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Dietary Intake of Total and Inorganic Arsenic by Adults in Arsenic-Contaminated Area of Ron Phibun District, Thailand

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Abstract Ron Phibun District, approximately 800 km south of Bangkok, is the site of tin mines operated almost 100 years ago. As a result of mining activities, arsenic contaminated the soil and groundwater of the district. The specific aim of this study was to estimate the dietary intakes of total and inorganic arsenic in 20 adults (10 males and 10 females) residing in Ron Phibun District by a duplicate food approach for 7-consecutive days. The weekly intake rates of inorganic arsenic ranged from 5.54 to 13.3 μg/kg BW for males and 6.11–12.1 μg/kg BW for females.

Keywords Total arsenic · Inorganic arsenic · Duplicate food · Ron Phibun district

Ron Phibun District is a district in Nakorn Si Thammarat Province approximately 800 km south of Bangkok. Ron Phibun District is the site of several former tin mines operated almost 100 years ago. As a result of processing operation, arsenic contaminated the soil and groundwater over the area of 500 square kilometers of the district (JICA 2002). Although the mines are no longer in operation, concern still remains over the potential adverse effects of arsenic through the consumption of contaminated food and groundwater. Arsenic concentrations were reported to be as high as 14,200 μg/g in soil (Visoottiviseth et al. 2002) and 5,114 μg/L in groundwater (Williams et al. 1996). The first case of arsenical skin cancer was reported in 1987, which initiated several studies in the district. Since the first report of skin cancer, people residing in the district were informed not to use groundwater for consumption. At present, they use groundwater for laundry and agricultural purposes but use commercial water and rainwater for consumption and cooking. Concentrations of arsenic in rainwater samples were reported to be 0.26–2.32 μg/L (Wongsanoon et al. 2001), which were below the limit of 10 μg/L established by the World Health Organization.

Inorganic arsenic [As(III) and As(V)] are the most toxic forms of arsenic. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has established the provisional tolerable weekly intake (PTWI) for inorganic arsenic rather than total arsenic (WHO 1989). It has been known that food is an important source of arsenic exposure in humans (WHO 2001). The specific aim of this study was to estimate daily and weekly intake rates of total and inorganic arsenic by adults residing in arsenic-contaminated Ron Phibun District using a duplicate food approach for 7-consecutive days.

Materials and Methods

All participants in this study were farmers who were born, lived, and worked in Ron Phibun District. The farmer was chosen in this study, as approximately 85% of the residents in Ron Phibun District were farmers (JICA 2002). The participants were 10 males and 10 females with an age range of 20–48 years. None of participants had gastrointestinal disorders or drank alcoholic beverages. No participants were vegetarians or were on special diets. All

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participants were paid for duplicate food samples and participating in this project. Weights of participants were recorded at Day 1 being 44–75 kg. Duplicate samples of food and beverages of each participant were collected in polypropylene containers from the breakfast of Day 1 through the evening meal of Day 7. Food samples were frozen and sent daily via air freight to the laboratory in Bangkok. In the laboratory, daily food and beverage samples of individual participants were pooled, weighed, homogenized, freeze-dried, weighed, and kept at 4°C until analysis. Duplicate food samples were collected between January and March 2008.

Sample preparation for determination of total arsenic was described by Munoz et al. (1999). An aliquot of lyophilized sample (0.25 ± 0.01 g) was mixed with 1 mL of ashing mixture (20% w/v $Mg(NO_3)_2$ $6H_20$ and 2% w/v MgO) and 5 mL of 50% (v/v) HNO_3. The mixture was evaporated on a hot plate to dryness and mineralized at 450°C in a furnace. The resulting white ash was dissolved in 5 mL of 6 N HCl and 5 mL of a reducing solution (5% w/v KI and 5% w/v ascorbic acid). The solution was left for 30 min and 10 mL of 50% v/v HCl was added to the solution. The solution was then filtered through a Whatman filter paper No. 1 into a 25-mL volumetric flask and adjusted to volume with 50% v/v HCl. The resulting solution was used for determination of total arsenic.

Inorganic arsenic was determined by the method described by Munoz et al. (1999). An accurate weight (0.5 ± 0.01 g) of lyophilized food sample was placed in a 50-mL screw-capped centrifuge tube; 4.1 mL of water was added to the sample and mixed until completely moistened. In order to hydrolyze As(III) from thiol group of proteins, 18.4 mL of concentrated HCl was added to the moistened sample, shaken for 1 h., and left overnight (12–15 h). Reducing agent (1 mL of 1.5% (w/v) hydrazine sulfate and 2 mL of hydrobromic acid) was added to the sample tube and vortexed. Ten milliliters of chloroform was added into the tube, shaken, and centrifuged. The chloroform phase was aspirated into another centrifuge tube. The extraction process was repeated twice. The chloroform phase was filtered through a syringe filter with 25 mm. PTFE membrane, pore size 0.45 μm (Chrometech, USA), to another tube. Inorganic arsenic in the chloroform phase was extracted with 10 mL of 1 N HCl and centrifuged. The aqueous phase was aspirated into a beaker. The extraction process was repeated one more time. The amount of inorganic arsenic in the combined aqueous acid phase was quantified as described in the determination of total arsenic with the addition of 2.5 mL of ashing mixture and 10 mL of 50% (v/v) HNO_3.

