J Occup Health 2016; 58: 196-200

Journal of Occupational Health

Original

A biological indicator of inorganic arsenic exposure using the sum of urinary inorganic arsenic and monomethylarsonic acid concentrations

Akihisa Hata[1], Hidetoshi Kurosawa[2,3], Yoko Endo[4], Kenzo Yamanaka[2], Noboru Fujitani[1] and Ginji Endo[5]

[1]Department of Medical Risk Management, Graduate School of Risk and Crisis Management, Chiba Institute of Science, Chiba, Japan, [2]Laboratory of Environmental Toxicology and Carcinogenesis, School of Pharmacy, Nihon University, Chiba, Japan, [3]Criminal Investigation Laboratory, Metropolitan Police Department, Tokyo, Japan, [4]Research Center for Occupational Poisoning, Kansai Rosai Hospital, Japan Labor Health and Welfare Organization, Hyogo, Japan and [5]The Institute for Science of Labour, Kawasaki, Japan

Abstract: Objectives: The sum of urinary inorganic arsenic (iAs), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA) concentrations is used for the biological monitoring of occupational iAs exposure. Although DMA is a major metabolite of iAs, it is an inadequate index because high DMA levels are present in urine after seafood consumption. We estimated the urinary iAs+MMA concentration corresponding to iAs exposure. **Methods:** We used data from two arsenic speciation analyses of urine samples from 330 Bangladeshi with oral iAs exposure and 172 Japanese workers without occupational iAs exposure using high-performance liquid chromatography with inductively coupled plasma mass spectrometry. **Results:** iAs, MMA, and DMA, but not arsenobetaine (AsBe), were detected in the urine of the Bangladeshi subjects. The correlation between iAs+MMA+DMA and iAs+MMA was obtained as log (iAs+MMA) = 1.038 log (iAs+MMA+DMA) − 0.658. Using the regression formula, the iAs+MMA value was calculated as 2.15 and 7.5 μg As/*l*, corresponding to 3 and 10 μg As/m^3 of exposures, respectively. In the urine of the Japanese workers, arsenic was mostly excreted as AsBe. We used the 95th percentile of iAs+MMA (12.6 μg As/*l*) as the background value. The sum of the calculated and background values can be used as a biological indicator of iAs exposure. **Conclusion:** We propose 14.8 and 20.1 μg As/*l* of urinary iAs+MMA as the biological indicators of 3 and 10 μg As/m^3 iAs exposure, respectively.
(J Occup Health 2016; 58: 196-200)
doi: 10.1539/joh.15-0241-OA

Key words: Arsenic, Biological monitoring, Occupational exposure, Oral exposure, Speciation

Introduction

Arsenic comes from mineral weathering and volcanic activity and is carried to the sea by groundwater and rivers. Inorganic arsenic (iAs) is found in drinking water, and an organic arsenic compound in addition to iAs is found in food. iAs species are metabolized to monomethylarsonic acid (MMA) and subsequently to dimethylarsinic acid (DMA) in humans[1)]. The total concentration of iAs and its metabolites, MMA and DMA, excreted in urine has been used for the biological monitoring of occupational iAs exposure by the American Conference of Governmental Industrial Hygienists (ACGIH) and Deutsche Forschungsgemeinschaft (DFG)[2,3)]. In addition to DMA, methylated arsenics such as arsenobetaine (AsBe), arsenocholine (AsCho), and arsenosugars (AsSugs) are abundant in seafood products[1,4)]. Although AsBe is only minimally metabolized in mammals[5)], AsCho and AsSug are extensively metabolized to AsBe and DMA[6,7)], respectively. Therefore, large amounts of AsBe and DMA and small amounts of MMA and iAs have been observed in the urine of seafood consumers without occupational iAs exposure[8-11)]. In our previous reports, for instance, the me-

Received August 28, 2015; Accepted January 12, 2016
Published online in J-STAGE March 24, 2016
Correspondence to: A. Hata, Department of Medical Risk Management, Graduate School of Risk and Crisis Management, Chiba Institute of Science, 15-8 Shiomicho, Choshi City, Chiba 288-0025, Japan (e-mail: ahata@cis.ac.jp)

