High-sensitivity quantitative analysis reveals the non-linear relationship between the dose and deposition of diphenylarsinic acid in the rat central nervous system following its subchronic exposure

doi: 10.1016/j.ntt.2017.12.004

Neurotoxicology and Teratology 65 (2018) 26–33

Contents lists available at ScienceDirect

Neurotoxicology and Teratology

journal homepage: www.elsevier.com/locate/neutera

High-sensitivity quantitative analysis reveals the non-linear relationship between the dose and deposition of diphenylarsinic acid in the rat central nervous system following its subchronic exposure

Tomoyuki Masuda[a,b,*], Kazuhiro Ishii[a,*], Tomohiro Nakayama[c], Nobuaki Iwasaki[c], Yasuyuki Shibata[d], Akira Tamaoka[a]

[a] Department of Neurology, Faculty of Medicine, University of Tsukuba, Ibaraki 305-8575, Japan
[b] Doctoral and Master's Programs in Kansei, Behavioral and Brain Sciences, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Ibaraki 305-8577, Japan
[c] Department of Pediatrics, Ibaraki Prefectural University of Health Sciences, Ibaraki 300-0331, Japan
[d] Center for Environmental Measurement and Analysis, National Institute for Environmental Studies, Ibaraki 305-8506, Japan

ARTICLE INFO

Keywords:
LC–MS/MS
Cerebrum
Rodent
Arsenic compound

ABSTRACT

In the year 2003, the residents of Kamisu, Japan, were exposed to pentavalent organic arsenic diphenylarsinic acid (DPAA[V]) via their normal drinking water. Following the exposure, they developed cerebellar and brainstem symptoms. Although the relatively high dose of DPAA(V) is assumed to have caused their symptoms, the relationship between the exposed dose of DPAA(V) and the level of their deposition in the central nervous system (CNS) remains unclear. Using liquid chromatography–tandem mass spectrometry, we examined the deposition of DPAA(V) and its pentavalent metabolites in the CNS tissues of Crl:CD(SD) rats following the administration of DPAA(V) for 28 days. We found that the concentrations of DPAA(V) in the CNS were very high, given a dose of 5.0 mg/kg/day. However, very low concentrations of DPAA(V) were detected at a dose of 0.3 or 1.2 mg/kg/day, suggesting the absence of a linear dose-response relationship between the dose and deposition of DPAA(V). We also found that this non-linear relationship was commonly observed in various non-CNS tissues, including the excretory system. Our study showed for the first time the exact relationship between the dose and tissue deposition of the organic arsenic following its subchronic administration.

1. Introduction

In the year 2003, unexplained symptoms were reported by patients in Kamisu, Ibaraki Prefecture, Japan. Organic arsenic compounds were identified in the wells of their drinking water as the cause of their symptoms (Ishii et al., 2004). The organic arsenic detected in the drinking well water was determined to be diphenylarsinic acid (DPAA[V]), a pentavalent organic arsenic compound used in the synthesis of chemical weapons during World War II (Kurata, 1980). Further analyses revealed that DPAA(V) had leaked from concrete-like blocks to the bottom of the aquifer and thus had contaminated the drinking well water (Watanabe et al., 2011). The water from one of the wells contained as much as 4.5 mg As/L of DPAA(V), which is 450 times higher than the concentration of the drinking water quality standards approved by the Ministry of Health, Labour and Welfare, Japan (Kinoshita et al., 2005; Shibata et al., 2005).

Organic arsenic monomethylarsonic acid (MMA[V]) and dimethylarsinic acid (DMA[V]) are known to be metabolites of inorganic arsenic (iAs). The metabolism and genotoxicity of these two organic arsenic compounds have been well studied since the early 1980s (Cohen et al., 2006). However, DPAA(V) is not a natural product. Therefore, our knowledge of its toxicokinetics has been quite limited. Residents exposed to DPAA(V) experienced progressive cerebellar, brainstem, and temporal and occipital lobe symptoms, but not peripheral neuropathies (Ishii et al., 2004). Analyses of the cerebral blood flow with single photon emission computed tomography revealed a decrease in the blood flow in the occipital lobe and cerebellar vermis in DPAA(V)-exposed residents (Ishii and Tamaoka, 2015).

To date, the relationship between the exposed dose of DPAA(V) and the level of its deposition in the central nervous system (CNS) has been unclear. Regarding other organic arsenic compounds, there has been one report using rodents to examine the relationship between subchronic exposure and tissue concentration. They measured the concentration of DMA(V) in body fluids and 4 tissues at 14 days after the

* Corresponding authors at: Department of Neurology, Faculty of Medicine, University of Tsukuba, Ibaraki 305-8575, Japan.
E-mail addresses: tmasu@md.tsukuba.ac.jp (T. Masuda), kazishii@md.tsukuba.ac.jp (K. Ishii).

https://doi.org/10.1016/j.ntt.2017.12.004
Received 1 August 2017; Received in revised form 25 November 2017; Accepted 5 December 2017
Available online 07 December 2017

Table 1
DPAA(V) concentration in the CNS tissues after oral administration of DPAA(V).

		DPAA(V) after 0.3–5.0 mg/kg/day of DPAA(V) administration (average ng As/g ± SEM)							
		0.3 mg/kg/day (male)	0.3 mg/kg/day (female)	0.3 mg/kg/day (average)	1.2 mg/kg/day (male)	1.2 mg/kg/day (female)	1.2 mg/kg/day (average)	5.0 mg/kg/day (male)	5.0 mg/kg/day (female)	5.0 mg/kg/day (average)
Central nervous system	Frontal-parietal lobe	58.9 ± 4.99 (3)	52.4 ± 4.00 (3)	55.7 ± 3.20 (6)	261 ± 12.2 (3)	185 ± 8.30 (3)	223 ± 18.1 (6)	8456 ± 520 (3)	9027 ± 420 (3)	8742 ± 325 (6)
	Temporal-occipital lobe	51.7 ± 3.45 (3)	41.7 ± 0.760 (3)	47.0 ± 2.75 (6)	204 ± 27.7 (3)	170 ± 12.2 (3)	187 ± 15.4 (6)	8062 ± 713 (3)	7417 ± 414 (3)	7740 ± 396 (6)
	Cerebellum	61.7 ± 1.23 (3)	53.7 ± 2.75 (3)	57.7 ± 2.25 (6)	274 ± 8.58 (3)	225 ± 5.04 (3)	249 ± 11.8 (6)	9359 ± 432 (3)	9286 ± 645 (3)	9323 ± 347 (6)
	Brainstem	76.5 ± 3.28 (3)	60.2 ± 4.03 (3)	68.3 ± 4.31 (6)	302 ± 25.4 (3)	255 ± 24.3 (3)	278 ± 18.9 (6)	11,824 ± 1179 (3)	11,325 ± 555 (3)	11,574 ± 593 (6)
	Spinal cord	74.2 ± 3.25 (3)	56.5 ± 3.38 (3)	65.4 ± 4.48 (6)	280 ± 24.3 (3)	236 ± 11.7 (3)	258 ± 15.6 (6)	9758 ± 1306 (3)	9603 ± 292 (3)	9681 ± 600 (6)
Central nervous system (average)		64.6 ± 2.00 (15)	52.9 ± 2.17 (15)	58.8 ± 2.93 (30)	264 ± 19.2 (15)	214 ± 10.4 (15)	239 ± 14.8 (30)	9492 ± 814 (15)	9332 ± 371 (15)	9412 ± 402 (30)

