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Are Chinese consumers at risk due to exposure to metals in crayfish? A bioaccessibility-adjusted probabilistic risk assessment

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ABSTRACT

Freshwater crayfish, the world's third largest crustacean species, has been reported to accumulate high levels of metals, while the current knowledge of potential risk associated with crayfish consumption lags behind that of finfish. We provide the first estimate of human health risk associated with crayfish (*Procambarus clarkii*) consumption in China, the world's largest producer and consumer of crayfish. We performed Monte Carlo Simulation on a standard risk model parameterized with local data on metal concentrations, bioaccessibility (φ), crayfish consumption rate, and consumer body mass. Bioaccessibility of metals in crayfish was found to be variable (68–95%) and metal-specific, suggesting a potential influence of metal bioaccessibility on effective metal intake. However, sensitivity analysis suggested risk of metals via crayfish consumption was predominantly explained by consumption rate (explaining >92% of total risk estimate variability), rather than metals concentration, bioaccessibility, or body mass. Mean metal concentrations (As, Cd, Cu, Ni, Pb, Se and Zn) in surveyed crayfish samples from 12 provinces in China conformed to national safety standards. However, risk calculation of φ-modified hazard quotient (HQ) and hazard index (HI) suggested that crayfish metals may pose a health risk for very high rate consumers, with a HI of over 24 for the highest rate consumers. Additionally, the φ-modified increased lifetime risk (ILTR) for carcinogenic effects due to the presence of As was above the acceptable level (10^{-5}) for both the median (ILTR $= 2.5 \times 10^{-5}$) and 90th percentile (ILTR $= 1.8 \times 10^{-4}$), highlighting the relatively high risk of As in crayfish. Our results suggest a need to consider crayfish when assessing human dietary exposure to metals and associated health risks, especially for high crayfish-consuming populations, such as in China, USA and Sweden.

1. Introduction

Freshwater crayfish (Decapoda: Parastacidae and Astacidae) are a significant worldwide aquatic food (FAO, 2014; NBSC, 2013), with a long history of human consumption (Patoka et al., 2014). In some European countries, long-standing cultural traditions are still linked with crayfish consumption, especially in Scandinavia (Taugbøl and Skurdal, 1999). In the USA, crayfish used as food is popular in Wisconsin, Minnesota, Arkansas, Mississippi, and Texas, and especially popular in Louisiana, where more than 90% of US crayfish are processed (Holdich, 1993; Richert and Sneddon, 2007; USITC, 2003). In China, crayfish have been widely used as food since the 1980s (Mu et al., 2007).

Crayfish are also widely reported to accumulate high concentrations of metals (Alcorlo et al., 2006; Anderson et al., 1997a, 1997b; Kouba et al., 2010; Kuklina et al., 2014). Due to their omnivory as well as necrophagia and life spans up to six years, crayfish bioaccumulate multiple toxins and heavy metals in their shells and flesh. Hazards associated with crayfish consumption have been reported, including Haff disease (Xie et al., 2010; Zhang et al., 2012), human paragonimiasis (Lane et al., 2009), *Vibrio* infections (Anda et al., 2001; Bean et al., 1998; Kay et al., 2012), intoxication by natural or man-made toxins (Vasconcelos, 1999; Vasconcelos et al., 2001), and effects resulting from exposure to organic contaminants (Gewurtz et al., 2000; Levengood and Schaeffer, 2011; Schilderman et al., 1999). Multiple studies have demonstrated heavy metal contamination in crayfish in

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Abbreviations: HQ, hazard quotient; HI, hazard index; ILTR, increased lifetime risk; FAO, Food and Agriculture Organization; NBSC, National Bureau of Statistics of China; USITC, United States International Trade Commission; MLYR, middle and lower reaches of the Yangtze River; ICP-MS, inductively coupled plasma-mass spectroscopy; CRM, certified reference material; USEPA, United States Environmental Protection Agency; RfD, reference dose; EDI, estimated daily intake; CSF, cancer slope factor; IR, ingestion rate; BW, body weight; ED, exposure duration; EF, exposure frequency; AT, averaging time; NCRPM, National Council on Radiation Protection and Measurements; MCS, Monte Carlo Simulation; *pd*, probability distribution; USFDA, United States Food and Drug Administration.