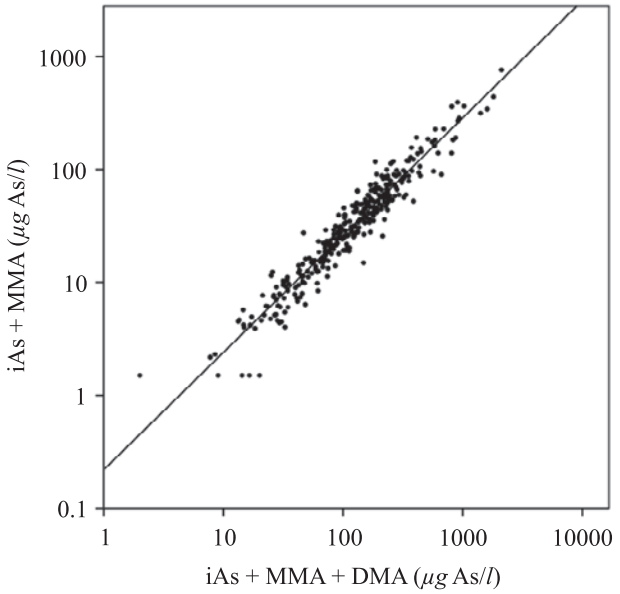

Fig. 1. Result of the regression analysis between x = [inorganic arsenic (iAs) + monomethylarsonic acid (MMA) + dimethylarsinic acid (DMA)] and y = [iAs+MMA], log y = 1.038 log x – 0.658 (r = 0.962, $p < 0.0001$).

dian urinary concentrations of DMA, AsBe, and total arsenic concentration of 172 male workers without arsenic exposure were 41.1, 74.5, and 132.2 μg As/*l*, respectively[11].

Because DMA is a major metabolite of iAs but is also excreted in urine after seafood consumption[12-14], the use of urinary DMA level for the biological monitoring of occupational iAs exposure is inadequate.

Therefore, to provide a possible method for the biological monitoring of occupational iAs exposure, we used the data of two arsenic speciation analyses of urine[11,15]. We attempted to use the sum of the urinary levels of iAs and MMA as an indicator of iAs exposure by using urinary samples from Bangladeshi who did not eat seafood but had chronic iAs exposure from drinking water. In addition, we used the 95th percentile value of urinary levels obtained from healthy Japanese workers without occupational iAs exposure as the background level as well as the DFG definition[16].

Subjects and Methods

Subjects

We used the following two different populations: 1) the residents of 17 villages in the Pabna District of Bangladesh who were chronically exposed to inorganic arsenic through drinking water; and 2) Japanese male workers without occupational iAs exposure. The profiles of these subjects were described in our previous reports[11,15]. The 330 Bangladeshi participants (165 men and 165 women) had a mean age of 38.8±11.0 years (range, 20-77 years), whereas the 172 Japanese participants were healthy male workers with a mean age of 46.5±13.6 years (range, 18-74 years) who had no occupational arsenic exposure for at least 6 months. Urine samples were collected and stored as described in our previous reports[11,15]. These studies were approved by the relevant ethics committees.

Arsenic speciation analysis

A urinary arsenic speciation analysis was performed using high-performance liquid chromatography (HPLC) with inductively coupled plasma mass spectrometry. The details, including the chemicals used, sample preparation, analytical conditions, and analytical procedure validation, are described in our previous reports[10,11].

Extrapolation of urinary arsenic concentration resulting from oral exposure to inhalation exposure

The Japanese government ministry defines the sum of iAs and MMA levels in the urine as the iAs exposure indicator, and its measurement is the second item of the special health examination of hazardous chemicals[17]. The Japanese Administrative Level is set at 3 μg As/m^3 of iAs exposure[17]. An extrapolation is performed under the following assumptions used by ACGIH[2]: 1) pulmonary ventilation is 10 m^3/8 h; 2) absorption is 60%; 3) 60% of the absorbed dose is recovered in urine; 4) urinary arsenic concentration remains consistent during the day; and 5) daily urine output is 1.2 *l*.

Calculations were performed using the SPSS statistical package (PASW SPSS Statistic version 18; IBM Japan, Tokyo, Japan)

Results

In a Bangladeshi study[15], arsenite (AsIII), arsenate (AsV), MMA, and DMA were detected in urine samples, whereas AsBe, which is excreted as a result of seafood ingestion, were not detected. The unidentified arsenic compounds are together termed "others." The median concentrations (95th percentile) of AsIII, AsV, MMA, DMA, and others were 16.8 (82.2), 1.8 (9.2), 13.7 (75.4), 88.6 (381.2), and <0.5 (1.9) μg As/*l*, respectively. DMA was the most abundant compound, followed by AsIII, MMA, AsV, and others.