The number between brackets indicates the number of samples examined.

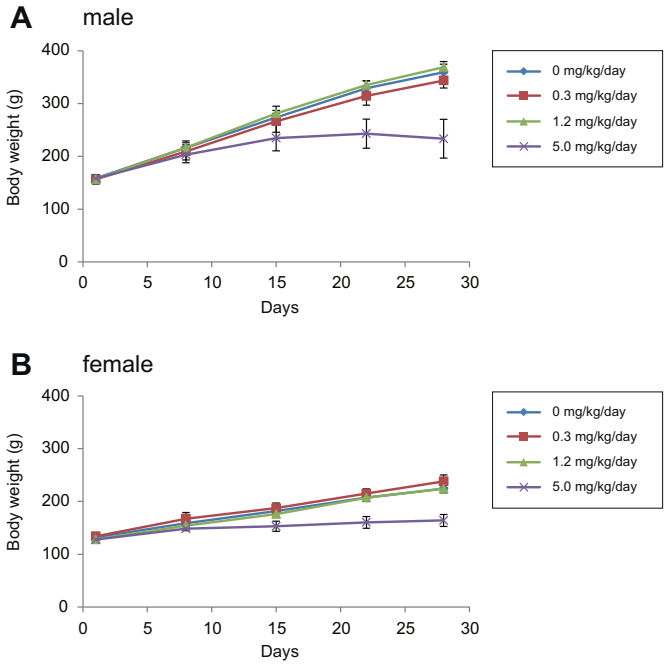

Fig. 1. Effect of 28-day exposure to DPAA(V) on body weight of rats. Body weights of male (A) and female (B) rats were measured on days 1, 8, 15, 22, and 28 during their exposure to 0–5.0 mg/kg/day of DPAA(V). Symbols show mean values, and vertical lines indicate SEM. $n = 3$, for each group.

administration of DMA(V) and found that the relationship between the dose of DMA(V) administered and its amount in tissues was nearly linear (Adair et al., 2007).

In the present study, we orally administered DPAA(V) to rats at incremental doses for 28 days. After the administration, the amounts of DPAA(V) deposition in 11 tissues were then measured by liquid chromatography–tandem mass spectrometry (LC–MS/MS). Furthermore, we measured the concentrations of pentavalent phenylarsonic acid (PAA[V]) and phenylmethylarsinic acid (PMAA[V]), which are known to be major metabolites of DPAA(V).

2. Materials and methods

2.1. Arsenic

Phenyl arsenic compounds (DPAA[V], diphenylmethylarsine oxide [DPMAO], PMAA[V], phenyldimethylarsine oxide [PDMAO], and PAA[V]) were purchased from Tri Chemical Laboratories (Yamanashi, Japan) and stored at 4 °C in the dark. Stable radioactive isotopes (^{13}C-DPAA[V], ^{13}C-DPMAO, ^{13}C-PMAA[V], ^{13}C-PDMAO, and ^{13}C-PAA[V]) were produced by Hayashi Pure Chemical Industries (Osaka, Japan).

2.2. Animals

Twenty-eight Crj:CD(SD) rats (6 weeks old; 12 males and 12 females) were obtained from Charles River Laboratories Japan, Inc. (Kanagawa, Japan). The administration of DPAA(V) to the rats and collection of their tissues were done at LSI Medience Corporation (Tokyo, Japan). All of the experiments were conducted according to the Guidelines for Proper Conduct of Animal Experiments, which were approved by the Science Council of Japan.

2.3. Experiments and the sampling of tissues

The rats were divided into four groups; each group consisted of 3 males and 3 females. Based on the previous evaluation that estimated

