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Research Note

Distribution of Heavy Metals in Muscles and Internal Organs of Korean Cephalopods and Crustaceans: Risk Assessment for Human Health

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ABSTRACT

Samples of seven species of cephalopods and crustaceans were collected from major fish markets on the Korean coast and analyzed for mercury (Hg) using a direct Hg analyzer and for the metals cadmium (Cd), lead (Pb), chromium, silver, nickel, copper, and zinc using inductively coupled plasma mass spectrometry. The distributions of heavy metals in muscles, internal organs, and whole tissues were determined, and a risk assessment was conducted to provide information concerning consumer safety. The heavy metals accumulated to higher levels ($P < 0.05$) in internal organs than in muscles for all species. The mean concentrations of Cd, which had the highest concentrations of the three hazardous metals (Cd, Pb, and Hg), in all internal organs (except those of blue crab) exceeded the regulatory limits set by Korea and the European Union. The Cd concentrations in all whole tissues of squid and octopus (relatively large cephalopods), red snow crab, and snow crab exceeded the European Union limits. The estimated dietary intake of Cd, Pb, and Hg for each part of all species accounted for 1.73 to 130.57%, 0.03 to 0.39%, and 0.93 to 1.67%, respectively, of the provisional tolerable daily intake adopted by the Joint Food and Agriculture Organization and World Health Organization Expert Committee on Food Additives; the highest values were found in internal organs. The hazard index (HI) is recognized as a reasonable parameter for assessing the risk of heavy metal consumption associated with contaminated food. Because of the high HI (>1.0) of the internal organs of cephalopods and the maximum HI for whole tissue of 0.424, consumers eating internal organs or whole tissues of cephalopods could be at risk of high heavy metal exposure. Therefore, the internal organs of relatively large cephalopods and crabs (except blue crab) are unfit for consumption. However, consumption of flesh after removing internal organs is a suitable approach for decreasing exposure to harmful metals.

Fishery products are an important global food resource. According to the Food and Agriculture Organization of the United Nations (FAO) *(14)*, Korea was the world's seventh largest consumer of fishery products in 2009, accounting for 56.1 kg per person per year, which is threefold higher than the global average consumption. The FAO *(15)* also reported that Korea ranked 3rd (292,405 tons) and 14th (135,720 tons) in the world for the production of cephalopods and crustaceans, respectively, in 2012. These products are exported mainly to the United States, China, Japan, and the European Union (EU) *(27)*.

Heavy metals such as cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb), and zinc (Zn) reach the marine environment through rivers and marine operations, such as the exploitation of offshore resources and disposal of dredged materials *(33, 36)*. In general, heavy metals accumulate in marine organisms from the aquatic environment and increase the contaminant load for humans consuming these organisms *(10, 32, 38)*.

Mollusks and crustaceans tend to contain high concentrations of heavy metals *(6, 21)*. Similar to other mollusks, cephalopods rapidly accumulate high levels of Cd, Cu, Hg, and other metals *(13, 37)*. Several studies have been performed to evaluate heavy metals in many species of crustaceans and cephalopods *(3, 6, 9, 21, 22, 26, 31)*. Some hazardous metals (e.g., Cd, Pb, and Hg) pose a risk to humans, even in trace amounts, via the consumption of contaminated organisms *(10, 31)*. To protect public health, the Korean government has established regulatory limits for three metals in the edible components of crustaceans and cephalopods *(25)*. Most of the internal organs of crustaceans and cephalopods are not officially considered edible by Korean health authorities; however, the internal organs of some species are considered edible in many cultures, including Korea. Heavy metal pollution can be hazardous to humans; therefore, the concentrations of heavy metals must be checked regularly in edible and ''nonedible'' tissues to provide information concerning consumer safety.

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In the present study, we determined the concentrations of heavy metals in muscle, internal organs (or digestive gland), and whole tissue of crustaceans and cephalopods collected from three fish markets on the Korean coast and evaluated the anatomical distribution of heavy metals in these samples. The estimated dietary intake (EDI) of heavy metals via consumption of each portion of crustacean and cephalopod was compared with the provisional tolerable daily intake (PTDI) established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) *(16–18)* or the U.S. Environmental Protection Agency (EPA) *(41)*. An assessment of the potential health risk associated with these heavy metals in different portions of these animals was performed using the target hazard quotient (THQ) and the hazard index (HI).