No significant differences were found in the ratios of iAs, MMA, and DMA between sexes or among subjects of different ages. Therefore, we combined the data for men and women. Fig. 1 presents the scatter plots of x = iAs+MMA+DMA and y = iAs+MMA on both logarithmic coordinates. The regression formula was log y = 1.038 log x − 0.658, whereas the correlation coefficient (r) was 0.962 (p<0.0001).

In a Japanese study[11], the AsBe concentration was the highest and more than half the subjects preferred eating

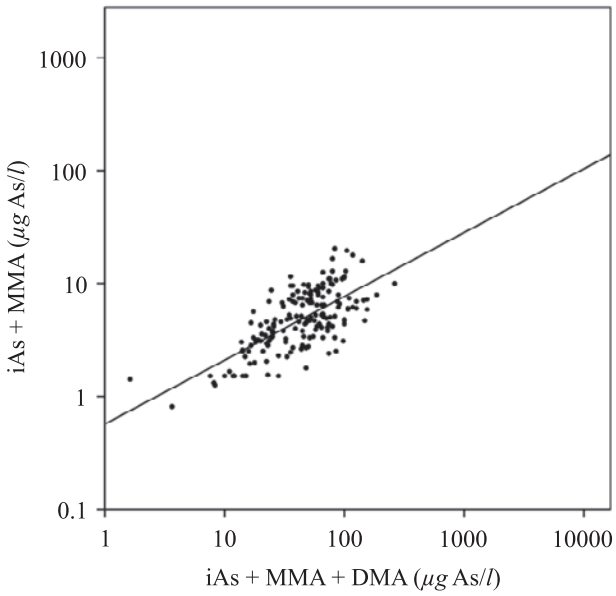

Fig. 2. The scatter plots of the data obtained from the Japanese workers; x = iAs + MMA + DMA and y = iAs+MMA on both logarithmic coordinates. The regression formula was $\log y = 0.725 \log x - 0.547$ ($r = 0.698$, $p < 0.0001$).

seafood than meat[10]. The median concentrations (95th percentile) of AsIII, AsV, MMA, DMA, AsBe, and others were 1.5 (5.4), <0.5 (1.7), 2.3 (6.2), 41.1 (109.2), 74.5 (243.7), and 4.1 (23.7) μg As/*l*, respectively. The median and 95th percentile of the urinary iAs+MMA concentrations were 4.4 and 12.6 μg As/*l*, respectively. Fig. 2 presents the scatter plots of the data obtained from the Japanese workers. The regression formula was $\log y = 0.725 \log x - 0.547$, and the correlation coefficient (r) was 0.698 ($p < 0.0001$).

We estimated the sum of the urinary iAs and MMA concentrations at 3 μg As/m^3 of exposure using the assumptions described in the Subjects and Methods section. A worker absorbs 18 μg of arsenic per day, of which 10.8 μg is excreted in the urine. A urinary concentration of 9 μg As/*l* is substituted for x in the equation $\log y = 1.038 \log x - 0.658$. The value of 2.15 μg As/*l* is obtained as y (iAs+MMA). When we consider the sum of the 95th percentile of the Japanese workers' iAs and MMA concentration of 12.6 μg As/*l* to be the background level, 14.8 μg As/*l* is assumed to be the biological exposure index corresponding to the Japanese Administrative Level of 3 μg As/m^3. Similarly, 20.1 μg As/*l* is assumed to be 10 μg As/m^3 of iAs exposure.

Discussion

For the biological monitoring of exposure to iAs, the seafood intake should not interfere with the urinary concentrations of iAs metabolites[2,3]. ACGIH and DFG use the total concentration of urinary iAs+MMA+DMA, excluding AsBe, for the biological monitoring of occupational exposure to iAs[2,16], because AsBe is the main arsenic compound in fish and shellfish[4,18]. ACGIH reported that the urinary arsenic concentrations in the general population were approximately 10 μg As/*l* in European countries and the United States and approximately 50 μg As/*l* in Japan[2]. DFG defines the Biologische Arbeitsstoff-Referenzwerte (BARs, biological reference values for workplace substances) as the background levels present at a particular time in a reference population of persons of working age who are not occupationally exposed to the substances; the BARs are based on the 95th percentile without taking the effects on health into consideration. DFG set BARs for As^{3+}, As^{5+}, MMA, and DMA as 0.5, 0.5, 2, and 10 μg As/*l*, respectively[16]. These BARs are consistent with the 95th percentile of urinary levels obtained from 82 German adults with no occupational exposure[19].