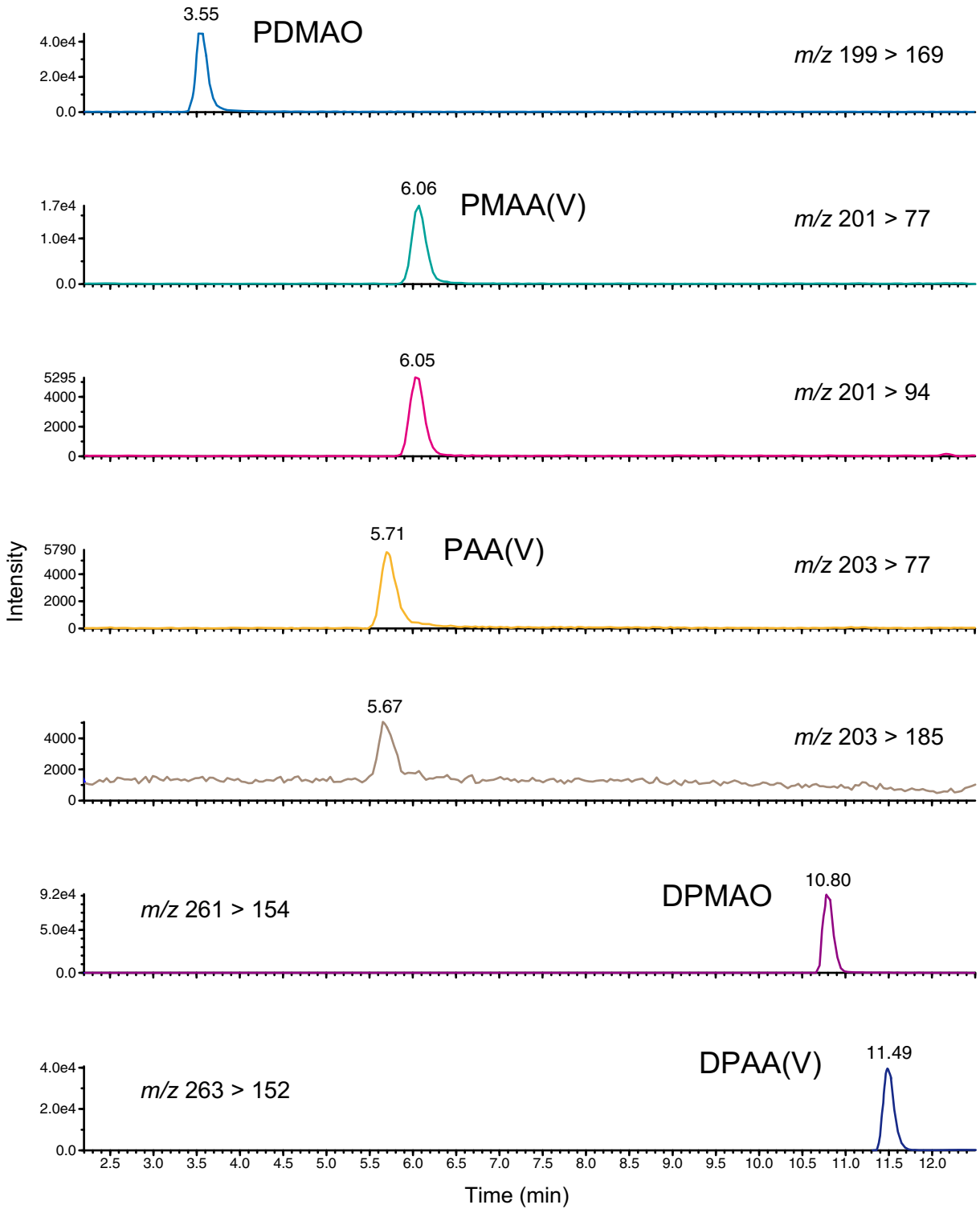

Fig. 2. A selected reaction monitoring chromatogram for phenyl arsenic compounds in aqueous solution. DPAA(V), DPMAO, PAA(V), PMAA(V), and PDMAO solutions (4 μL each) were injected into an LC–MS/MS system fitted with an Atlantis T3 column.

the exposed dose for the Kamisu cohort who developed CNS symptoms to be 0.62–0.93 mg/kg/day (Ishii et al., 2014), the rats were orally administered 0.3, 1.2, and 5.0 mg/kg/day of DPAA(V) in this study. DPAA(V) dissolved in distilled water was administered through an oral gastric tube for 28 days to 3 groups at each dose (see Table 1). Supplemental water was not administered to the control group.

After the animals were euthanized, tissue specimens (11 total) were excised and collected (Table 1). We selected these tissues with a focus on the CNS based on our previous findings using cynomolgus monkeys (Masuda et al., 2017). Five tissues were derived from the CNS (frontal-parietal lobe, temporal-occipital lobe, cerebellum, brainstem, and spinal cord). We also collected samples from the peripheral nervous system (PNS; sciatic nerve), excretory system (liver and kidneys), lymphatic system (spleen), respiratory system (lungs), and the muscular system (iliopsoas muscle). The samples were then frozen in liquid nitrogen and stored at − 80 °C.

