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Metabolism and disposition of arsenic species from controlled dosing with sodium arsenite in adult and neonatal rhesus monkeys. VI. Toxicokinetic studies following oral administration

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ABSTRACT

Arsenic is a common toxic contaminant in food and drinking water. Metabolic activation of arsenic species produces reactive trivalent intermediates that can disrupt cellular regulatory systems by covalent binding to thiol groups. Arsenic exposures have been associated with human diseases including cancer, diabetes, lung and cardiovascular disorders and there is accumulating evidence that early life exposures are important in the etiology. Previous toxicokinetic studies of arsenite ingestion in neonatal CD-1 mice showed consistent evidence for metabolic and physiologic immaturity that led to elevated internal exposures to trivalent arsenic species in the youngest mice, relative to adults. The current study in rhesus monkeys showed that metabolism and binding of trivalent intermediates after arsenite ingestion were similar between adult monkeys and CD-1 mice. Unlike neonatal mice, monkeys from the age of 5–70 days showed similar metabolism and binding profiles, which were also similar to those in adults. The absence of evidence for metabolic immaturity in monkeys suggests that toxicological effects observed in mice from early postnatal exposures to arsenic could over-predict those possible in primates, based on significantly higher internal exposures.

1. Introduction

Arsenic, in its pristine inorganic (As^i) and organic metabolite forms, is a ubiquitous contaminant in water and food (Takami and Frankenberger, 1992). Epidemiological studies have associated As exposures with many important human maladies, including cancer, diabetes, cardiovascular and pulmonary disease (European Food Safety Authority, 2009; U.S. Environmental Protection Agency, 2010; World Health Organization, 2011; U.S. Food and Drug Administration, 2016). The strength of associations is greatest in populations from the developing world where water supplies can be heavily contaminated (Mukherjee et al., 2006) and estimated daily intake of As^i can reach as high as 50 μg/kg bw. Reliance on As^i-contaminated drinking water-based epidemiological findings introduces uncertainty in the risk assessment of As in foods due to: 1) the large dose extrapolation required for daily intake from food in developed countries, which is estimated at below 1 μg/kg bw; and 2) the similar consumption levels of As^i and organic As species in foods (European Food Safety Authority, 2009; World Health Organization, 2011; U.S. Food and Drug Administration, 2016).

Beyond the direct toxic effects of As exposure, there is accumulating evidence from epidemiological studies and animal models of elevated risks for diseases in adulthood associated with early life exposures to As^i (reviewed in Farzan et al., 2013; Thomas, 2013; Tolins et al., 2014; Tsuji et al., 2015; Bommarito and Fry, 2016; Young et al., 2018). In general, the concept of developmental origins of health and disease (DOHaD) can be summarized as follows: exposure to carcinogens or other toxicants during fetal and neonatal development can confer additional susceptibility to disease, relative to adults, based on: 1) metabolic/physiologic immaturity that leads to elevated internal exposures to toxic species; and 2) unique molecular targets for toxicant action present during developmental programming (e.g., stem cell differentiation and epigenetic processes; Grandjean et al., 2015). This additional susceptibility could be manifested as increased incidences or decreased latencies of disease later in life. Indeed, the early life exposure in transplacental and "whole-life" CD-1 mouse models for As^i carcinogenesis (Waalkes et al., 2003; Tokar et al., 2011) was the breakthrough necessary to recapitulate the carcinogenic effects of As^i seen in humans and afforded a means to investigate mechanisms and dose-response to complement epidemiologically based approaches (Steinmaus et al., 2014).

Previously, we investigated the metabolism and disposition of

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ingested As^i during adult, fetal, and neonatal periods in the CD-1 mouse to understand any metabolic factors involved in the dramatically increased cancer susceptibility (Twaddle et al., 2018a, 2018b, 2018c, 2019a). While internal exposures to putatively toxic As species in the fetus were below those in the mother, directly dosed neonatal mice showed consistent evidence for metabolic and excretory immaturity that led to enhanced formation and binding of trivalent As species in target tissues. This toxicokinetic evidence was interpreted as support for the hypothesis that early life exposures to As^i could be more potent than the corresponding adult exposures, particularly when coupled with the unique set of molecular targets expressed during development. While the metabolic vulnerability of young CD-1 mice probably contributes to the carcinogenic potency of As^i in the "whole-life" exposure model, there is little quantitative information on human metabolism with which to evaluate the relevance for risk assessment.

Developing non-human primate models are often used to bridge gaps in extrapolation to human infants because of the many known similarities in physiology, metabolism, and pharmacology (Chellman et al., 2009). The current study used rhesus monkeys (*Macaca mulatta*) with an oral dose of sodium arsenite (50 μg/kg bw), which was specifically selected to minimize non-linear effects on metabolism and toxicokinetics reported previously in mouse studies (Twaddle et al., 2018a) and to overlap the high range of human exposures. Individual neonates were dosed starting on post-natal day 5 (PND 5), then on PND 35, and PND 70. These serial measurements and evaluations of toxicokinetics were compared directly with those from adult rhesus monkeys using the identical dosing, analytical methodology, and toxicokinetic procedures, which were also used previously in mice (Twaddle et al., 2018c, 2019a). The results showed major differences between developing mice and monkeys that should be useful in the interpretation of results from the "whole-life" mouse model for As^i carcinogenesis and other animal models of human diseases.

2. Methods

2.1. Reagents and standards

Hydrogen peroxide (30%) was purchased from Fisher Optima (Thermo Fisher Scientific, Waltham, MA); ammonium phosphate dibasic from Sigma-Aldrich (St. Louis, MO); MilliQ-H_2O (18 MΩ) from Millipore (Billerica, MA); and 30 kDa molecular weight cutoff centrifuge filters (30 kD MWCO) from EMD Millipore (Darmstadt, Germany). Blood was collected in EDTA-coated plasma separator tubes.

