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Probabilistic Risk Assessment of Cancer from Exposure Inorganic Arsenic in Duplicate Food by Villagers in Ronphibun, Thailand

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Abstract

Ronphibun district is a district in Nakorn Si Thammarat province, within southern Thailand. This district is the site of several former tin mines that were in operation 100 years ago. Arsenic contamination caused by past mining activities remains in the area. The specific purpose of this study was conducted to assess cancer risk in people living within Ronphibun district from exposure to inorganic arsenic via duplicate food using probabilistic risk assessment. A hundred and fifty duplicate food samples were collected from participants. Inorganic arsenic concentrations are determined by hydride generation atomic absorption spectrometry. Inorganic arsenic concentrations in duplicate food ranged from 0.16 to 0.42 µg/g dry weight. The probabilistic carcinogenic risk levels were 6.76×10^{-4} and 1.74×10^{-3} based on the 50th and 95th percentile, respectively. Risk values for people in Ronphibun from exposure to inorganic arsenic remained higher than the acceptable target risk. Sensitivity analysis indicted that exposure duration and concentrations of arsenic in food were the two most influential of cancer risk estimates.

Keywords: probabilistic risk assessment; inorganic arsenic; duplicate food; Ronphibun district

1. Introduction

Ronphibun district is a district in Nakorn Si Thammarat province, within southern Thailand. The district is the site of several former tin mines that were in operation 100 years ago. Although the mines are no longer in operation, concern still remains over the potential adverse effects of consumption of arsenic in contaminated food and groundwater. Arsenic concentrations were reported to be as high 14,200 µg/g in soil (Visoottiviseth *et al*., 2002) and 5,114 µg/L in well water (Williams *et al*., 1996). Health problems caused by consumption of arsenic contaminated food and water were first reported in 1987. More than 1,000 cases of arsenic based health problems were reported in 1992 (MPH, 2003). Since the 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. It has been known that food is an important source of arsenic exposure in humans. Arsenic concentrations may differ between uncooked and cooked food. Therefore, tests to assess risk by food consumption should take into account ready-to-eat foods. To determine the actual intake, duplicate food sampling method is required. Other sampling methods can not take into account the effects of the cooking process or the cooking water. At present, assessing risks on human health is based on exposure to inorganic arsenic (ATSDR, 2007). A number of studies in Ronphibun have reported the concentration of arsenic in food based on total arsenic rather than inorganic arsenic compounds. Additionally, health risk assessment was only calculated with deterministic method (MPH, 2003). Risk assessment method could be applied for both carcinogenic and non-carcinogenic effects. This research focused on cancer risk only. The objective of this study was to conduct a cancer risk assessment from consuming inorganic arsenic contaminated in food collected by duplicate sampling method in adults living in Ronphibun district using probabilistic approach.

2. Materials and Methods

2.1. Sampling Method

The participants in this study were 25 males and 25 females, ranging from 24 to 68 years old. All

participants were farmers who were lived and worked in this area. No participants had gastrointestinal disorders or consumed alcoholic beverages. The participants were briefed at beginning and throughout the study. The briefing included detailed information on the goal and the background of the study with instructions on how best to collect the duplicate portion of the diet including how to complete the supplied questionnaire. This questionnaire contained questions on sex, age, occupational, details about the type and quantity of food and beverages within the sampling period, body weight, exposure duration, and exposure frequency. Administration of questionnaires was produced by staff for this research. Duplicate food samples of each participant were collected in polypropylene containers from the breakfast of day 1 through the evening meal of day 3. Food samples were frozen and sent daily 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 4ºC until analysis. The samples were collected between March and August 2008.

2.2. Chemical Analysis

Inorganic arsenic was determined by the method described by Munoz *et al*. (1999). An accurate weight (0.5 ± 0.01g) 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 hydrochloric acid 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 0.45µm (Chrometech, U.S.A.), to another tube. The inorganic arsenic in the chloroform phase was extracted with 10 ml of 1 N hydrochloric acid 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 with the addition of 2.5 ml of ashing mixture and 10 ml of 50% (v/v) nitric acid. Atomic absorption spectrometer Perkin Elmer AAnalyst 300 equipped with an autosampler AS90 and flow injection system Finorganical Arsenic 400 was used to determine inorganic arsenic concentration 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 x 0.7 cm i.d., heating electrothermal, cell temperature 900 ºC, carrier gas flow rate argon, 50-100 ml/min, reducing agent (0.2% (w/v) sodium borohydride in 0.05% (w/v) sodium hydroxide solution) flow rate 5-7 ml/min, and hydrochloric acid 9 - 11 ml/min.