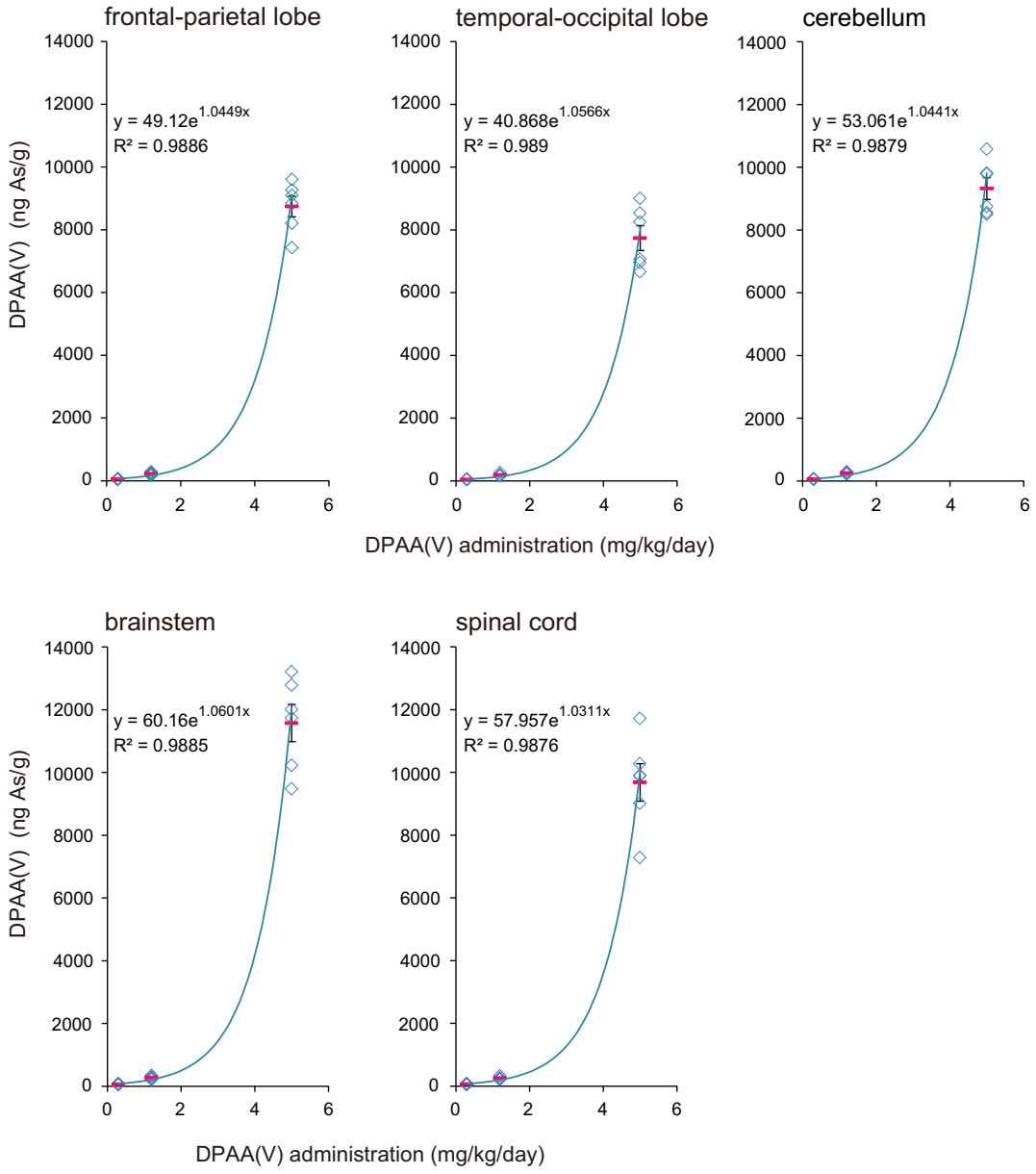

Fig. 3. The curves fit for exponential approximation show a correlation between the administered doses and the average concentrations of DPAA(V) (ng As/g) in the CNS tissue specimens The approximation curve obtained from 3 measured points shows the correlation between the administered doses and the average concentrations of DPAA(V) (ng As/g) in each CNS tissue specimen after administering DPAA(V) (0.3–5.0 mg/kg/day) for 28 days. The vertical line shows the concentration of DPAA(V), while the horizontal one shows the amount of DPAA(V) administered. The average values ± SEM are shown using horizontal lines.

2.4. Extraction of phenyl arsenic compounds

A Teflon homogenizer (Ikemoto Scientific Technology Co., Ltd., Tokyo, Japan) was used to process tissue samples, and 20% of each homogenate was prepared in 50 mM ammonium acetate solution. The separation of fluids from the tissues was performed using an ultra-centrifuge (105,000 × *g*) at 4 °C. Subsequently, 0.1 mL of bovine serum albumin (80 mg/mL; Takara Bio Inc., Shiga, Japan), 1 mL of 4 M NaOH, and 0.9 mL of H_2O were added to 0.1 g of the precipitates prepared above. The radioactive isotopes ^{13}C-DPAA(V), ^{13}C-DPMAO, ^{13}C-PMAA(V), ^{13}C-PDMAO, and ^{13}C-PAA(V) (each 100 ppb) were added to the solutions and incubated at 90 °C for 3 h. After adding diethyl ether (Wako Pure Chemical Industries, Ltd.: > 99.5%), the mixed solution was centrifuged for 5 min to extract phenyl arsenic compounds with diethyl ether. Using a stream of dry N_2, the diethyl ether was removed from the phenyl arsenic compounds. After the addition of nitric acid (Wako Pure Chemical Industries, Ltd.), the phenyl arsenic compounds were completely dissolved in 10 mL of H_2O.

2.5. Analysis of phenyl arsenic

Agilent 1200 Series (Agilent Technologies, Santa Clara, CA) coupled with 4000 Q TRAP LC–MS/MS System (AB Sciex, Framingham, MA) were used in this study. Chromatographic separation was achieved using Atlantis T3 columns (150 × 2.1 mm i.d., 3-μm thickness, Waters Corporation, Milford, MA) at 40 °C. The sample solutions (4 μL) were injected into the column. In the columns, elution was performed using a linear gradient of 0.1% formic acid (Wako Pure Chemical Industries, Ltd., 99.0%) in water [A] with 0.1% formic acid in methanol-water (1:9, v/v) [B] as follows: 80 to 10% [A]: 20 to 90% [B] (7.5 min), 10% [A]: 90% [B] (2-min hold), 10 to 80% [A]: 90 to 20% [B] (0.5 min), 80% [A]: 20% [B] (10-min hold). The flow rate was maintained at 0.2 mL/min. Electrospray ionization was performed in positive ionization mode. The $[M + H]^+$ ions of the targeted compounds (DPAA[V],

Table 2
DPAA(V) concentration in various non-CNS tissues after oral administration of DPAA(V).

		DPAA(V) after 0.3–5.0 mg/kg/day of DPAA(V) administration (average ng As/g ± SEM)							
		0.3 mg/kg/day (male)	0.3 mg/kg/day (female)	0.3 mg/kg/day (average)	1.2 mg/kg/day (male)	1.2 mg/kg/day (female)	1.2 mg/kg/day (average)	5.0 mg/kg/day (male)	5.0 mg/kg/day (female)	5.0 mg/kg/day (average)
Peripheral nervous system	Sciatic nerve	18.0 ± 4.60 (3)	19.0 ± 5.83 (3)	18.5 ± 3.33 (6)	120 ± 37.4 (3)	78.0 ± 25.6 (3)	98.8 ± 22.3 (6)	4591 ± 1326 (3)	4069 ± 1033 (3)	4330 ± 761 (6)
Excretory system	Liver	49.9 ± 6.06 (3)	27.9 ± 1.19 (3)	38.9 ± 5.66 (6)	199 ± 28.5 (3)	117 ± 9.67 (3)	158 ± 22.9 (6)	4970 ± 409 (3)	4153 ± 438 (3)	4562 ± 324 (6)
	Kidney	68.3 ± 1.18 (3)	129 ± 6.63 (3)	98.8 ± 14.0 (6)	231 ± 41.0 (3)	416 ± 36.6 (3)	324 ± 48.0 (6)	6786 ± 1522 (3)	5470 ± 491 (3)	6128 ± 774 (6)
Excretory system (average)		59.1 ± 2.94 (6)	78.6 ± 3.19 (6)	68.8 ± 4.77 (12)	215 ± 34.7 (6)	266 ± 23.1 (6)	241 ± 21.9 (12)	5878 ± 954 (6)	4811 ± 463 (6)	5345 ± 531 (12)
Lymphatic system	Spleen	25.1 ± 1.92 (3)	23.1 ± 1.89 (3)	24.1 ± 1.29 (6)	114 ± 3.10 (3)	85.1 ± 10.1 (3)	100 ± 8.00 (6)	7387 ± 1499 (3)	7216 ± 548 (3)	7301 ± 715 (6)
Respiratory system	Lung	27.8 ± 1.30 (3)	21.3 ± 1.55 (3)	24.6 ± 1.72 (6)	104 ± 12.0 (3)	75.0 ± 2.14 (3)	89.5 ± 8.47 (6)	6846 ± 1475 (3)	5789 ± 721 (3)	6318 ± 771 (6)
Muscular system	Iliopsoas muscle	8.54 ± 0.610 (3)	6.87 ± 0.330 (3)	7.71 ± 0.490 (6)	36.1 ± 2.61 (3)	24.2 ± 3.31 (3)	30.1 ± 3.25 (6)	1932 ± 108 (3)	1596 ± 127 (3)	1764 ± 106 (6)

The number between brackets indicates the number of samples examined.