Atomic absorption spectrometer Perkin Elmer AAnalyst 300 equipped with an autosampler AS90 and flow injection system Finorganical Arsenic 400 was used to determine total and inorganic arsenic concentrations in the final solutions. The atomic absorption spectrophotometric conditions were: wavelength 193.7 nm, slit width 0.70 nm, EDL current 380 mA, and loop sample 0.5 mL. The hydride generation conditions were: quartz cell 16 cm path length ×0.7 cm i.d., heating electrothermal, cell temperature 900°C, carrier gas flow rate argon, 50–100 mL/min, reducing agent (0.2% (w/v) $NaBH_4$ in 0.05% (w/v) sodium hydroxide solution) flow rate 5–7 mL/min, and HCl 9–11 mL/min.

The accuracy of determination of total arsenic was assessed by analyzing NIST Standard Reference Materials SRM 1568a (rice flour), 1573a (tomato leaves), and 2977 (mussel tissue). Since no commercial standard reference materials for inorganic arsenic are available, the amount of inorganic arsenic in SRM 1566a (oyster tissue) and 1568a (rice flour) were determined and compared with the previously reported values.

For determination of the limit of quantitation (LOQ) level for total arsenic, food samples (0.25 g) were fortified with arsenic [As(III): As(V) 1: 1 w/w] at concentrations of 1, 2.5, and 5 μg/g; blank samples were not fortified with arsenic. Fortified and blank samples were quantified as described in the determination of total arsenic. For determination of the LOQ level for inorganic arsenic, food samples (0.5 g) were fortified with a mixture of inorganic arsenic [As(III): As(V) 1: 1 w/w] at concentrations of 0.25, 0.5, and 1.0 μg/g; blank samples were not fortified with arsenic. Fortified and blank samples were quantified as described in the determination of inorganic arsenic.

The differences of each parameter between males and females were analyzed using Student's t test. Statistical significance was considered when $p < 0.05$.

Results and Discussion

Calculation for the LOQ of the method was based on the standard deviation of y-intercepts (σ) and the mean of slopes (S) of regression lines using equation LOQ = 10 σ/S (US-FDA 1996). LOQs for total and inorganic arsenic were 0.016 and 0.036 μg/g (dry weight), respectively. Concentrations of total arsenic found in the three standard reference materials were: SRM 1568a (rice flour) 0.27 ± 0.01 μg/g (n = 7; certified value: 0.29 ± 0.03 μg/g), SRM 1573a (tomato leaves) 0.106 ± 0.006 μg/g (n = 6; certified value: 0.112 ± 0.004 μg/g), and SRM 2977 (mussel tissue) 8.62 ± 0.31 μg/g (n = 7; reference value: 8.83 ± 0.91 μg/g). The levels of inorganic arsenic found in SRMs 1566a (oyster tissue) and 1568a (rice flour) were 0.601 ± 0.037 μg/g (n = 4) and 0.103 ± 0.017 μg/g (n = 6), which agreed well with the previously reported values of 0.647 ± 0.027 μg/g

(Munoz et al. 1999) and 0.110 ± 0.027 μg/g (Munoz et al. 2002), respectively.

Daily weights of lyophilized duplicate food samples ranged from 267 to 543 g for males and 226–443 g for females (Table 1). Statistical analysis showed that the daily amounts of food consumed by males were higher than those consumed by females. Concentrations of total and inorganic arsenic in daily food samples for males ranged from 0.221 to 1.04 μg/g and 0.082 - 0.342 μg/g, respectively. These values were comparable to those for females ranging from 0.267 to 1.03 μg/g and 0.070 to 0.367 μg/g, respectively. The percentages of inorganic arsenic with respect to total arsenic in food consumed by males and females were 14.1–62.4 and 14.6–59.1, respectively.