NIST-certified solutions (standard reference materials, SRMs) of arsenate were purchased from SPEX (Metuchen, NJ). MMA^V (disodium methyl arsenate hexahydrate) and DMA^V (dimethylarsinic acid) were purchased from Chem Service (West Chester, PA), sodium arsenite was purchased from Lab Chem (Zelienople, PA), and all solutions were prepared by accurately weighing a portion and diluting with MilliQ-H_2O. All dilutions were prepared in dark, polypropylene bottles and stored at 4 °C. All standards were prepared on the basis of elemental As concentration (75 g/mol) and analyzed by infusion into the ICP/MS (inductively coupled plasma mass spectrometry) to ensure an equal As concentration, using arsenate as the reference (NIST SRM 1640A, trace elements in natural water; Gaithersburg, MD).

2.2. Animal handling procedures

Procedures involving care and handling of non-human primates were reviewed and approved by Institutional Animal Care and Use Committee at Alpha Genesis, Inc. (Yemassee, SC) where the dosing and sampling were conducted, and reviewed at the National Center for Toxicological Research (NCTR, Jefferson, AR) where the analyses were performed. Previous work established the low-level contamination of chow-based animal diets by various As species (Twaddle et al., 2018a). Accordingly, adult monkeys that had been maintained on chow diets were placed on a fruit-only diet approximately 24 h prior to dosing in order to minimize background exposure to As; however, low residual levels of DMA^V were still observed in most pre-dose serum samples (Table S1). Adult male rhesus monkeys of unspecified age with body weights (bw) of 8.13–9.91 kg were obtained from the Alpha Genesis colony (Table S2) and dosed by gavage. It was not practicable to control the diet consumed by the neonatal monkeys during PND 5–70 because rhesus monkeys nurse exclusively for the first 2 weeks of life and weaning typically starts around 4 months of age (Cawthon Lang, 2005). Neonatal monkeys were also dosed by gavage, initially on postnatal day (PND) 5, and subsequently on PND 35, and finally on PND 70 using procedures described previously (Doerge et al., 2010, 2016). Body weights are reported in Table S3. The mean and ranges of body weights for these neonatal monkeys are similar to those reported in previous studies that used the same ages (Doerge et al., 2010, 2016).

Group sizes used reflected a balance between funding availability and statistical power. A group size of n = 3 was deemed the minimum needed to evaluate differences between toxicokinetic parameters derived from individual adult monkey time-concentration profiles. Similarly, an equal numbers of male and female neonatal monkeys were targeted, n = 3 each at all ages tested (PND 5, 35, and 70) to examine the possibility of sex-differences. When none were observed, the data were combined to yield a minimum of n = 6. Two neonatal monkeys died from failure to thrive before all three dosing events could be conducted. All time point data collected were included in neonatal group comparisons, leading to a range of n = 6–8. Insufficient sample volumes precluded analysis of some PND 5 (n = 5 analyzed) and PND 70 (n = 4 analyzed) neonatal monkey erythrocytes.

Monkeys were dosed with sodium arsenite by gavage throughout (10 μg/ml as As equivalents, administered at a volume of 5 ml/kg bw for a dose of 50 μg/kg bw or 667 nmol/kg bw). In all cases, serial blood samples (~0.2 ml) were collected from the saphenous vein of the leg at each of the designated time points (0.5, 1, 2, 4, 6, 8, 12, 24, and 48 h). Pre-dose blood was drawn from each monkey for use as the 0 time point. Blood samples were collected in EDTA tubes (3 ml purple top; Becton, Dickinson and Co., Franklin Lakes, NJ) and gently mixed at room temperature so that plasma and erythrocyte fractions could be separated by centrifugation. Samples were frozen and stored at −80 °C until shipment on dry ice to the NCTR where they were stored at −60 °C until analyzed. As previously reported, repeated analysis of selected mouse blood samples stored at −60 °C over the course of several months showed no evidence for significant changes in the determined concentrations of any As species (Twaddle et al., 2018c).

The dosing solutions were prepared by accurately weighing sodium arsenite, diluting with MilliQ H_2O into a dark, polypropylene bottle, and storing at 4 °C until use. Stability of the aqueous arsenite dosing solution has been determined to be greater than 12 months at ambient conditions (Twaddle et al., 2018a). The dosing solution concentrations were verified using LC-ICP/MS by the NCTR laboratory and dosing solutions were sent to Alpha Genesis just prior to dosing.

2.3. Sample preparation procedures

Plasma was processed as previously described (Twaddle et al., 2018a), but analysis of non-human primate erythrocytes required some modifications. Initial experiments conducted with rhesus monkey erythrocytes using methodology developed for the mouse, both from external spikes and incurred samples, showed that a lower sample volume and increased volume of H_2O_2 were required to ensure complete oxidation of trivalent As species, which was monitored by the conversion of spiked arsenite to arsenate. The optimized method, which included decreasing the volume of the monkey erythrocytes to 10 μL and increasing the volume of H_2O_2 to 400 μL (added in 100 μL aliquots), was sufficient to quantitatively convert arsenite into arsenate. For non-human primate erythrocyte samples treated with H_2O_2, a 10 μL aliquot of sample was added to a 15 mL polypropylene centrifuge tube

containing 90 μL H_2O and mixed. An aliquot of 100 μL of H_2O_2 was added to the tube and allowed to sit for 5 min. The tubes were gently tapped on the bench to aid in bubble disruption and this was repeated for 3 additional 100 μL additions of H_2O_2. After the final addition of H_2O_2, the sample was allowed to sit for approximately 15 min. An aliquot of reacted sample was transferred to the pre-rinsed 30kD MWCO centrifuge filter and spun for 15 min at 14000 × *g* at 15 °C. The resulting filtrate was transferred to polypropylene vials and 50 μL injections were made into the LC-ICP system. Because of the increased dilution of the sample, the LOD for DMA, MMA, and As5 are each roughly 14 nM.