^{13}C-DPAA[V], DPMAO, ^{13}C-DPMAO, PMAA[V], ^{13}C-PMAA[V], PDMAO, ^{13}C-PDMAO, PAA[V], ^{13}C-PAA[V]) were selected as the precursor ions. Selected reaction monitoring (SRM) was used to quantify each compound. The precursor and product ions were monitored. The collision energies of the compounds were as follows: m/z 263 > 152, 35 eV for DPAA(V); m/z 275 > 158, 35 eV for ^{13}C-DPAA(V); m/z 261 > 154, 35 eV for DPMAO; m/z 273 > 166, 35 eV for ^{13}C-DPMAO; m/z 203 > 77, 30 eV for PAA(V); m/z 209 > 83, 30 eV for ^{13}C-PAA(V); m/z 201 > 77, 50 eV for PMAA(V); m/z 207 > 83, 50 eV for ^{13}C-PMAA(V); m/z 199 > 169, 30 eV for PDMAO; and m/z 205 > 175, 30 eV for ^{13}C-PDMAO. To determine the compounds with greater precision, we monitored the other product ions as well: m/z 203 > 185 for PAA(V); m/z 201 > 94 for PMAA(V). The MS/MS parameters for the analysis were as follows: spray voltage, 4500 V; curtain gas pressure, 20 Pa; vaporizing temperature, 700 °C; collision gas pressure, 8 Pa. Data acquisition and instrument control were performed using the Analyst ver. 1.4 software (AB Sciex).

2.6. Statistical analyses

The Tukey–Kramer method was used for multiple group comparisons. The Kruskal-Wallis test was used for nonparametric statistics. Statistical analyses were performed using the JMP software program (version 5.12-J, SAS Institute Inc., Cary, NC, USA). p-Values of < 0.05 were considered statistically significant. The JMP software program (version 5.12-J) was also used to perform a curvilinear regression analysis, which allowed us to approximate the quadratic curves.

3. Results

3.1. General observations and weight changes

The 5.0 mg/kg/day of DPAA(V)-exposed group seemed to be less active than the control group. Additional symptoms including tremors and gait disturbance were observed in this group, as well as a decrease in mean body weight gain regardless of gender (Fig. 1). However, the body weight in the 5.0 mg/kg/day of DPAA(V)-exposed group was not significantly different from that in the other groups after 28-day exposure both genders (Kruskal-Wallis test, $p = 0.0765$–0.0809).

3.2. Liquid chromatography–tandem mass spectrometry

DPAA(V) and its related metabolites were identified in the tissue samples by LC–MS/MS using the isotope dilution method. These phenyl arsenic compounds were well separated under the LC conditions using Atlantis T3 columns. The $[M + H]^+$ ions of the target compounds were carefully observed by electrospray ionization. The sample solutions (4 μL) were injected into the LC–MS/MS system to achieve optimal detection sensitivity. During SRM, the product ions and collision energies were selected by optimizing the conditions in the product ion scan spectra of the target precursor ions. A typical SRM chromatogram for the control is shown in Fig. 2. Calibration curves were prepared for the peak area ratios of the target compounds of the corresponding ^{13}C–labeled internal standards on the respective SRM chromatograms. If positive peaks were observed in SRM, the presence of the corresponding product ion peaks was tested for PAA(V), PMAA(V), and PDMAO. This provides a definitive confirmation of the presence of PAA(V), PMAA(V), and PDMAO, as these highly hydrophilic compounds typically suffer from severe interference from the biological matrix components in sample extracts during LC separation and MS/MS detection.

3.3. Quantitative analysis of DPAA(V) in the CNS tissues

The exposed dose for the Kamisu residents who experienced CNS neuropathies has been estimated at 0.62–0.93 mg/kg/day (Ishii et al.,