By contrast, the urinary levels of arsenic of the Japanese subjects who were not occupationally exposed to iAs but were exposed to organic arsenic from food were reported to be much higher than those reported in the German data. Mohri *et al.*[8] reported that the mean daily intake of dietary arsenic was 182 μg As (range, 27-376) for 4 Japanese volunteers. The mean amount of total arsenic eliminated daily in urine was 148 μg As (range, 50-416) and composed of 1.4% iAs, 3.5% MMA, and 33.6% DMA. Yamauchi *et al.*[9] reported that the mean urinary total arsenic level in 56 healthy Japanese volunteers was 129±92.0 μg As/*l*, composed of 6.7% iAs, 2.2% MMA, and 26.7% DMA. Urinary arsenic levels in Japanese people are almost equal to or higher than 50 μg As/*l* of iAs+MMA+DMA[8-11]. The mean concentration of iAs+MMA in urine reported by Mohri *et al.* and Yamauchi *et al.* were calculated to be 7.3 μg As/day and 11.5 μg As/*l*, respectively. These values are within the range of the median and 95th percentile levels (4.4 and 12.6 μg As/*l*, respectively) reported in our previous results[11].

AsSugs are the major arsenic compounds in seaweeds[18,20,21]. In particular, many studies have reported detecting DMA in urine after consumption of seaweed[12,13,22,23]. AsSugs, the majority of which become DMA, are broken down in the body and then excreted[7,24-26]. Li *et al.*[18] reported that iAs were detected at levels below 2% of the total arsenic in fish or shellfish samples of Chinese seafood. However, they were not detected in algae samples. Buchet *et al.*[12] found no statistically significant difference in the urinary MMA concentration between subjects who regularly consumed seafood and those who did not. Apostoli *et al.*[27] observed that iAs and MMA had shorter biological half-lives than DMA. Thus, it can be concluded that seafood consumption has only a small effect on the urinary concentrations of iAs and MMA.

A simulation study presumed that 25 μg As/g of creatinine as the sum of arsenic metabolites corresponds to 10 μg As/m^3 of inhalation exposure[2]. In this simulation, the background level was assumed to be zero.

DFG recommends 50 μg As/*l* as the exposure equivalent for carcinogenic substances at 0.01 mg As/m^3 in the air[16]. Because the background level (As^{3+}+As^{5+}+MMA+DMA) established by DFG is 13 μg As/*l*, the urinary excretion from inhalation exposure at 10 μg As/m^3 was considered to be 37 μg As/*l*.

The Bangladeshi subjects in this study had no interference from seafood ingestion; therefore, they were considered to have a zero background level, and the regression formula was based on a zero background level.

Under the regression formula $\log y = 1.038 \log x - 0.658$, when we use x = 37 μg As/*l* of urinary iAs+MMA+DMA corresponding to iAs exposure at 10 μg As/m^3, urinary iAs+MMA level is assigned as y = 9.3 μg As/*l*. Using the assumptions by ACGIH as mentioned in the Methods section, the urinary iAs+MMA level was estimated to be 7.5 μg As/*l* at 10 μg As/m^3 of exposure. These concentrations were considered to be nearly comparable.

We estimated the background level of the subjects exposed to arsenic from seafood but not occupationally exposed to iAs to be 12.6 μg As/*l* (the 95th percentile of urinary iAs+MMA)[11]. We propose 14.8 and 20.1 μg As/*l* of urinary iAs+MMA for the biological monitoring of 3 and 10 μg As/m^3 of iAs exposure, respectively.

This study has two limitations. First, the Bangladeshi subjects were exposed to iAs not by inhalation but through drinking water. Farmer and Johnson[28] reported that the ranges in the average urinary arsenic speciation pattern in workers who occupationally inhaled inorganic arsenic were 11%-14% AsIII, 1%-6% AsV, 14%-18% MMA, and 63%-70% DMA. When we compared the urinary analysis results from various populations exposed to iAs-contaminated water using HPLC separation, the proportions of urinary iAs, MMA, and DMA were 11.4%-34.0%, 7.5%-26.9%, and 47.7%-78.8%, respectively[15]. Although a report that used a physiologically based pharmacodynamic model suggested that the ratio of iAs was slightly higher in those exposed to arsenic through the respiratory tract than those exposed orally[29], the proportions obtained by workers who inhaled arsenic were similar to those in Asian people with oral iAs exposure. Moreover, the iAs absorption and excretion rates from repeated oral exposure was estimated to be approximately 60%[30], and ACGIH extrapolated these rates to estimate urinary concentration resulting from inhalation exposure[2]. Because the normal range for 24-hour urine volume was reported to be 0.8-2.0[31] or 1.0-1.5 *l*/day[32], the excretion volume of urine set at 1.2 *l*/day under this assumption is almost the median of that of healthy adults. Thus, we can use the data of the Bangladeshi residents who were orally exposed to arsenic for the Japanese workers who were exposed to arsenic through the respiratory tract.