All erythrocyte samples were analyzed with and without H_2O_2 treatment using LC-ICP/MS to separate and quantify soluble pentavalent As species and arsenite (Twaddle et al., 2018a). Repeated analysis of selected mouse blood samples stored at −60 °C over the course of several months showed no evidence for significant changes in the determined concentrations of trivalent and pentavalent As species, as reported previously (Twaddle et al., 2018b).

2.4. Liquid chromatography

Ion exchange LC was performed using a Thermo UltiMate 3000 HPLC system (Thermo Scientific, Germering, Germany) consisting of a pump and autosampler. A Hamilton PRP-X100 column (4.1 × 250 mm, 10 μ particle size, Hamilton, Reno, NV), with an isocratic mobile phase consisting of 98% 10 mM ammonium phosphate (pH 8.25, prepared daily) and 2% methanol (Thermo Fisher Scientific) at a flow rate of 1 ml/min, was used for analyte separation/speciation.

2.5. Mass spectrometry

A Thermo X-Series II ICP-MS (Thermo Electron, Bremen, Germany), equipped with a microflow nebulizer and Peltier-cooled spray chamber maintained at 2 °C (PC3, Elemental Scientific, Omaha, NE), was operated in KED mode to monitor elemental As (*m/z* 75) while mitigating potential mass interference (i.e., ArCl).

2.6. Sample calibration curve

Quantification of each sample set used a series of As standards (arsenite, arsenate, MMA^V, DMA^V) in 10 mM ammonium phosphate (pH 8.25) at defined concentrations to prepare a daily calibration curve. Typically, these standards consisted of a blank along with 3–5 concentrations over a range of 0.05–20 ng/mL As. Linear responses were consistently observed ($R^2 > 0.999$). A typical sample set consisted of calibration standards, a buffer blank, matrix blanks, matrix spikes at multiple concentrations, and incurred samples. Standards were interspersed throughout the sample set to monitor ICP/MS and chromatographic performance. The column effluent was directed through a 10-port switching valve (Rheodyne/IDEX, Lake Forest, IL) that was used to introduce a post-column standard addition of arsenate to provide signal normalization throughout every sample set, as described previously (Twaddle et al., 2018a).

2.7. Method validation

Method validation consisted of spiking plasma or erythrocytes with 3 concentrations of mixed As standards and preparing each concentration in quadruplicate (e.g., 0.1, 1.0, and 10.0 ng/mL) as described previously (Twaddle et al., 2018a). Similarly, plasma and erythrocytes were spiked at 5, 10, and 100 ng/g with mixed As standards and mixed with 50 μL-equivalent aliquots analyzed in quadruplicate on separate days as described previously (Twaddle et al., 2018a).

Method detection limits for As species, which reflect the presence of low levels of DMA in blood from untreated monkeys (Table S1) that were subtracted from post-dosing samples, were approximately 1 nM in blood (50 μL).

2.8. Toxicokinetic analysis

Individual plots of serial serum concentrations of As species vs. time following arsenite administration to each monkey were analyzed using model-independent pharmacokinetic analysis (PK Solutions 2.0 software, Summit Research Services, Montrose, CO). Natural log-linear plots were fit to up to three kinetic phases corresponding to elimination, distribution, and absorption. The first-order elimination rate constants were determined from the terminal slope of the respective curve and the first-order absorption or appearance rate constants were determined after subtracting the contribution from the terminal elimination phase of the respective curve. The half-times for absorption of arsenite ($t_{1/2Abs}$), appearance for all metabolites ($t_{1/2App}$), and elimination ($t_{1/2Elim}$) were determined from the respective rate constants using the relationship $t_{1/2} = \ln 2/k$. Internal exposures were determined as $AUC_{0-\infty}$, by using the trapezoidal rule, or as C_{max} (maximal blood concentration), which was determined by visual inspection. T_{max}, the time at which C_{max} was observed, was also determined by visual inspection.

2.9. Statistical analysis

Pharmacokinetic parameters, which were determined from time-concentration profiles of individual monkeys, are shown as means ± SD for n = 3–8 monkeys. Statistical comparisons of adult and neonatal monkey data were conducted by using either one-way repeated-measures analysis of variance (RM-ANOVA) or one-way analysis of variance (ANOVA). Pairwise comparisons were made by using the Student-Newman-Keuls method. When necessary, the data were ln transformed before the analysis to maintain an equal variance or normal data distribution. Significance was associated with p-values < 0.05.

3. Results

3.1. Background exposure

Previous studies in untreated mice showed that blood and tissues contained measurable levels of DMA, despite attempts to reduce background blood levels of As species from the vendor vivarium's chow-based diet as much as practicable by switching upon arrival at the NCTR to another lower As diet (Twaddle et al., 2018a). For these studies, adult monkeys were transferred from a chow diet to a fresh fruit-based diet for a day before dosing in an attempt to reduce background levels. No comparable dietary intervention was feasible for the neonatal monkeys. Nonetheless, plasma and erythrocytes from untreated monkeys (pre-dose) contained detectable levels of only DMA^V (Table S1). The individual background values were subtracted from levels determined after dosing in order to more accurately derive toxicokinetic parameters that are dependent on complete elimination (e.g., $t_{1/2Elim}$, $AUC_{0-\infty}$, and clearance).

3.2. Plasma toxicokinetics in adult monkeys after oral dosing with sodium arsenite

Gavage treatment of adult male rhesus monkeys with 50 μg/kg bw doses of sodium arsenite (i.e., 667 nmol As/kg bw) led to its rapid appearance in plasma ($t_{1/2Abs}$ = 0.6 ± 0.4 h, Table 1) with maximal plasma concentrations observed at 0.5–4 h (T_{max}). Arsenite was apparently rapidly oxidized to arsenate pre-systemically since its plasma levels generally exceeded those of the parent compound (Fig. 1 and Table 1). DMA^V was the major component observed in plasma after 0.5 h, with T_{max} observed at 2–4 h (Fig. 1 and Table 1). Of the total AUC for As species present in plasma, DMA^V represented 94%, arsenate was ~4%, MMA^V and arsenite were both ~1% (Table 1). The elimination of DMA^V from plasma was slower than the parent compound, with observable levels present at 48 h (Fig. 1 and Table 1). No evidence was

Table 1
Toxicokinetic parameters in plasma from adult male monkeys dosed orally with 50 μg/kg bw sodium arsenite (As equivalents; values represent means from n = 3 individual profiles ± SD).