MATERIALS AND METHODS

Reagents and standard solutions. Suprapure grade nitric acid (Merck, Darmstadt, Germany) was used for sample preparation. Deionized water (DIW) was passed through a Milli-Q water purification system (Millipore, Billerica, MA). Working standard solutions of Cd, Pb, Cr, silver (Ag), Ni, Cu, and Zn were prepared by diluting 1,000 mg/liter standard solutions (Merck) in DIW and used as calibration standards. MESS-3, a marine sediment certified reference material, was purchased from the National Research Council of Canada (Ottawa, Ontario, Canada) and used as a calibration standard for Hg.

Sample collection. Specimens of four species of cephalopod—squid (*Todarodes pacificus*), octopus (*Enteroctopus dofleini*), small octopus (*Octopus minor*), and webfoot octopus (*Octopus ocellatus*)—and three species of crustacean—snow crab (*Chionoecetes opilio*), red snow crab (*Chionoecetes japonicus*), and blue crab (*Portunus trituberculatus*)—were collected between March and November in 2011 at three major fish markets located on the eastern (Pohang), western (Gunsan), and southern (Yeosu) coasts of Korea. The fish markets were located where the animals were harvested on each coast and were for sale for human consumption. The samples were transported to the laboratory in coolers. These species were selected because they are very popular foods in Korea. Although the internal organs of the selected species were typically considered nonedible, excluding the blue crab where the entire body is marinated in soy sauce and the small cephalopods (e.g., webfoot octopus) that are boiled whole, some consumers in Korea occasionally eat the boiled whole tissue of other selected species, including the internal organs.

Sample preparation. Upon arrival at the laboratory, the collected samples were immediately separated according to species and washed with tap water and DIW. When possible, at least five specimens per species were examined. The shells of crustaceans and the skins of cephalopods were removed. The specimens were dissected and separated into muscle and internal organs (or digestive glands). The separated samples were weighed, homogenized, and then stored below -20°C until analyzed. The homogenized samples were freeze-dried with a vacuum freeze dryer (FDU-2100, EYELA, Tokyo, Japan) and ground into powder for analysis. About 1.0 g of the powdered sample was placed in a 60-ml digestion vessel (Savillex, Eden Prairie, MN), and 20 ml of nitric acid was added. The vessel was covered and left overnight at room temperature. The samples were then digested with a heating digester (Digi PREP HP, SCP Science, Champlain, NY). The digested samples were allowed to cool to room temperature, dissolved in 2% nitric acid, filtered (glass wool), and made up to 100 ml with 2% nitric acid to analyze all heavy metals except Hg. Approximately 0.1 g of homogenized sample was used for Hg analysis. The heavy metal concentrations in the whole tissue were calculated according to concentrations in the muscle and internal organs. The muscle of cephalopods consists of arms, fins, and mantle.

Heavy metal analysis. All digested samples were analyzed for Ag, Cd, Cr, Cu, Ni, Pb, and Zn in triplicate using an inductively coupled plasma mass spectrometer (ElAN DRC II, PerkinElmer, Waltham, MA). Total Hg in the homogenized samples was measured directly in triplicate using a combustion gold amalgamation method with a direct mercury analyzer (DMA-80, Milestone, Milano, Italy). The blanks, calibration standards, and certified reference materials were analyzed using the same methods. The concentrations of heavy metals were expressed in micrograms per gram of sample wet weight.

The accuracy of the heavy metal analysis method was assessed using the SRM-1566b (oyster tissue) certified reference material provided by the National Institute of Standards and Technology (Gaithersburg, MD). The quantitative recoveries of the heavy metals in the oyster tissue certified reference material (n = 5) ranged from 91.1 to 107.7%, i.e., 96.1% for Cd, 91.2% for Hg, 103.6% for Pb, 91.1% for Ag, 107.7% for Ni, 97.8% for Cu, and 95.6% for Zn. These recoveries were within the acceptable values recommended by AOAC International *(2)*, which are 70 to 125%, 75 to 120%, and 80 to 115% for 0.01, 1.0, and 10 μg/g, respectively.

Statistical analysis. Statistical evaluation was conducted using an analysis of variance with the general linear model procedure (SAS version 9.2, SAS Institute, Cary, NC). Duncan's multiple range test was applied to determine the significance of differences between the mean concentrations of heavy metals in samples.