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the USA (Mason et al., 2000; Besser et al., 2007; Hothem et al., 2007; Moss et al., 2010), Italy (Bellante et al., 2015; Bruno et al., 2006), Spain (Devesa et al., 2002; Alcorlo et al., 2006; Vioque-Fernandez et al., 2007) and China (Huang, 2009; Wu et al., 2010; Peng et al., 2015). However, most of the previous studies were based on localized sampling, especially in contaminated areas. Levels of metal contamination in crayfish intended for human consumption at regional or national scales have rarely been reported.

China is the world's largest producer and consumer of crayfish (FAO, 2014; Mu et al., 2007; NBSC, 2013). Of the more than 500,000 t of reported crayfish production and consumption annually worldwide (McClain and Romaire, 2007), China alone is responsible for 529,000 t per year, exporting about 26,000 t, mainly into the USA and Europe (Mu et al., 2007; NBSC, 2013; Schuler et al., 2000). The majority of crayfish production is used for human consumption within China (Mu et al., 2007; Qiu et al., 2012; Yue et al., 2009). Dietary metal exposure has also been shown to pose health risk to consumers in China. In particular, arsenic (As) exposure from consumption of rice and other foods poses incremental lifetime cancer risk increases to 1 to 2 per 1000 Chinese individuals (Li et al., 2011). Given the abovementioned hazards and wide population exposure, the health risk of crayfish consumption in China warrants study. Among the few studies focused on health risk due to metal exposure via crayfish consumption (Schuler et al., 2000; Wu et al., 2010), a small-scale survey in Shanghai City showed that lead (Pb) intake due to crayfish consumption could pose risk to human health (Wu et al., 2010). However, data are limited at regional or national scales in China on metal concentrations and dietary bioavailability in crayfish, as well as dietary consumption rates.

When assessing metal exposure via diet, total metal burden often provides a conservative (i.e., high) estimate of exposure due to the fact that only a fraction of metal is bioavailable (Oomen et al., 2002; Versantvoort et al., 2005). In vitro bioaccessibility tests, validated against in vivo studies (Li et al., 2014a; Li et al., 2014b; Schroder et al., 2004), provide a reliable method to assess metal bioavailability, which aids in dietary exposure and risk assessment (Amiard et al., 2008).

We report on the first large-scale assessment of health risk due to heavy metals in crayfish in China. Our study includes regions throughout China, but focuses on the main region of crayfish consumption, the middle and lower reaches of the Yangtze River delta area. Cooked red swamp crayfish (*Procambarus clarkii*) were collected from restaurants in 23 cities in China, and determined for metal concentrations (As, Cd, Cu, Ni, Pb, Se and Zn). Effective metal intake (i.e., metal bioaccessibility) was estimated using an in vitro digestion model and considered in a probabilistic risk calculation. Risk indices include increased lifetime cancer risk (ILTR) for As, and the hazard quotient (HQ) for all examined metals. Finally, sensitivity analysis was performed to compare the relative importance of metal concentration, bioaccessibility, ingestion rate, and consumer body weight for risk via crayfish consumption.

2. Materials and methods

2.1. Crayfish sampling

We obtained 210 red swamp crayfish (*P. clarkii*) samples from 23 cities (12 provinces, Supplemental information Fig. S1), in which crayfish consumption has been frequently reported. Samples were obtained in September, 2012, during the peak season of crayfish consumption (i.e., June to September, Peng et al., 2015). Among the selected cities, 15 were located in the middle and lower reaches of the Yangtze River (MLYR), China's main crayfish producing and consuming area (Mu et al., 2007), and 8 were located in other regions of the country (Fig. S1). A single site (restaurant) was sampled for crayfish in each city, except Nanjing, for which three separate restaurants were sampled.

