SUPPLEMENTAL INFORMATION for

In Vitro Biotransformation of Dimethylarsinic Acid and Trimethylarsine Oxide by Anaerobic Microflora of Mouse Cecum Analyzed by HPLC-ICP-MS and HPLC-ESI-MS

Kevin M. Kubachka,^a Michael C. Kohan,^c Sean D. Conklin,^b Karen Herbin-Davis,^d John T. Creed,^a and David J. Thomas^d

 ^aUS EPA, ORD, NERL, Microbiological and Chemical Exposure Assessment Research Division, Cincinnati, OH 45268, USA. E-mail: creed.jack@epa.gov; Fax: +1-513-569-7757; Tel: +1-513-569-7617
^bOak Ridge Postdoctoral Research Fellow
^cUS EPA, ORD, NHEERL, Environmental Carcinogenesis Division, Research Triangle Park, NC 27711, USA
^dUS EPA, ORD, NHEERL, Experimental Toxicology Division, Research Triangle Park, NC 27711, USA

Four supplemental figures are included in this document with their respective captions:

Figure SI-1 Figure SI-2 Figure SI-3 Figure SI-4



Time (min)

Figure SI-1: Example HPLC-ICP-MS chromatograms (m/z 75) for various mixtures of arsenic standard. A) HPLC-ICP-MS using *Separation 1* for the separation of TMAS standard (upper trace) with TMAO as an impurity and DMDTA standard (lower trace) with DMTA^V and DMA^V as impurities. * denotes elution time of As^{III}, As^V, and MMA^V elute. B) HPLC-ICP-MS using *Separation 2* for the separation of As^{III}, DMA^V, MMA^V, DMTA^V, As^V. C) HPLC-ICP-MS using *Separation 3* for the separation of a standard mixture of DMDTA, DMTA^V, and TMAS. ** denotes elution time of As^{III}, MMA^V, DMA^V, and As^V. The conditions for each separation are listed in **Table 1**.

Intestinal ceca of B6C3F₁ male mice were removed under sterile anaerobic conditions Mixed with VPI buffer (0.1g CaCl₂, 0.2g MgSO₄, 0.5g KH₂PO₄, 5.0g NaHCO₃ and 1.0g NaCl I⁻¹) at 0.03g cecal contents per mL of buffer

Supplementation Table												
Cecum Samples (cecum + VPI buffer)		Incubation Time (Hours at 37 °C) X = 3 replicates										
Supplemen- tation level	DMA ^V Supplementation					TMAO Supplementation						
	ng As g⁻¹	0	1	6	18	24	ng As g⁻¹	0	0.5	1	6	24
I	0	Х		Х		Х	0	Х				Х
I	20	Х	Х	Х	Х	Х	17	Х	X	Х	Х	Х
III	200	Х	Х	Х	Х	Х	170	Х	X	Х	Х	Х
N	1000	Х				Х	830	Х				Х
Controls (no cecum, VPI buffer) at t = 0 and 24 only. All controls supplemented at all four levels (n = 3).												

Diluted appropriately with 20mM $(NH_4)_2CO_3$ at pH – 9.0 to minimize conversion of oxide to sulfide while awaiting analysis

Thawed and vortexed, then centrifuged at 10400 x g for 10 min

Flash frozen using liquid N₂, stored at -72 °C until analysis

Figure SI-2: Summary of the experimental design including: cecal content preparation, supplementation levels (DMA^V and TMAO), anaerobic incubation period, and sample preparation prior to analysis.



Figure SI-3: Time dependent metabolism of DMA^V (A) 20 ng As g⁻¹, B) 1000 ng As g⁻¹(B1, major metabolites, B2, minor metabolites) in incubated reaction mixtures containing the anaerobic microflora from a mouse cecum. Data obtained by HPLC-ICP-MS analysis using *Separation 1*. Error bars represent 1 σ in the positive direction. Time dependence for concentrations of sum of all arsenic species (-- \blacksquare --), DMA^V (- \Box -), DMTA^V (- Δ -), and DMDTA (- \bigcirc -), iAs (- \blacktriangle -) (>95% As^V) and TMAS (- \blacklozenge -).



Figure SI-4: Time dependent metabolism of TMAO (**A**) 17 ng As g^{-1} , **B**) 830 ng As g^{-1}) in incubated reaction mixtures containing the anaerobic microflora from a mouse cecum. Data obtained by HPLC-ICP-MS analysis using *Separation 1*. Error bars represent 1σ in the positive direction.

Time dependence for concentrations of sum of all arsenic species (-- \blacksquare --), TMAS (- \bullet -). TMAO (- \neg --), and iAs (- \bullet --) (>95% As^V).