Second, the biological half-lives of iAs and MMA are shorter than that of DMA[27,33,34]. The sampling times of the sum of iAs, MMA, and DMA were proposed to be at the end of the workweek by ACGIH and after several shifts by DFG. Therefore, the urinary sampling time of the sum of iAs and MMA should be applied to the end of the workshift.

Acknowledgments: We would like to give heartfelt thanks to Dr. Mohamed Ahsan Habib whose cooperation was invaluable throughout the course of our study. This work was supported in part by a Grant-in-Aid for Scientific Research (C) (No. 26460176) from the Ministry of Education, Culture, Sports, Science and Technology of Japan; a Grant-in-Aid for Young Scientists (B) (26860442) from the Ministry of Education, Culture, Sports, Science and Technology of Japan; and a grant from the Food Safety Commission, Cabinet Office, Government of Japan (Research Program for Risk Assessment Study on Food Safety, No. 1407).

References

1) International Programme on Chemical Safety (IPCS). Arsenic and arsenic compounds. 2nd ed. Geneva: WHO; 2001.
2) American Conference of Governmental Industrial Hygienists (ACGIH). Arsenic and soluble inorganic compounds [CD-ROM]. Cincinnati: ACGIH; 2001.
3) Deutsche Forschungsgemeinschaft (DFG). Arsenic and inorganic arsenic compounds. In: Drexler H, Greim H, editors. Essential BAT Value Documentations. Weinheim: Wiley-VCH; 2006. p. 77-84.
4) Hirata S, Toshimitsu H, Aihara M. Determination of arsenic species in marine samples by HPLC-ICP-MS. Anal Sci 2006; 22: 39-43.
5) Vahter M, Marafante E, Dencker L. Metabolism of arsenobetaine in mice, rats and rabbits. Sci Total Environ 1983; 30: 197-211.
6) Marafante E, Vahter M, Dencker L. Metabolism of arsenocholine in mice, rats and rabbits. Sci Total Environ 1984; 34: 223-240.
7) Francesconi KA, Tanggaar R, McKenzie CJ, Goessler W. Arsenic metabolites in human urine after ingestion of an arsenosugar. Clin Chem 2002; 48: 92-101.
8) Mohri T, Hisanaga A, Ishinishi N. Arsenic intake and excretion by Japanese adults: a 7-day duplicate diet study. Food Chem Toxicol 1990; 28: 521-529.
9) Yamauchi H, Mashiko M, Saitoh J, Yamamura Y. Intake of different chemical species of dietary arsenic by the Japanese, and their blood and urinary arsenic levels. Appl Organomet Chem 1992; 6: 383-388.
10) Hata A, Endo Y, Nakajima Y, et al. HPLC-ICP-MS speciation analysis of arsenic in urine of Japanese subjects without occupational exposure. J Occup Health 2007; 49: 217-223.