As Species	$t_{1/2Elim}$ (h)	$t_{1/2Abs\ or\ App}$ (h)	$AUC_{0-\infty}$ (nM x h)	C_{max} (nM)	T_{max} (h)
DMA^V	9.3 ± 0.49	0.6 ± 0.3	3230 ± 623	397 ± 159	2.7 ± 1.2
MMA^V	2.2 ± 1.2	–	39 ± 17	8.7 ± 4.2	2.7 ± 1.2
Arsenate	1.7 ± 0.42	0.3 ± 0.3	145 ± 34	53 ± 9.9	0.7 ± 0.3
Arsenite	7.2 ± 7.4	0.6 ± 0.4	56 ± 12	15 ± 7.9	2.2 ± 1.8

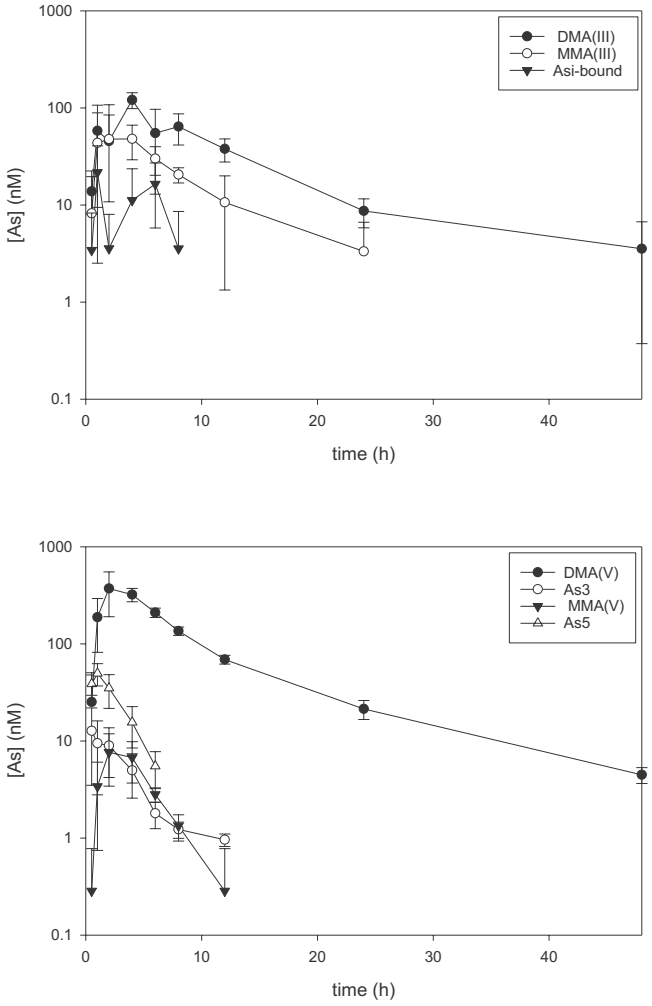


Fig. 1. Time course data for "free" As species in plasma (bottom panel) and bound trivalent As species in erythrocytes (top panel) from adult male rhesus monkeys dosed orally with 50 μg/kg bw sodium arsenite (As equivalents; values plotted represent means of n = 3 individual plasma and erythrocyte measurements ± SD). The LOD was approximately 1 nM in blood (50 μL).

observed in the ICP/MS chromatograms to support formation of trimethylarsine oxide (TMAO) or any other As-containing metabolite (not shown). A pilot study of plasma samples collected after arsenite dosing, conducted with and without H_2O_2, showed that DMA^{III} and MMA^{III} apparently were bound to plasma proteins, similar to what was seen previously in mice (Twaddle et al., 2018c); however, such binding contributed only a small amount to the total present (e.g., DMA^{III} levels were below 20% of DMA^V, not shown).

3.3. Erythrocyte toxicokinetics in adult monkeys after oral dosing with sodium arsenite

Previous mouse studies established the utility of the erythrocyte fraction as an accessible source for measuring systemic trivalent As species to complement the measurements of pentavalent As species in monkey plasma (Twaddle et al., 2018a, 2018c) and provide a means to estimate tissue levels (Twaddle et al., 2018b). Measurements of "free" As species were conducted in monkey erythrocyte samples with and without H_2O_2 and the difference was used to quantify the bound trivalent As species (Twaddle et al., 2018a). Evidence for binding of trivalent DMA, MMA, and arsenite to thiols present in the erythrocytes was consistently observed following arsenite dosing (Fig. 1, Table 2). DMA^{III} was the predominant bound species present (i.e., 67% of total AUC), MMA^{III} was 23%, and bound As^i was 10%; Fig. 1 and Table 2). Elimination of the bound As species proceeded in the order of As^i > MMA^{III} > DMA^{III} (i.e., increasing $t_{1/2Elim}$, Table 2).

Table 2
Toxicokinetic parameters in erythrocytes from adult male rhesus monkeys dosed orally with 50 μg/kg bw sodium arsenite (As equivalents; values represent means from n = 3 individual profiles ± SD).