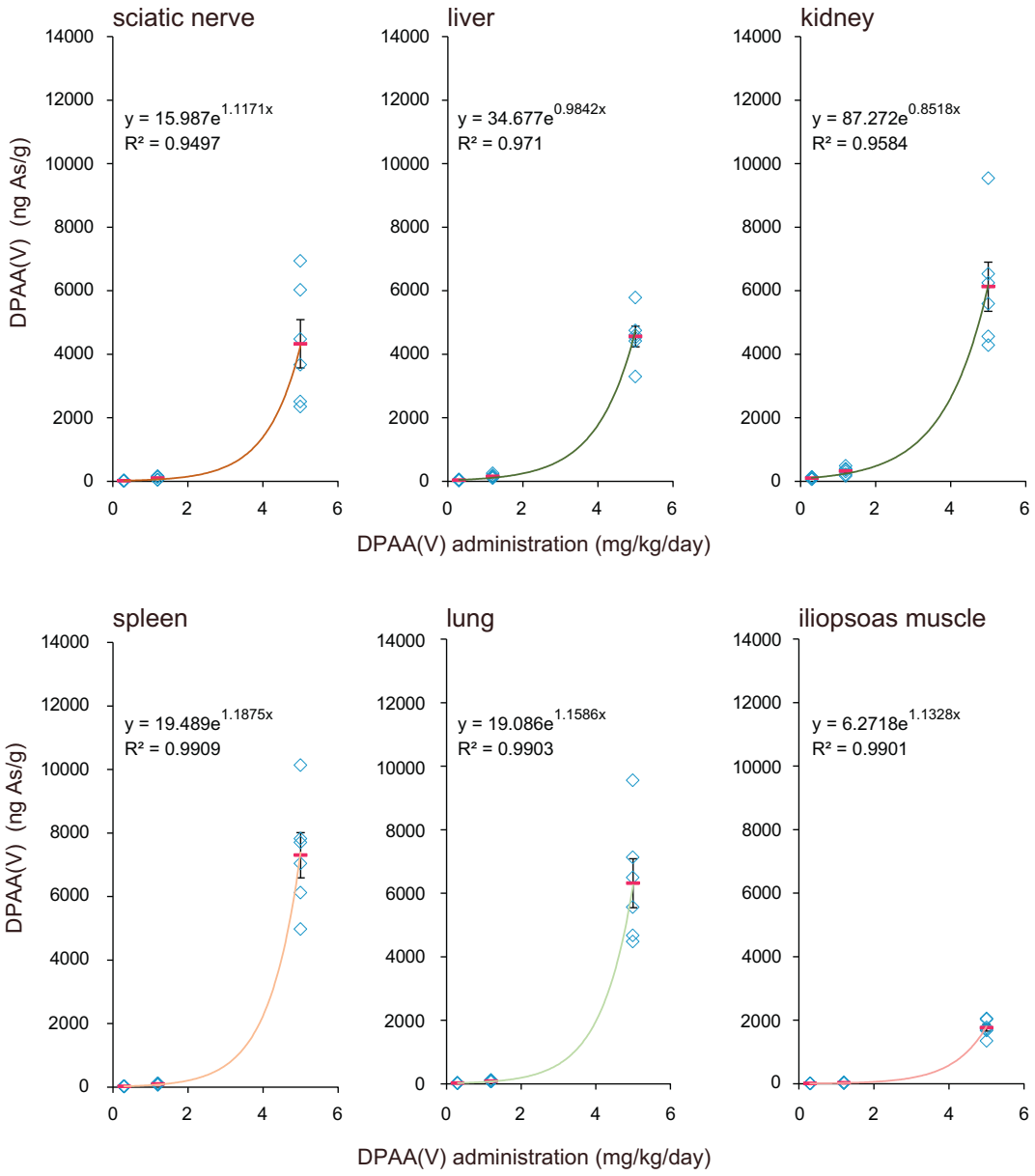

Fig. 4. The curves fit for exponential approximation show a correlation between the administered doses and the average concentrations of DPAA(V) (ng As/g) in various non-CNS tissues. The approximation curve obtained from 3 measured points shows the correlation between the administered doses and the average concentrations of DPAA(V) (ng As/g) in each tissue after administering DPAA(V) (0.3–5.0 mg/kg/day) for 28 days. The vertical line shows the concentration of DPAA(V), while the horizontal one shows the amount of DPAA(V) administered. The average values ± SEM are shown using horizontal lines.

2014). Based on this previous evaluation, rats were administered either 0.3, 1.2, or 5.0 mg/kg/day of DPAA(V) orally in the present study. The control group was administered water without DPAA(V). After administering DPAA(V) for 28 days, the CNS tissues (frontal-parietal lobe, temporal-occipital lobe, cerebellum, brainstem, and spinal cord) were collected, and the concentration of DPAA(V) was measured via LC–MS/MS (Table 1). The relationship between the exposed dose of DPAA(V) and its concentration is shown in Fig. 3. No DPAA(V) was detected in the tissues analyzed from rats in the control group (data not shown).

The concentrations of DPAA(V) in all of the CNS tissues showed a gradual increase with the dose that was administered. However, although DPAA(V) concentrations rose in the group administered 5.0 mg/kg/day of DPAA(V), it was not in a dose-dependent manner (Fig. 3). Namely, the relationship between doses and tissue concentrations of DPAA(V) was not linear in any of the CNS tissue specimens.

3.4. Quantitative analysis of DPAA(V) in various non-CNS tissues

To examine whether the rapid increase of DPAA(V) concentrations at 5.0 mg/kg/day of DPAA(V) administration may be characteristic of the CNS tissues, we selected tissues other than CNS ones (sciatic nerve, liver, kidneys, spleen, lungs, iliopsoas muscle) and measured their DPAA(V) concentrations after administering DPAA(V) for 28 days (Table 2). We found the same trend in the relationship between doses and tissue concentrations of DPAA(V), i.e. the absence of a linear dose-response relationship following administration (Fig. 4). In addition, we compared the concentration of DPAA(V) in the CNS tissues with those in the other tissues. At 5.0 mg/kg/day of DPAA(V) administration, we found that the average concentration of DPAA(V) in the CNS tissues was significantly higher than those in the other tissues ($^*p < 0.001$; Fig. 5).

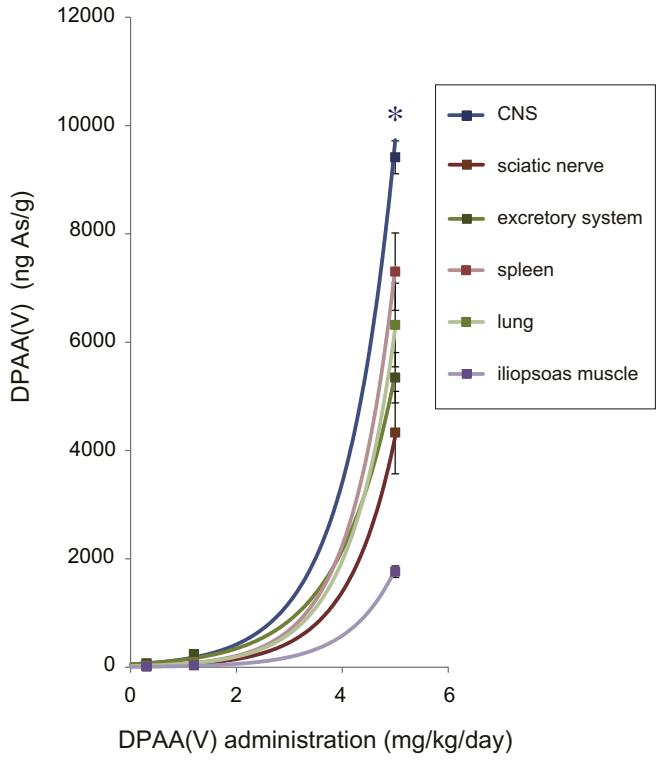

Fig. 5. Comparison of the curves fit for exponential approximation of all of the tissues. Each approximation curve shows the correlation between the administered doses and the average concentrations of DPAA(V) (ng As/g) in each tissue. The vertical line shows the concentration of DPAA(V), while the horizontal one shows the amount of DPAA(V) administered. The average values ± SEM are shown using horizontal lines. $^{*}p < 0.001$ when compared with the other tissues.