RESULTS AND DISCUSSION

Anatomical distribution of heavy metals in cephalopods and crustaceans. The concentrations and distributions of the heavy metals in the muscle, internal organs, and whole tissue samples of cephalopods and crustaceans are shown in Figure 1. The heavy metals accumulated to significantly higher levels ($P < 0.05$) in internal organs than in muscles for all species. The mean concentrations in the whole tissue of cephalopods decreased in the order of Zn (71.72 μg/g) > Cu (24.148 μg/g) > Cd (1.423 μg/g) > Ni (0.303 μg/g) > Ag (0.125 μg/g) > Cr (0.045 μg/g) > Pb (0.032 μg/g) > Hg (0.029 μg/g); however, the difference between Pb and Hg was not significant. The metal concentration ratio of internal organs to muscles in cephalopods was relatively high for Cd and Ag but low for Hg, Cr, and Zn (Table 1).

Three heavy metals—Cd, Hg, and Pb—are harmful to humans, even at trace concentrations *(10)*. Korea has established regulatory limits for these three hazardous metals to protect those that consume fishery products *(25)*. The mean concentrations of Cd in the internal organ (nonedible) samples of cephalopod species, which were the highest of these hazardous metals, were 2.741 to 9.810 μg/g, with the highest concentrations found in squids (Fig. 1).