As mentioned above, the daily amounts of food consumed by males were significantly higher than those by females, while the concentrations of total and inorganic arsenic in food for both ganders were comparable. As a result, the daily amounts of total and inorganic arsenic consumed by males were significantly higher than those by females. The amounts of total and inorganic arsenic consumed by individual males ranged from 68.2 to 564 μg/day and 29.6–146 μg/day, while the values for individual females were 72.6–390 and 15.8–103 μg/day, respectively. However, when the body weights of individual participants were used to calculate the daily intake rates of total and inorganic arsenic, the average daily intake rates of total and inorganic arsenic in males were comparable to those in females. The daily intake rate of inorganic arsenic were between 0.49 and 1.96 μg/kg BW for males, and 0.31–2.01 μg/kg BW for females. In this study, daily intake rates of inorganic arsenic in either males or females did not exceed the provisional tolerable daily intake (PTDI) of 2.1 μg/kg BW recommended by the JECFA (WHO 1989).

The weekly intake rate of inorganic arsenic for each participant was the summation of daily intake rates over the 7-day experimental period; the values ranged from 5.54 to 13.3 μg/kg BW for males, and 6.11–12.1 μg/kg BW for females. There were no weekly intake rates of inorganic arsenic of any participant exceeding the PTWI of 15 μg/kg BW established by the JECFA (WHO 1989). Table 2 shows a comparison of arsenic intake studied by the duplicate food approach for 7 days in various countries. Wilhelm et al. (2003) reported dietary daily intakes of total arsenic by 14 German children ranging from 0.60 to 98.0 μg/day. Jorhem et al. (1998) reported an average daily intake of total arsenic in 15 Swedish adults of 60.0 ± 0.04 μg/day. Mohri et al. (1990) estimated daily intake of total and inorganic arsenic in four Japanese adults living in Fukuoka being 27.0–376 and 1.80–22.6 μg/day, respectively. When compared to the values reported from other countries, the daily amounts of total and inorganic

Table 1 Daily food intake, dietary arsenic concentration, arsenic daily intake, and arsenic daily intake rate by 20 participants

	n	Food intake (μg/g dw)	Arsenic concentration (μg/g dw)		% Inorganic arsenic in food	Daily intake (μg/day)		Daily intake rate (μg/kg BW/day)	
			Total	Inorganic		Total	Inorganic	Total	Inorganic
Male	70	401 ± 69.1 (267–543)	0.820 ± 0.185 (0.221–1.04)	0.241 ± 0.063 (0.082–0.342)	30.3 ± 8.52 (14.1–62.4)	334 ± 104 (68.2–564)	96.6 ± 28.8 (29.6–146)	5.07 ± 1.43 (1.12–7.61)	1.48 ± 0.43 (0.49–1.96)
Female	70	311 ± 44.7* (226–443)	0.776 ± 0.191 (0.267–1.03)	0.223 ± 0.065 (0.070–0.367)	29.6 ± 8.84 (14.6–59.1)	240 ± 62.9* (72.6–390)	68.1 ± 17.9* (15.8–103)	4.86 ± 1.28 (1.42–7.50)	1.38 ± 0.37 (0.31–2.01)
Overall	140	356 ± 73.6 (226–543)	0.798 ± 0.188 (0.221–1.04)	0.232 ± 0.065 (0.070–0.367)	30.1 ± 8.66 (14.1–62.4)	287 ± 97.7 (68.2–564)	82.4 ± 27.8 (15.8–146)	4.97 ± 1.36 (1.12–7.61)	1.43 ± 0.40 (0.31–2.01)

Numbers in parentheses are ranges

* Significantly different from the male ($p < 0.05$)

Table 2 Arsenic intakes studied by the duplicate food approach for 7-consecutive days from various countries

Country	n	Daily intake (μg/day)		Reference
		Total	Inorganic	
Germany	14	6.90 ± 12.4 (0.60–98.0)	ND	Wilhelm et al. (2003)
Sweden	15	60.0 ± 0.04 (<50–180)	ND	Jorhem et al. (1998)
Japan	4	182 ± 114 (27.0–376)	10.3 ± 5.50 (1.80–22.6)	Mohri et al. (1990)
Thailand	20	287 ± 97.7 (68.2–564)	82.4 ± 27.8 (15.8–146)	Present study

Numbers in parentheses are ranges

ND = not determined

arsenic consumed by the 20 adults residing in Ron Phibun District were relatively high. This can be explained that all of the reports from those countries were studied in uncontaminated areas.

High intake rates of total and inorganic arsenic by adults living in Ron Phibun District may be partly due to possible uses of contaminated well water for cooking and the consumption of vegetables and fruits locally grown in the contaminated soil. It has been shown that several edible plants grown in contaminated soils accumulated high levels of arsenic (Abedin et al. 2002; Gulz et al. 2005; Helgesen and Larsen 1998). To avoid consumption of food products of plant origin containing high levels of arsenic, further investigation is being studied to characterize the concentrations of total and inorganic arsenic in individual vegetables and fruits grown in Ron Phibun District.

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