As Species	$t_{1/2Elim}$ (h)	$AUC_{0-\infty}$ (nM x h)	C_{max} (nM)	T_{max} (h)
DMA^{III}	19 ± 16	1508 ± 312	126 ± 3.6	4.7 ± 1.2
MMA^{III}	4 ± 6	510 ± 229	63 ± 30	3.7 ± 2.5
As^i-bound	2 ± 2	218 ± 203	62 ± 67	2.0 ± 1.7

3.4. Plasma toxicokinetics in neonatal monkeys after oral dosing with sodium arsenite

The plasma toxicokinetics of arsenite and its metabolites were also evaluated in neonatal monkeys, with dosing of each individual on PND 5 (Fig. 2 and Table 3), then later on PND 35 (Fig. S1 and Table 4), and PND 70 (Fig. S2 and Table 5). In general, there were few differences observed in the kinetic parameters, either between neonatal monkeys of different ages or between neonatal and adult monkeys. RM-ANOVA with pair-wise comparisons showed that the DMA^V AUC and C_{max} parameters for PND 35 monkeys were significantly different from PND 5, and DMA^V C_{max} was significantly different from PND 70 also; however, the absence of a consistent age-associated trend in the internal exposure parameters over the ages used, and the absence of confirmatory changes in other neonatal kinetic parameters, made these observations inconclusive. For this reason, the individual neonatal monkey data were combined, as an average of the three dosing times, for subsequent comparisons with adults. Comparison of plasma toxicokinetic parameters between the combined neonatal group and the adult group showed several significant differences using ANOVA with pair-wise comparisons, including plasma AUC and C_{max} values for MMA^V, arsenite, and arsenate (Fig. 3). Notable were the insignificant differences between adult vs. neonatal monkeys for internal exposure to the major metabolite, DMA^V.

3.5. Erythrocyte toxicokinetics in neonatal monkeys after oral dosing with sodium arsenite

The binding of arsenite and its trivalent metabolites was also evaluated in erythrocytes from neonatal monkeys on PND 5 (Fig. 2 and Table 6), PND 35 (Fig. S1 and Table 7), and PND 70 (Fig. S2 and

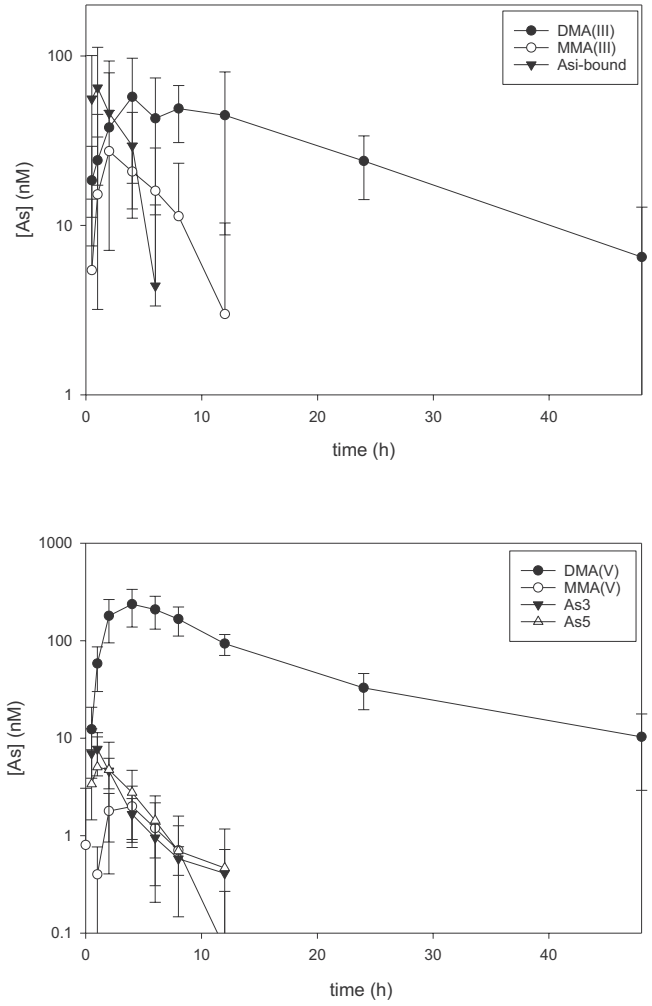


Fig. 2. Time course data for "free" As species in plasma (bottom panel) and bound trivalent As species in erythrocytes (top panel) from neonatal rhesus monkeys (PND 5) dosed orally with 50 μg/kg bw sodium arsenite (As equivalents; values plotted represent means of n = 8 individual plasma measurements and n = 5 individual erythrocyte measurements ± SD). The LOD was approximately 1 nM in blood (50 μL).

Table 8). No significant differences were observed in the AUC and C_{max} values between neonatal monkeys of different ages using RM-ANOVA with pair-wise comparisons (Tables 6–8); however, neonatal and adult monkeys showed significant differences for AUC and C_{max} values for bound DMA^{III} and MMA^{III}, but not As^{i}, using ANOVA with pair-wise comparisons (Fig. 3). In both cases, the adult values exceeded those for neonatal monkeys. The percentages of total AUC for bound trivalent As species were similar across ages, with respective neonatal values for DMA^{III}, MMA^{III}, and As^{i} of 73–86%, 6–13%, and 8–14% vs. 67%, 23%, and 8% in adult monkey erythrocytes.

4. Discussion

4.1. Toxicokinetics of As species in adult monkeys following gavage administration of sodium arsenite

Previous pilot and toxicokinetic studies of bolus oral dosing with sodium arsenite in adult female CD-1 mice provided a framework for sample collection and analysis to generate toxicokinetic data for pentavalent and bound trivalent As species in blood and tissues (Twaddle et al., 2018a, 2018c). The current study in non-human primates provides complementary information from measurements in blood alone, as used previously in pregnant mice (Twaddle et al., 2018b). Tissue measurements were not included in this monkey study because they were deemed unlikely to produce sufficient additional information to justify terminal procedures. Gavage treatment of adult monkeys with sodium arsenite led to extensive methylation, predominately to the terminal metabolite, DMA^{V}, which represented 94% of combined plasma AUCs for As species (Scheme). In addition, smaller amounts of the intermediate, MMA^{V}, the oxidation product, arsenate, and the substrate, arsenite, were observed in plasma (Fig. 1). Similarly, DMA^{III} was the predominant species present bound to erythrocyte thiols after oral dosing with arsenite, with smaller amounts of bound MMA^{III} and As^{i} (Fig. 1).