11) Suzuki Y, Shimoda Y, Endo Y, Hata A, Yamanaka K, Endo G. Rapid and effective speciation analysis of arsenic compounds in human urine using anion-exchange columns in HPLC-ICP-MS. J Occup Health 2009; 51: 380-385.
12) Buchet JP, Lison D, Ruggeri M, Foa V, Elia G. Assessment of exposure to inorganic arsenic, a human carcinogen, due to the consumption of seafood. Arch Toxicol 1996; 70: 773-778.
13) Heinrich-Ramm R, Mindt-Prufert S, Szadkowski D. Arsenic species excretion after controlled seafood consumption. J Chromatogr B Analyt Technol Biomed Life Sci 2002; 778: 263-273.
14) Morton J, Mason H. Speciation of arsenic compounds in urine from occupationally unexposed and exposed persons in the U.K. using a routine LC-ICP-MS method. J Anal Toxicol 2006; 30: 293-301.
15) Hata A, Yamanaka K, Habib MA, Endo Y, Fujitani N, Endo G. Arsenic speciation analysis of urine samples from individuals living in an arsenic-contaminated area in Bangladesh. Environ Health Prev Med 2012; 17: 235-245.
16) Deutsche Forschungsgemeinschaft (DFG). Assessment values in biological material. In: Hartwig A, editor. List of MAK and BAT values 2015. Weinheim, Germany: Wiley-VCH; 2015. p. 224-253.
17) Ministry of Health, Labour and Welfare in Japan. An ordinance to revise the portions of the Ordinance on Industrial Safety and Health. (Ordinance of the Ministry of Health, Labour and Welfare No. 158 of 2008); 2008.
18) Li W, Wei C, Zhang C, Van Hulle M, Cornelis R, Zhang X. A survey of arsenic species in Chinese seafood. Food Chem Toxicol 2003; 41: 1103-1110.
19) Heitland P, Koster HD. Fast determination of arsenic species and total arsenic in urine by HPLC-ICP-MS: concentration ranges for unexposed German inhabitants and clinical case studies. J Anal Toxicol 2008; 32: 308-314.
20) Llorente-Mirandes T, Ruiz-Chancho MJ, Barbero M, Rubio R, Lopez-Sanchez JF. Determination of water-soluble arsenic compounds in commercial edible seaweed by LC-ICPMS. J Agric Food Chem 2011; 59: 12963-12968.
21) Morita M, Shibata Y. Chemical form of arsenic in marine macroalgae. Appl Organometal Chem 1990; 4: 181-190.
22) Van Hulle M, Zhang C, Schotte B, et al. Identification of some arsenic species in human urine and blood after ingestion of Chinese seaweed Laminaria. J Anal At Spectrom 2004; 19: 58-64.
23) Ma M, Le XC. Effect of arsenosugar ingestion on urinary arsenic speciation. Clin Chem 1998; 44: 539-550.
24) Wei C, Li W, Zhang C, Van Hulle M, Cornelis R, Zhang X. Safety evaluation of organoarsenical species in edible Porphyra from the China Sea. J Agric Food Chem 2003; 51: 5176-5182.
25) Raml R, Goessler W, Traar P, Ochi T, Francesconi KA. Novel thioarsenic metabolites in human urine after ingestion of an arsenosugar, 2',3'-dihydroxypropyl 5-deoxy-5-dimethylarsinoyl-beta-D-riboside. Chem Res Toxicol 2005; 18: 1444-1450.
26) Raml R, Raber G, Rumpler A, Bauernhofer T, Goessler W, Francesconi KA. Individual variability in the human metabolism of an arsenic-containing carbohydrate, 2',3'-dihydroxypropyl 5-deoxy-5-dimethylarsinoyl-beta-D-riboside, a naturally occurring arsenical in seafood. Chem Res Toxicol 2009; 22: 1534-1540.
27) Apostoli P, Bartoli D, Alessio L, Buchet JP. Biological monitoring of occupational exposure to inorganic arsenic. Occup Environ Med 1999; 56: 825-832.
28) Farmer JG, Johnson LR. Assessment of occupational exposure to inorganic arsenic based on urinary concentrations and speciation of arsenic. Br J Ind Med 1990; 47: 342-348.
29) Mann S, Droz PO, Vahter M. A physiologically based pharmacokinetic model for arsenic exposure. II. Validation and application in humans. Toxicol Appl Pharmacol 1996; 140: 471-486.
30) Buchet JP, Lauwerys R, Roels H. Urinary excretion of inorganic arsenic and its metabolites after repeated ingestion of sodium metaarsenite by volunteers. Int Arch Occup Environ Health 1981; 48: 111-118.
31) Urine 24-hour volume: MedlinePlus Medical Encyclopedia [database on the Internet]. U.S. National Library of Medicine. [Online]. [cited 2015 Aug. 4].
32) Imai T. Production of urine and excretion. In: Hongo T, Hiroshige T, Toyoda J, editors. Standard physiology. 6th ed. Tokyo: Igaku Shoin; 2006. p. 776-783.
33) Tam GK, Charbonneau SM, Bryce F, Pomroy C, Sandi E. Metabolism of inorganic arsenic (74As) in humans following oral ingestion. Toxicol Appl Pharmacol 1979; 50: 319-322.
34) Buchet JP, Lauwerys R, Roels H. Comparison of the urinary excretion of arsenic metabolites after a single oral dose of sodium arsenite, monomethylarsonate, or dimethylarsinate in man. Int Arch Occup Environ Health 1981; 48: 71-79.