Exposure was assessed as consumed, rather than from contamination of the raw commodity. Therefore, all samples were of cooked crayfish intended for human consumption, obtained from local restaurants. Crayfish were cooked by either stewing or frying. Cooking methods had no significant effect on metal (except for Zn) bioaccessibility (nested ANOVA, site nested within cooking, $p > 0.05$; Supplemental information Table S1), and thus the effects of cooking methods on the risk of metals were not considered in this study. We did not distinguish farm-raised versus wild-caught crayfish in this study. However, the majority of crayfish consumed in China are farm-raised (Mu et al., 2007; Yue et al., 2009). All samples were vacuum-packed, stored on ice, and transported to the lab within 3 days. After tail length determination (5.4 ± 0.8 cm, mean ± SD), tail muscle (edible part of crayfish) was dissected, weighed (3.0 ± 1.1 g), rinsed with ultrapure water and freeze-dried. Samples were ball-milled to a fine powder and stored at −20 °C until further analysis.

2.2. Determining metal concentrations in crayfish tail muscle

Subsamples of crayfish tail muscle were digested in concentrated trace metal grade nitric acid (HNO_3), and then analyzed for As, Cd, Cu, Ni, Pb, Se and Zn by inductively coupled plasma-mass spectroscopy (ICP-MS, Perkin Elmer/NexION300). The instrument detection limits were 0.08, 0.06, 0.43, 0.07, 0.48, 0.50 and 0.63 ng L^{-1} for Cd, Cu, Ni, Pb, As, Se and Zn, respectively. Quality control for digestion and ICP-MS analysis included blanks, certified reference material (CRM; scallop tissue, GBW10024), and duplicate measurement (RSD < 10%). Instrumental quality control included calibration checks and running a standard after every 30 samples. Additionally, internal standards were used to correct the sensitivity drift and matrix effects (Long and Martin, 1989): Bi, Ge, In and Sc were spiked into the digested samples (1 μg L^{-1}) before analysis. CRM (3 replicates) was digested and analyzed with each batch of samples. The recoveries (mean ± SD) of As, Cd, Cu, Ni, Pb, Se and Zn in the CRM were 98 ± 8%, 97 ± 6%, 99 ± 11%, 103 ± 11%, 96 ± 15%, 102 ± 10% and 97 ± 11%, respectively.

2.3. Bioaccessibility of metals in crayfish tail muscle

Metal bioaccessibility was measured in 24 samples from 8 restaurants in MLYR, including Suqian, Suzhou, Anqing, Hangzhou, Anji, and Xinyang cities, and 2 restaurants in Nanjing City (see Supplemental information Table S2). In this study, freeze-dried tissues were used for assessing metal bioaccessibility. Our preliminary experiments (using 10 crayfish individuals from 5 sampling sites) indicated that freeze-drying had insignificant effects on either concentration or bioaccessibility of metals. Dietary metal bioaccessibility was estimated by the in vitro digestion model developed by Versantvoort et al. (2005), simulating the digestion processes in the human mouth, stomach and small intestine. Briefly, digestion juices, including saliva, gastric juice, duodenal juice and bile juice, were prepared artificially and heated to 37 ± 2 °C before use (details described in Supplemental information Table S3). Crayfish subsamples were mixed with saliva ($pH = 6.8$) and incubated for 5 min, followed by addition of gastric juice ($pH = 1.3$) for 2 h (mixed pH = 2–3), and then, simultaneous administration of duodenal juice ($pH = 8.1$), bile ($pH = 8.2$) and 1 M $NaHCO_3$ solution for another 2 h. The final pH in the mixture was 6.5–7. The sample-to-fluid ratio was 1:95 (g mL^{-1}) at a volume ratio of 3 saliva:6 gastric juice:6 duodenal juice:3 bile:1 $NaHCO_3$ for the digestion juice (Oomen et al., 2003). A sample-to-fluid ratio of 1:95 instead of 1:98 (Oomen et al., 2003) was used in this study to facilitate calculating solution volumes. However, minor changes in sample-to-fluid ratios have insignificant effects on quantified metal bioaccessibility (Hamel et al., 1998; Oomen et al., 2003). Mixing was conducted at 55 rpm at 37 °C, followed by centrifugation (1640 ×g, 5 min) to separate the supernatants and pellets. Scallop tissue (GBW10024) was used as a CRM for quality control.

Both supernatants and residues were digested in concentrated HNO_3 and analyzed for metals by ICP-MS as described in Section 2.2.