4.2. Toxicokinetics of As species in neonatal monkeys following gavage administration of sodium arsenite

This study tested the hypothesis that metabolic and/or physiological immaturity could alter the metabolism and disposition of As^{i} and related species in such a way that elevated internal exposures to toxic intermediates could increase susceptibility to adverse effects, relative to adults. This hypothesis was tested over the developmental window from PND 5, the earliest age that the monkeys could be handled safely, to PND 70. This age range has been used previously to study the development of metabolic (e.g., Phase II enzyme ontology) and physiologic (e.g., renal excretion) factors relevant to toxicant exposures in human infants (e.g., bisphenol A; Doerge et al., 2010; soy infant formula; Doerge et al., 2016). These comparisons of neonatal toxicokinetics for bisphenol A in non-human primate vs. rodent models provided important insights for inter-species extrapolation to human infants using physiologically based pharmacokinetic modeling (Yang et al., 2015).

In general, there were few differences between the toxicokinetic parameters for As species derived from neonatal monkeys dosed orally with sodium arsenite, and no clear pattern of development over PND 5–70. This conclusion includes kinetic parameters (e.g., half-times for elimination and absorption) and internal exposure parameters (AUC and C_{max}) for arsenite and metabolites. Furthermore, there were only a few significant differences in As toxicokinetics between neonatal and adult monkeys, and none that showed evidence for elevated internal exposure to potentially toxic trivalent As species during the sensitive neonatal period. These findings suggest that metabolic differences alone should not be a source of additional susceptibility in neonatal monkeys to the toxic effects of As species, compared to adults. Such metabolic determinants are independent from the susceptibility that could arise from unique molecular targets that are present only during

Table 3
Toxicokinetic parameters in plasma from PND 5 neonatal monkeys dosed orally with 50 μg/kg bw sodium arsenite (As equivalents; values represent means from n = 8 individual profiles ± SD).

As Species	$t_{1/2Elim}$ (h)	$t_{1/2Abs\ or\ App}$ (h)	C_{max} (nM)	T_{max} (h)	$AUC_{0-\infty}$ (nM x h)
DMA^{V}	8.7 ± 2.8	1.2 ± 0.34	238 ± 97	4.3 ± 0.7	3197 ± 1015
MMA^{V}	3.7 ± 3.8	1.1 ± 0.46	2.1 ± 1.2	3.3 ± 1.0	15 ± 5.3
Arsenate	3.8 ± 2.5	0.61 ± 0.59	6.1 ± 4.7	2.0 ± 1.3	31 ± 20
Arsenite	8.8 ± 8.2	0.39 ± 0.19	8.5 ± 4.8	0.8 ± 0.3	29 ± 7.8

Table 4
Toxicokinetic parameters in plasma from PND 35 neonatal monkeys dosed orally with 50 μg/kg bw sodium arsenite (As equivalents; values represent means from n = 7 individual profiles ± SD).

As Species	$t_{1/2Elim}$ (h)	$t_{1/2Abs\ or\ App}$ (h)	C_{max} (nM)	T_{max} (h)	$AUC_{0-\infty}$ (nM x h)
DMA^V	9.0 ± 0.76	1.1 ± 1.0	503 ± 136	2.9 ± 1.1	4556 ± 457
MMA^V	2.9 ± 1.1	0.89 ± 0.41	4.9 ± 3.7	2.3 ± 0.8	27 ± 20
Arsenate	2.5 ± 1.7	0.51 ± 0.27	9.6 ± 6.1	1.6 ± 0.7	31 ± 22
Arsenite	2.2 ± 0.56	0.38 ± 0.36	7.4 ± 5.4	1.0 ± 0.7	24 ± 11

the perinatal developmental process (e.g., epigenetic processes; Grandjean et al., 2015).

4.3. Ontogeny of hemoglobin in rhesus monkeys and mice

An implicit element in comparisons of trivalent As species binding to erythrocytes is that the binding occurs mainly to equivalently accessible thiol groups on hemoglobin (Twaddle et al., 2018a). Time-concentration profiles in adult mice (Twaddle et al., 2018c) and monkeys (Fig. 1) indicated that following arsenite dosing, levels of erythrocyte-bound As species rose from low background levels, reached a maximum within a few hours, and subsequently declined to low levels within 24–48 h. It is noteworthy that these properties are not seen in As-treated rat erythrocytes, where enhanced binding and decreased clearance rates from a unique cysteine residue in rat hemoglobin lead to accumulation of bound As species (Lu et al., 2004). Electrophoretic analysis of rhesus monkey hemoglobins showed one fetal form and one adult form present at birth, and that by PND 35 virtually all was present as the adult form (Wu et al., 1977). The similarities in binding and kinetics of As species to neonatal and adult monkey erythrocytes reported here suggest that structural differences in hemoglobin did not affect the conclusions. In mice, it has been reported that from birth all hemoglobin is present in the adult form (Kitchen and Brett, 1974).

4.4. Comparison of toxicokinetics of As species between adult rhesus monkeys and CD-1 mice following gavage administration of sodium arsenite

Overall, the toxicokinetic behavior of As species in plasma and erythrocytes were similar between adult mice and monkeys after equivalent dosing with arsenite. This pattern observed from plasma measurements includes the predominant formation of DMA^V, with smaller amounts of MMA^V, and As^i species. The pattern in erythrocytes reflects covalent binding of $DMA^{III} > MMA^{III} > As^i$. While the AUC values for arsenite and its metabolites were consistently lower in the adult mouse (Twaddle et al., 2018c) vs. the adult monkey (Tables 1–2), the differences were consistent with the principle of allometry, which predicts that body weight differences alone affect internal toxicokinetic parameters (U.S. Environmental Protection Agency, 2011). Accordingly, the body weight difference between a 25 g mouse and an 8.79 kg monkey predict that an identical dose, based on the mass of compound administered per unit of body weight (i.e., mg/kg bw), would produce an AUC in the mouse 23% of that in a monkey (Table S4).