3.5. Sex differences in the tissue accumulation of DPAA(V)

We further analyzed the sex differences in the tissue accumulation of DPAA(V). In the 0.3 mg/kg/day of DPAA(V)-exposed group, the DPAA(V) concentrations in a part of the male tissues except for the sciatic nerve and the kidney were significantly higher than those in the female tissues. The significant differences in DPAA(V) concentration between the male and female tissues were as follow: the temporal-occipital lobe ($p = 0.0423$), brainstem ($p = 0.0365$), spinal cord ($p = 0.0200$), liver ($p = 0.0231$), kidneys ($p = 0.000900$), and lungs ($p = 0.0265$). In the 1.2 mg/kg/day of DPAA(V)-exposed group, the significant differences between the concentrations of male and female tissues were as follow: the frontal-parietal lobe ($p = 0.00660$), cerebellum ($p = 0.00820$), and kidneys ($p = 0.0282$). Except for the kidney, the DPAA(V) concentrations in the male tissues were significantly higher than those in the female tissues. In the group exposed to 5.0 mg/kg/day of DPAA(V), we could not detect any significant difference in the concentrations of DPAA(V) between male and female tissues.

3.6. Quantitative analysis of PMAA(V) and PAA(V) in the rat tissues

PMAA(V) and PAA(V) are known to be major metabolites of DPAA(V). Using LC–MS/MS, we measured the concentrations of PMAA(V) and PAA(V) in the same tissues from one male rat of each group. We detected very small amounts of PMAA(V) and PAA(V) in the 11 tissues after administering DPAA(V) (Table 3).

4. Discussion

In the present study, we measured the concentration of DPAA(V) in 5 CNS and 6 other non-CNS tissues after 28 days of DPAA(V) administration. When DPAA(V) was administered to rats at 5.0 mg/kg/day, the amount of DPAA(V) in each tissue rapidly increased as compared with that at 1.2 mg/kg/day, i.e. the relationship between the dose of DPAA(V) administered and its amount in tissues was nonlinear after high-dose DPAA(V) exposure.

There are two possible explanations for the rapid DPAA(V) accumulation after a high-dose exposure. Since DPAA(V) is known to be excreted in urine and feces via the liver and kidneys in mammals (Kobayashi et al., 2008; Kobayashi and Hirano, 2013), one possibility is renal impairment due to the exposure to DPAA(V). However, no chronic nephropathy was reported in the kidneys of DPAA(V)-exposed rats (Yamaguchi et al., 2017), suggesting that DPAA(V) may not play a role as a nephrotoxin. Thus, it is possible that DPAA(V) accumulated in the kidneys at concentrations that surpassed the rate of excretion by the kidneys.

The second potential explanation is the impairment of the liver function. Our histopathological observations in monkeys have shown that the subchronic administration of DPAA(V) induced hepatic changes, such as atypical ductular proliferation and cell infiltration in Glisson's capsules (unpublished observation). A separate study using rats also revealed that DPAA(V) is toxic in bile ducts when administered for 52 days (Yamaguchi et al., 2017). It is likely that the impairment of the liver function might induce a decrease in DPAA(V) excretion and subsequently cause DPAA(V) to accumulate rapidly in the tissues. To clarify the toxic mechanism of DPAA(V) in vivo, further analyses will be required including determination of the principal cause of the rapid

Table 3
PMAA(V) and PAA(V) concentration in the tissues of rats after oral administration of DPAA(V).

		PMAA(V) and PAA(V) after 0.3–5.0 mg/kg/day of DPAA(V) administration (average ng As/g ± SEM)					
		0.3 mg/kg/day		1.2 mg/kg/day		5.0 mg/kg/day	
		PMAA(V)	PAA(V)	PMAA(V)	PAA(V)	PMAA(V)	PAA(V)
Central nervous system	Frontal-parietal lobe	23.3	ND	22.1	ND	23.4	40.5
	Temporal-occipital lobe	ND	ND	ND	ND	17.1	17.7
	Cerebellum	ND	ND	ND	ND	16.1	37.9
	Brainstem	ND	ND	ND	ND	16.7	72.8
	Spinal cord	ND	ND	ND	ND	30.0	15.8
Peripheral nervous system	Sciatic nerve	ND	ND	ND	ND	18.4	4.07
Excretory system	Liver	ND	ND	1.94	1.63	64.1	20.6
	Kidney	ND	ND	1.93	1.81	93.2	41.3
Lymphatic system	Spleen	1.25	ND	5.60	ND	283	18.7
Respiratory system	Lung	ND	ND	2.82	ND	152	12.0
Muscular system	Iliopsoas muscle	ND	ND	ND	ND	32.7	5.38

ND; not detected.

DPAA(V) accumulation after high-dose exposure.

The present study showed that the concentrations of DPAA(V) in the CNS tissues at 5.0 mg/kg/day of DPAA(V) administration were significantly higher than those in the other tissues. Our previous results showed that DPAA(V) was detected in the cerebrospinal fluid obtained from both the Kamisu residents and DPAA(V)-exposed monkeys (Ishii et al., 2014; Masuda et al., 2017). These findings suggest that DPAA(V) can pass through the blood-brain barrier (BBB) in primates. Although we did not examine the DPAA(V) concentration in the cerebrospinal fluid in the present study, the amount of DPAA(V) in the rat CNS suggests that DPAA(V) may also pass through the BBB of rodents and enter the CNS. In the previous study, we showed that DPAA(V) was barely excreted from the CNS tissues after passing through the BBB in monkeys (Masuda et al., 2017). Along the same lines, it is highly likely that the low excretion capacity of DPAA(V) may result in a large amount of DPAA(V) deposition in the rat CNS.

The estimated dose of DPAA(V) exposure per day (0.93 mg/kg/day) in the Kamisu cohort (Ishii et al., 2014) is smaller than that in this study. However, the residents in Kamisu with neurologic symptoms had been drinking the contaminated water at that concentration daily for > 3 years, whereas we set the duration of exposure in our present study to a much shorter one (28 days). Considering the short duration of exposure, it is reasonable in this study to have prepared a larger dose for the daily exposure than in the Kamisu cohort. Prior to this study, a preliminary experiment of oral administration of DPAA(V) to rats at doses of 1.7–170 mg/kg/day for 14 days was carried out (data not shown). Based on the results of that preliminary experiment, we decided to set 5.0 mg/kg/day of DPAA(V) as a high dose; because we expected that rats would not die during the 28-day administration period. If the duration of exposure is not taken into consideration, the DPAA(V) doses of 0.3–1.2 mg/kg/day at which the linear DPAA(V) accumulation was shown correspond to the dose of daily exposure in Kamisu residents. The absence of remarkable symptoms in Kamisu residents other than the CNS ones predicted that there might be no rapid accumulation in the tissues other than the CNS by DPAA(V) exposure at 0.93 mg/kg/day. Results obtained from the doses of 0.3–1.2 mg/kg/day in this study support this prediction. Our previous results using monkeys showed that the clearance rate of DPAA(V) in the CNS is quite low compared with that in other tissues, resulting in the high accumulation of DPAA(V) in the CNS tissues for a long period (Masuda et al., 2017). Along the same line, in the case of Kamisu residents, the long-term exposure to relatively low doses of DPAA(V) might have induced the high accumulation of DPAA(V) only in the CNS, resulting in the onset of their neurological symptoms.