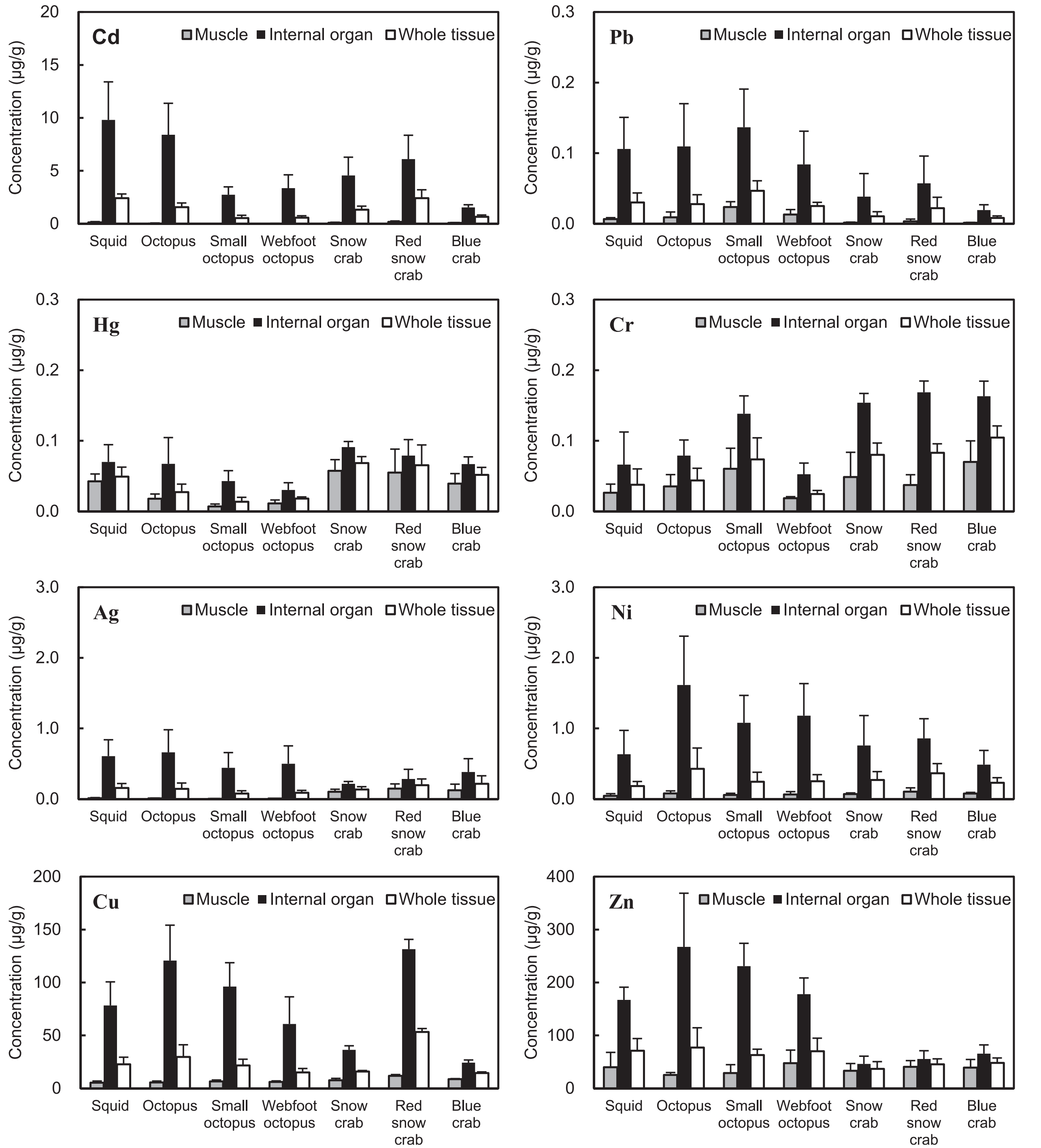

FIGURE 1. *Heavy metal concentrations and distributions in muscles, internal organs, and whole tissues of cephalopods and crustaceans collected from three fish markets on the Korean coast. Scale bar represents one standard deviation. Cd, cadmium; Pb, lead; Hg, mercury; Cr, chromium; Ag, silver; Ni, nickel; Cu, copper; Zn, zinc.*

These mean concentrations were lower than those reported previously in Korea (9.240 to 23.124 μg/g) *(23)*. Our results were also lower than the Cd concentrations in the digestive gland of cephalopods from other geographic areas, such as the northern Pacific Ocean (211.0 μg/g) *(7),* Argentinean waters (485.0 μg/g, dry weight) *(20),* Brazilian waters (18.5 to 1,002.9 μg/g) *(9),* and the southern Indian Ocean (215.0 to 369.0 μg/g, dry weight) *(4)*. All internal organ samples had mean Cd concentrations above the regulatory limit (2.0 μg/g) for the edible portions of cephalopods set by Korea *(25)* and the Codex Alimentarius Commission *(8)*. However, the mean Cd concentrations in all muscle samples were 0.012 to 0.129 μg/g, with the maximum found in squids, which is ~15-fold lower than the regulatory limits set by Korea and the Codex Alimentarius Commission. The mean (±SD) Cd concentrations in all tested whole tissue samples of squid (2.415 ± 0.395 μg/g) and octopus (1.568 ± 0.393 μg/g), which are relatively large cephalopod species, exceeded the regulatory limits set by Korea (2.0 μg/g) *(25)* and the EU (1.0 μg/g) *(11),* respectively, but the

TABLE 1. *Ratios of heavy metals in internal organs versus muscle in cephalopods and crustaceans (crabs) from three fish markets on the Korean coast*

Common name	No. of samples	Heavy metal ratio (internal organ/muscle)[a]							
		Cd	Pb	Hg	Cr	Ag	Ni	Cu	Zn
Cephalopods	30								
Squid	8	76.2	16.1	1.6	2.5	60.7	14.3	14.5	4.2
Octopus	8	314.1	12.0	3.8	2.2	82.6	20.3	21.4	10.6
Small octopus	7	223.7	5.8	6.1	2.3	110.3	19.2	14.6	8.0
Webfoot octopus	7	234.5	6.5	2.7	2.8	75.0	18.1	10.0	3.7
Crustaceans	21								
Snow crab	7	48.5	56.7	1.6	3.2	2.1	10.9	4.8	1.4
Red snow crab	7	34.4	12.0	1.4	4.5	1.9	8.3	11.1	1.4
Blue crab	7	18.5	31.1	1.7	2.3	3.1	6.3	2.8	1.7

[a] The ratio was calculated as the heavy metal concentration in a gram of internal organ divided by that in a gram of muscle. Cd, cadmium; Pb, lead; Hg, mercury; Cr, chromium; Ag, silver; Ni, nickel; Cu, copper; Zn, zinc.

mean Cd concentrations in other octopus species were below the regulatory limits.

Cephalopods are widespread in numerous marine habitats from coastal waters to very deep ocean environments and include benthic (e.g., octopus), nectobenthic (cuttlefish), and oceanic (squid) species *(6)*. In this study, the pelagic cephalopod (squid) contained significantly higher ($P < 0.05$) Cd concentrations than did the benthic species (octopi). Cephalopods feed on a wide range of other marine animals, including crustaceans, mollusks, fish, and other cephalopod species *(30, 35)*, and bioaccumulation of pollutants can occur through the food chain. Squids also have higher feeding rates than do octopi *(34)*. Koyama et al. *(26)* reported that a cephalopod species (*Sepioteuthis lessoniana*) accumulated Cd from food rather than from the surrounding seawater. Therefore, diet is the main pathway of Cd uptake in cephalopods, and the digestive gland is the main retention organ.