In both species, the time courses for plasma and erythrocyte levels were consistent with the reactions shown in Scheme: 1) high systemic fluxes of pentavalent and trivalent As metabolites through the action of arsenite-methyltransferase (As3MT; Dheeman et al., 2014); 2) extensive binding of trivalent As species to thiol groups in tissues and erythrocyte (Twaddle et al., 2018a, 2018c); 3) facile mobilization of bound trivalent As species by physiological levels of GSH (Twaddle et al., 2018a); 4) reduction of pentavalent As species to the trivalent analogs by cellular

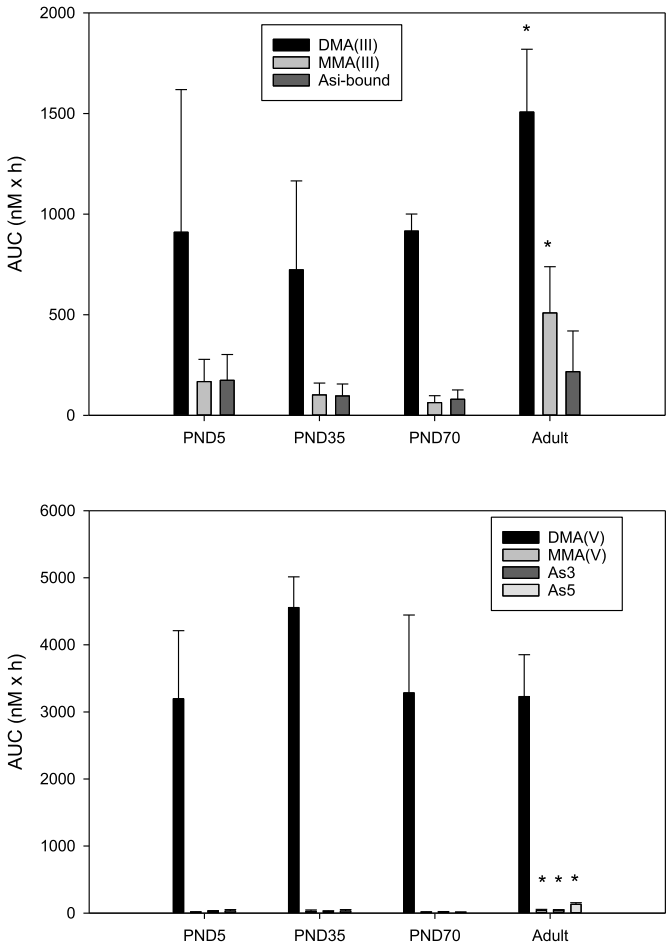


Fig. 3. Comparison of plasma AUCs (areas under time-concentration curves) for "free" As species in plasma (bottom panel) and AUCs for bound trivalent As species in erythrocytes produced after oral administration with sodium arsenite (50 μg/kg bw, As equivalents; values plotted represent means ± SD). Note that * signifies a significant difference between the adult and neonatal groups using ANOVA ($p < 0.05$).

Table 5
Toxicokinetic parameters in plasma from PND 70 neonatal monkeys dosed orally with 50 μg/kg bw sodium arsenite (As equivalents; values represent means from n = 6 individual profiles ± SD).

As Species	$t_{1/2Elim}$ (h)	$t_{1/2Abs\ or\ App}$ (h)	C_{max} (nM)	T_{max} (h)	$AUC_{0-\infty}$ (nM x h)
DMA^V	9.2 ± 3.3	0.52 ± 0.18	391 ± 129	2.3 ± 0.8	3284 ± 1160
MMA^V	1.8 ± 1.0	0.53 ± 0.36	4.8 ± 3.8	1.6 ± 0.5	14 ± 5.8
Arsenate	1.2 ± 1.1	0.23 ± 0.12	3.0 ± 1.8	1.1 ± 0.5	9.4 ± 8.5
Arsenite	2.2 ± 1.2	0.27 ± 0.18	4.9 ± 3.3	0.6 ± 0.2	15 ± 8.8

Table 6
Toxicokinetic parameters in erythrocytes from PND 5 rhesus monkeys dosed orally with 50 μg/kg bw sodium arsenite (As equivalents; values represent means from n = 5 individual profiles ± SD).

As Species	$t_{1/2Elim}$ (h)	$AUC_{0-\infty}$ (nM x h)	C_{max} (nM)	T_{max} (h)
DMA^{III}	4.9 ± 3.5	911 ± 708	64 ± 27	8.0 ± 2.4
MMA^{III}	0.87 ± 0.68	168 ± 111	26 ± 10	3.6 ± 2.6
As^{i}-bound	1.0 ± 1.0	175 ± 128	55 ± 40	2.0 ± 1.2

Table 7
Toxicokinetic parameters in erythrocytes from PND 35 rhesus monkeys dosed orally with 50 μg/kg bw sodium arsenite (As equivalents; values represent means from n = 5 individual profiles ± SD).

As Species	$t_{1/2Elim}$ (h)	$AUC_{0-\infty}$ (nM x h)	C_{max} (nM)	T_{max} (h)
DMA^{III}	8.1 ± 6.0	724 ± 442	67 ± 44	7.4 ± 9.5
MMA^{III}	1.8 ± 1.0	102 ± 59	23 ± 11	2.2 ± 1.1
As^{i}-bound	1.7 ± 1.3	97 ± 60	39 ± 21	1.7 ± 1.4

Table 8
Toxicokinetic parameters in erythrocytes from PND70 rhesus monkeys dosed orally with 50 μg/kg bw sodium arsenite (As equivalents; values represent means from n = 4 individual profiles ± SD).