In this study, we analyzed DPAA(V) accumulation in the tissues following its administration to equal numbers of male and female rats. Several tissues showed sex differences in DPAA(V) accumulation in each group, and the results obtained were divided into cases where either male or female concentrations were higher. There is room for argument about the results obtained in this research because we used a limited number of samples. Further detailed analysis of sex differences in DPAA(V) accumulation may be required in the future.

To our knowledge, the present study is the first report that comprehensively analyzed the relationship between the dose and tissue condensation of compounds of organic arsenic(V) via the high-sensitivity quantitative method of LC–MS/MS. We hope our comprehensive results will be utilized as the fundamental data of tissue distributions of DPAA(V) and thus may contribute to the future treatment of DPAA(V)-exposed patients and the prevention of arsenic poisoning in humans.

Conflict of interest

The authors declare no conflicts of interest in association with the present study.

Transparency document

The Transparency document associated with this article can be found, in online version.

Acknowledgements

This work was supported by JSPS KAKENHI Grant Nos. JP20390039, JP16590811 and JP15K08148 from the program Grants-in-Aid for Scientific Research of the MEXT, Japan, and a Grant-in-Aid for Research on Study (toxic study group) of the Health Effects of DPAA supported by the Ministry of the Environment, Japan.

References

Adair, B.M., Moore, T., Conklin, S.D., Creed, J.T., Wolf, D.C., Thomas, D.J., 2007. Tissue distribution and urinary excretion of dimethylated arsenic and its metabolites in dimethylarsinic acid- or arsenate-treated rats. Toxicol. Appl. Pharmacol. 222, 235–242. http://dx.doi.org/10.1016/j.taap.2007.04.012.

Cohen, S.M., Arnold, L.L., Eldan, M., Lewis, A.S., Beck, B.D., 2006. Methylated arsenicals: the implications of metabolism and carcinogenicity studies in rodents to human risk assessment. Crit. Rev. Toxicol. 36, 99–133.

Ishii, K., Tamaoka, A., 2015. Ten-years records of organic arsenic (diphenylarsinic acid) poisoning: epidemiology, clinical feature, metabolism, and toxicity. Brain Nerve 67, 5–18. http://dx.doi.org/10.11477/mf.1416200081.

Ishii, K., Tamaoka, A., Otsuka, F., Iwasaki, N., Shin, K., Matsui, A., Endo, G., Kumagai, Y., Ishii, T., Shoji, S., Ogata, T., Ishizaki, M., Doi, M., Shimojo, N., 2004. Diphenylarsinic acid poisoning from chemical weapons in Kamisu, Japan. Ann. Neurol. 56, 741–745. http://dx.doi.org/10.1002/ana.20290.

Ishii, K., Itoh, Y., Iwasaki, N., Shibata, Y., Tamaoka, A., 2014. Detection of diphenylarsinic acid and its derivatives in human serum and cerebrospinal fluid. Clin. Chim. Acta 431, 227–231. http://dx.doi.org/10.1016/j.cca.2014.01.029.

Kinoshita, K., Shida, Y., Sakuma, C., Ishizaki, M., Kiso, K., Shikino, O., Ito, H., Morita, M., Ochi, T., Kaise, T., 2005. Determination of diphenylarsinic acid and phenylarsonic acid, the degradation products of organoarsenic chemical warfare agents, in well water by HPLC-ICP-MS. Appl. Organomet. Chem. 19, 287–293. http://dx.doi.org/10.1002/aoc.789.

Kobayashi, Y., Hirano, S., 2013. The role of glutathione in the metabolism of diphenylarsinic acid in rats. Metallomics 5, 469–478. http://dx.doi.org/10.1039/C2MT20228J.

Kobayashi, Y., Negishi, T., Mizumura, A., Watanabe, T., Hirano, S., 2008. Distribution and excretion of arsenic in cynomolgus monkey following repeated administration of diphenylarsinic acid. Arch. Toxicol. 82, 553–561. http://dx.doi.org/10.1007/s00204-007-0270-x.

Kurata, H., 1980. Lessons learned from the destruction of the chemical weapons of the Japanese Imperial Forces. In: Stockholm International Peace Research Institute (Ed.), Chemical Weapons: Destruction and Conversion. Taylor and Francis, London, pp. 77–93.

Masuda, T., Ishii, K., Seto, Y., Hosoya, T., Tanaka, R., Nakayama, T., Iwasaki, N., Shibata, Y., Tamaoka, A., 2017. Long-term accumulation of diphenylarsinic acid in the central nervous system of cynomolgus monkeys. Arch. Toxicol. 91, 2799–2812. http://dx.doi.org/10.1007/s00204-016-1928-z.

Shibata, Y., Tsuzuku, K., Komori, S., Umedzu, C., Imai, H., Morita, M., 2005. Analysis of diphenylarsinic acid in human and environmental samples by HPLC-ICP-MS. Appl. Organomet. Chem. 19, 276–281. http://dx.doi.org/10.1002/aoc.792.

Watanabe, S., Egusa, N., Hirata, T., Yokoyama, N., Yamazato, Y., Morita, M., 2011. Numerical simulation on groundwater contamination by organoarsenic compounds in Kamisu City, Ibaraki Prefecture, Japan. Jiban-Kogaku J. 6, 383–394. http://dx.doi.org/10.3208/jgs.6.383.

Yamaguchi, T., Gi, M., Yamano, S., Fujioka, M., Tatsumi, K., Kawachi, S., Ishii, N., Doi, K., Kakehashi, A., Wanibuchi, H., 2017. A chronic toxicity study of diphenylarsinic acid in F344 rats in drinking water for 52 weeks. Exp. Toxicol. Pathol. 69, 1–7. http://dx.doi.org/10.1016/j.etp.2016.10.002.