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 values previously reported. For determination of the limit of quantitation (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

Table 1. Summary statistics of input parameters in the Monte Carlo analysis

Parameter	Symbol	Unit	Descriptive Statistics[a]	Distribution Pattern[b]
Inorganic arsenic concentration	C_{iAs}	µg/g	0.28 ± 0.09 (0.14 - 0.42)	Lognormal (0.35, 0.18)
Ingestion rate	IR	g/day	368 ± 65.2 (273 - 629)	Lognormal (173.07, 50.54)
Exposure duration	ED	years	28 ± 5.8 (3 - 65)	Inverse Gaussian (39.55, 190.06)
Exposure frequency	EF	day/year	352 ± 16.4 (200 - 365)	Triangular (200, 350, 365)
Body weight	BW	kg	58.2 ± 9.8 (42 - 89)	Normal (58.26, 9.99)
Averaging time	ATc	d	-	Constant (25,550)
Carcinogenic potency slope	CPS	$(mg/kg\ bw/day)^{-1}$	-	Constant (1.5)

a: mean ± SD. and numbers in parentheses are ranges,
b: normal (mean, standard deviation), lognormal (geometric mean, geometric standard deviation), inverse gaussian (mean, lambda), triangular (minimum, most likely, maximum)

not fortified with arsenic. Fortified and blank samples were quantified as described in the determination of inorganic arsenic.

2.3. Cancer Risk Calculation

The structure of a probabilistic model is similar to that of a deterministic model with all the operators that link the variables together except that each variable is represented by a distribution function instead of a single value. For cancer effect, risk is estimated as the incremental probability of an individual developing cancer over a lifetime as a result of exposure to the potential carcinogen. Carcinogenic risk (CR) is accepted in ranges 10^{-4} to 10^{-6} depending on a scale of the target population (US EPA, 2001). In this study, an acceptable cancer risk of 1.0×10^{-4} (one case per 10,000 population) was established for people within Ronphibun district because this target site has about 30,000 villagers. According to US Environmental Protection Agency guideline (US EPA, 2001), carcinogenic risk is calculated by the following Eq. (1):

$$CR = \frac{C_{iAs} \times IR \times ED \times EF \times CPS}{BW \times ATc} \times 10^{-3} \quad (1)$$

where; CR is carcinogenic risk, C_{iAs} is the concentration of inorganic arsenic in duplicate food (µg/g dry weight), IR is the ingestion rate (g/day), ED is the exposure duration (years), EF is the exposure frequency (days/year), BW is the body weight (kg), ATc is the averaging time for cancer effects, equal to the life expectancy time (70 year x 365 day = 25,550 days), 10^{-3} is the unit conversion factor, CPS is the carcinogenic potency slope of the inorganic arsenic. Currently, CPS of ingested inorganic arsenic is 1.5 $(mg/kg\ body\ weight/day)^{-1}$ (ATSDR, 2007).

The probability distributions for input variables were interpolated with the software @RISK (version 4.5) in combination with Microsoft Excel (Palisade, 2004). Fitted distributions of the input variables were established by Anderson-darling method. A summary of the input parameters is shown in Table 1. Exposure and cancer risk distributions were run with 10,000 iterations of the model using Latin hypercube sampling and the results used to estimate various percentiles of carcinogenic risk using the Eq (1). These setting were sufficient to obtain stability of <5% difference in the 95th percentile risk estimate. Finally, sensitivity analysis was conducted by calculating input parameters with statistical distributions, Spearman's rank order correlation coefficient, between the input parameters and carcinogenic risk.

Table 2. Summary of probabilistic cancer risk of inorganic arsenic

Statistical Value	Cancer Risk
Min	2.75×10^{-5}
Mean	7.92×10^{-3}
SD.	5.09×10^{-4}
5th percentile	2.12×10^{-4}
25th percentile	4.08×10^{-4}
50th percentile	6.76×10^{-4}
75th percentile	9.52×10^{-4}
95th percentile	1.74×10^{-3}
Max	5.66×10^{-3}

3. Results and Discussion

3.1. Inorganic arsenic analysis

Calculation for the LOQ was based on U.S.FDA method (US FDA, 1996). LOQ for inorganic arsenic was 0.036 µg/g dry weight. Concentrations of inorganic arsenic found in SRM 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.