As Species	$t_{1/2Elim}$ (h)	$AUC_{0-\infty}$ (nM x h)	C_{max} (nM)	T_{max} (h)
DMA^{III}	1.7 ± 3.0	916 ± 85	79 ± 21	3.0 ± 2.4
MMA^{III}	1.1 ± 1.2	64 ± 35	21 ± 8.3	1.5 ± 0.6
As^{i}-bound	1.5 ± 1.4	80 ± 47	31 ± 10	1.5 ± 0.6

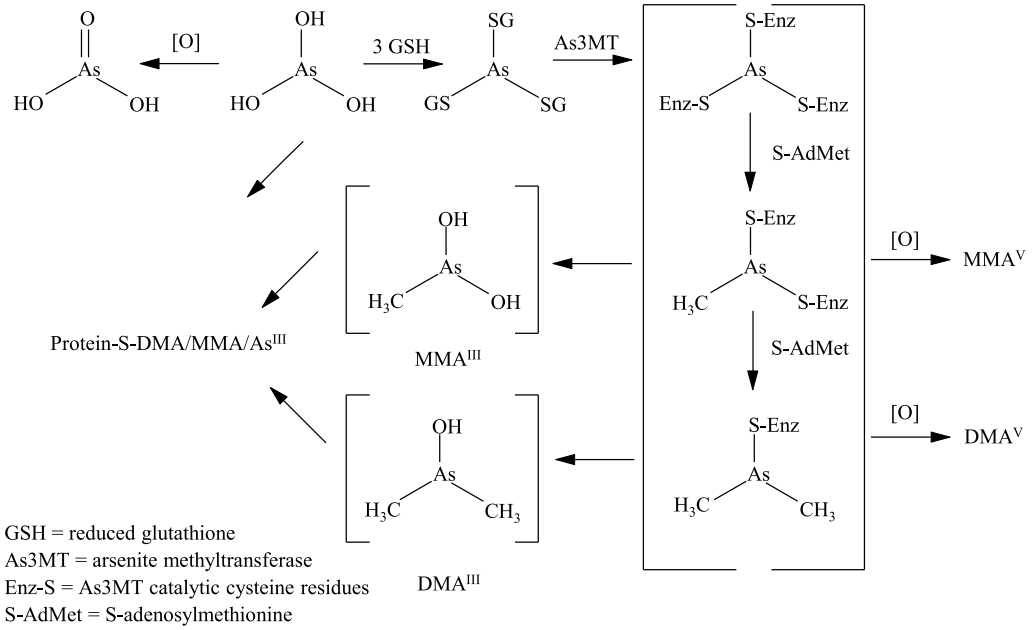
constituents (Delnomdedieu et al., 1994; Stýblo et al., 1997; Németi and Gregus, 2013; Twaddle et al., 2018a, 2018c, 2019b); and 5) urinary clearance of pentavalent As species, predominantly DMA^{V} (Twaddle et al., 2018a).

4.5. Comparison of toxicokinetics of As species between neonatal rhesus monkeys and CD-1 mice following gavage administration of sodium arsenite

Major differences were observed between neonatal monkeys and mice in the metabolism of arsenite and the disposition of reactive trivalent As intermediates in two studies using identical experimental designs in terms of dosing and analytical methodology. Previously, we reported significant and consistent age-related differences in plasma, erythrocyte, and liver endpoints between neonatal and adult CD-1 mice. These endpoints reflected metabolic activation and clearance in ways that increased internal exposures to reactive trivalent As intermediates in blood and liver in the youngest mice and reached adult levels by weaning (Twaddle et al., 2019a). Specifically, increased levels of arsenite (and arsenate) and elevated fluxes of MMA^{III} were consistent with saturation of As3MT capacity. Also, the lower total clearance of DMA^{V} in the youngest mice was consistent with lower renal excretory capacity. These findings were interpreted as strong evidence for immaturity of metabolic and physiologic processes in CD-1 mice that confer additional susceptibility to the toxic effects of As species, which depend on the binding of trivalent As species to thiol groups (Spuches et al., 2005; Shen et al., 2013) at the center of many cellular regulatory systems (Go and Jones, 2013). These prominent effects on metabolic activation and/or excretion of arsenite were not seen in developing monkeys. If anything, higher levels of binding for trivalent As species were seen in adult monkeys, a result inconsistent with predictions based on body weight differences alone (U.S. Environmental Protection Agency, 2011). Smaller neonatal monkeys are predicted, based on allometry, to require a larger dose in order to achieve equivalent internal exposure as a larger adult, which is determined by the relative body weights ($bw^{3/4}$; 50% dose reduction for adults vs. PND 5 in Table S4). The absence of evidence for metabolic/physiologic immaturity in neonatal monkeys, as seen in mice, provides important information about species differences for extrapolation of dose-response relationships from animal models to human risk assessment.

5. Conclusions

The current study in developing and adult monkeys did not recapitulate the deficiencies in metabolism and excretion of As species observed previously in neonatal mice treated in an identical manner. Since metabolism and disposition of As^{i} are central to its toxicity, the similarity in internal exposure to reactive trivalent As species in neonatal and adults monkeys suggests that toxicokinetic differences alone would not contribute to additional susceptibility during early neonatal



Scheme 1. Metabolic transformations of arsenite (As^{III}) by oxidation to arsenate (As^{V}), arsenite methyltransferase- (As3MT) catalyzed methylation to $MMA^{III/V}$ and $DMA^{III/V}$, and binding of trivalent As species to protein thiol groups.

life. The absence of evidence for metabolic immaturity in monkeys suggests that toxicological effects observed in mice from early postnatal exposures to arsenic could over-predict those possible in primates, based on significantly higher internal exposures and overall immaturity at birth. These findings could be useful in understanding the role of early life exposures to dietary As in important human diseases, including cancer.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2019.110760.

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