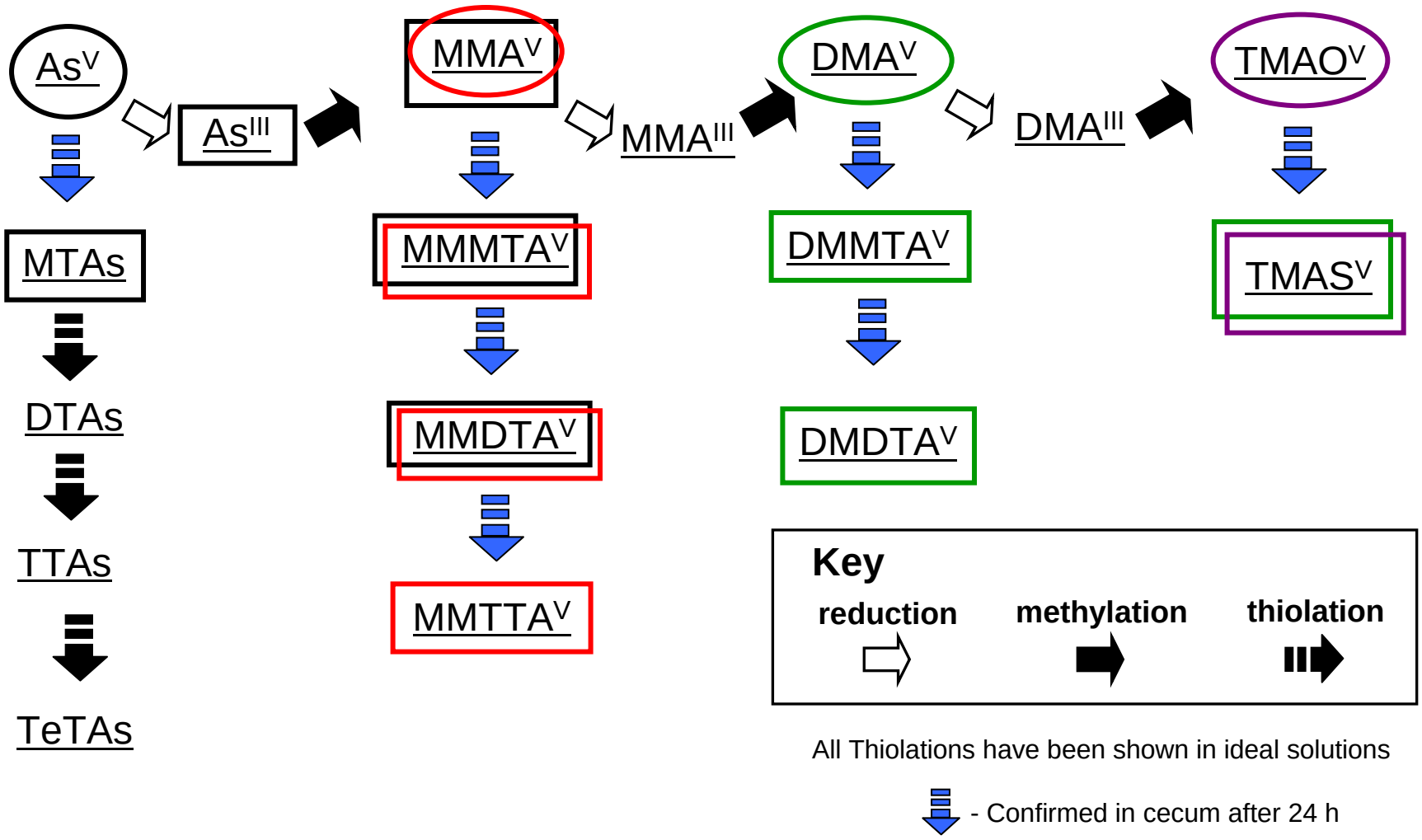
T = 0 h (A) and T = 24 h (B) using PRP-X100 separation.

# Time Dependent Arsenic Species Distribution

Cecum supplemented with $MMA^V$ (200 ng As $g^{-1}$)

**Preliminary Results**

# Summary


$As^{V}$
$As^{III}$
$MMA^{V}$
$MMA^{III}$
$DMA^{V}$
$DMA^{III}$
$TMAO^{V}$
MTAs
DTAs
TTAs
TeTAs
$MMMTA^{V}$
$MMDTA^{V}$
$MMTTA^{V}$
$DMMTA^{V}$
$DMDTA^{V}$
$TMAS^{V}$
Key
reduction
methylation
thiolation
All Thiolations have been shown in ideal solutions
- Confirmed in cecum after 24 h

# Acknowledgments

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  - US EPA, Cincinnati
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    - Dr. Jody Shoemaker

- Discussion:
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  - Dr. William Cullen