3.2. Probabilistic cancer risk

Body weights of the participants were normally distributed, which ranged from 42 to 89 kg. Concentration of inorganic arsenic in food samples ranged from 0.14 to 0.42 µg/g; lognormal distribution best fitted the concentration data. Daily weights of lyophilized duplicate food ranged between 273 and 629 g/day with the best fitted of lognormal distribution and other parameters are shown in Table 1. In this study, all samples of duplicate food and interview data were pooled into the statistical analysis. Monte Carlo simulation was carried out to estimate distributions of exposure and risk using the fitted distributions of the input variables in the carcinogenic risk equation (Eq. 1). US EPA (2001) suggests that the 50th percentile of cancer risk should be considered central tendency estimate and the 95th percentile of risk may be considered reasonable maximum estimate. The same percentiles were chosen in this study. From the Monte Carlo results, lifetime cancer risk from duplicate food intake by Ronphibun residents had the 50th percentile of 6.76×10^{-4} and the 95th percentile of 1.74×10^{-3}. In term of 6.76×10^{-4} means about 7 of

Table 3. Sensitivity analysis of input parameters of the cancer risk assessment

Exposure Parameter	Rank Correlation[a]	
	Value	Normalized r^2 x 100% for value
Exposure duration	0.83	71.89
Inorganic arsenic concentration	0.34	12.06
Exposure frequency	0.21	4.6
Body weight	-0.29	8.78
Ingestion rate	0.16	2.67

a: @RISK output includes Spearman's rank correlation, r, and normalized r^2 values calculated by dividing each r^2 value by the sum of all r^2 value.

10,000 people may be increased cancer effect from the background. The cancer risk from duplicate food intake was excess acceptable level of $1x10^{-4}$. The summaries of these results are shown in Table 2. Sensitivity analysis was performed to assess the effects of the main input variables on the final cancer risk outputs. Approximately 71.98% of influence on cancer risk resulted from exposure duration, 12.06% from arsenic concentration and 15.96% for the remaining three variables. Table 3 shows a sensitivity analysis of input parameters of the cancer risk assessment.

In 2003, The Ministry of Public Health reported that the cancer risk from consumption of food and water in Ronphibun district was $2.9x10^{-2}$ based on exposure duration of 20 years (MPH, 2003). Chantarawijit *et al*. (2000) presented that the cancer risk from arsenic via food consumption ranged from $4x10^{-3}$ to $8x10^{-4}$. When compared to the values reported from above studies, the cancer risk level in the present study was lower than previously reported values but exceeded the risk level of concern ($1x10^{-4}$). A notable difference between the present assessments and the previous assessment is the use of probabilistic method in the assessment. The results of high cancer risk estimate can be explained that the original problem of high arsenic accumulation in soil and water at this site have not completely managed to solve the problem. It may be partly due to possible uses of contaminated well water for cooking and the consumption of foods locally grown in the contaminated soil. Some foods may have highly accumulated arsenic and may thus represent a health risk. SEARO (2001) estimated that approximately 6,120 of 24,566 potentially exposed subjects in Ronphibun site were showing symptoms of arsenicosis. The metabolism of inorganic arsenic has an important role in its toxic effects. However, the exact mechanism of the action of inorganic arsenic is not known but several hypotheses have been proposed and the bioavailability of inorganic arsenic through consumption of cooked foods are not known. There is still a question about the risk to individuals who are exposed to inorganic arsenic, as well as the dose needed for adverse effects to develop. A definite understanding of the mechanism of action will allay uncertainties associated with the risk assessment for inorganic arsenic. It should be pointed out that the cancer risk estimate was based on two major assumptions. The first assumption was that averaging inorganic arsenic intake via a duplicate food to give a daily intake value for cancer risk calculation was valid. Second, the mechanism of carcinogenesis by inorganic arsenic was assumed to have no threshold dose. However, some research has indicated there is a threshold in carcinogenesis caused by inorganic arsenic (ATSDR, 2007). Finally, it is important to note that the estimates derived from duplicate food studies depend on the dietary habits of participants in local area and may not be generalized to other regions. This present result only concern the local residents in Ronphibun district, not extended to people living in other regions of Thailand. Foods are major potential sources of inorganic arsenic exposure in the arsenic affected area but it is difficult to identify the concentrations of inorganic arsenic in individual types of food in this study. Further studies are needed to better understand the levels of inorganic arsenic in different types of food.

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