The ratio of internal organ Cd to muscle Cd in cephalopods was 76.2 to 314.1; the highest ratio was found in octopus (Table 1). These ratios are lower than those for cephalopods in Korea (162.0 to 1,552) reported previously *(23)*. In the present study, the vast majority of Cd was present in the internal organs of cephalopods, which accounted for 95.9 to 98.6% of the total content. Bustamante et al. *(5)* also reported that the digestive gland stored the majority of Cd, reaching 98% in some cephalopod species.

The mean concentrations of Pb in each cephalopod ranged from 0.007 to 0.137 μg/g (Fig. 1), with the maximum in the internal organs of small octopus, but were far below the regulatory limits in the edible portions of cephalopods as stipulated by Korea (2.0 μg/g) *(25)* and the EU (1.0 μg/g) *(11)*. The ratios of internal organ Pb to muscle Pb in cephalopods ranged from 5.8 to 16.1; the highest ratio was in squid (Table 1). The internal organs of cephalopods contained 49.7 to 78.7% of the Pb, with the highest percentage found in the internal organs of squid.

The maximum concentration (0.070 ± 0.024 μg/g) of Hg was found in the internal organs of squid (Fig. 1), but this value was approximately sevenfold lower than the regulatory limits (0.5 μg/g) in the edible portion of cephalopods established by Korea *(25)* and the EU *(12)*. The internal organ to muscle Hg ratios in cephalopods ranged from 1.6 to 6.1; the highest ratio was in the small octopus (Table 1). The internal organs of cephalopods contained 34.0 to 54.3% of the total Hg, which was much higher than the percentage in cephalopods (15 to 40%) from the northeastern Atlantic reported by Bustamante et al. *(6)*.

Among the crustaceans (crabs), the mean concentrations of metals in the whole tissue samples decreased significantly ($P < 0.05$) in the order Zn (43.48 μg/g) > Cu (27.87 μg/g) > Cd (1.484 μg/g) > Ni (0.288 μg/g) > Ag (0.182 μg/g) > Cr (0.089 μg/g) > Hg (0.062 μg/g) > Pb (0.014 μg/g) (Fig. 1). The metal ratios for internal organs versus muscles in crabs was relatively high for Cd and Pb but low for Hg, Cr, Ag, and Zn (Table 1). The mean concentrations of Cd, which were the highest of the hazardous metals (Cd, Hg, and Pb), in the internal organ samples of snow crabs, red snow crabs and blue crabs were 4,556, 6.105, and 1.539 μg/g, respectively (Fig. 1). The Cd concentrations exceeded the regulatory limits (0.5 to 1.0 μg/g) in the edible portion of crustaceans (with the exception of brown crab meat) set by the EU (0.5 μg/g) *(11)* and Korea (1.0 μg/g, with the exception of blue crab) *(25)*. However, Cd in the internal organ samples of blue crabs was below the limit (5.0 μg/g) for blue crab (including the internal organs) set by Korea. The mean Cd concentrations in all muscle samples ranged from 0.083 to 0.177 μg/g, with the maximum found in red snow crabs, and were all considerably below the regulatory limits set by Korea and the EU. The mean Cd concentrations in the whole tissue samples of red snow crabs (2.423 μg/g) and snow crabs (1.329 μg/g) exceeded the regulatory limits established by Korea (2.0 μg/g) and the EU (1.0 μg/g), but the Cd concentration in the blue crab was below these limits. The ratio of internal organ to muscle Cd in crab species ranged from 18.5 to 48.5; the highest ratio was in snow crab (Table 1). The internal organs of crabs contained 92.9 to 95.5% of the total Cd, with the highest percentages found in

TABLE 2. *Estimated dietary intake of heavy metals via the consumption of cephalopods and crustaceans (crabs) in Korea*

Common name	ADI (μg/kg/day)[a]	Sample	EDI (μg/kg/day)[b]							
			Cd	Pb	Hg	Cr	Ag	Ni	Cu	Zn
Cephalopods	0.1232									
Squid	0.0950	Muscle	0.0122	0.0006	0.0040	0.0025	0.0009	0.0042	0.5139	3.7906
		Internal organ	0.9316	0.0101	0.0066	0.0063	0.0576	0.0602	7.4342	15.8653
		Whole tissue	0.2293	0.0029	0.0047	0.0036	0.0148	0.0174	2.1606	6.7458
Octopus	0.0050	Muscle	0.0001	<0.0001	0.0001	0.0002	<0.0001	0.0004	0.0285	0.1278
		Internal organ	0.0424	0.0006	0.0003	0.0004	0.0033	0.0082	0.6097	1.3499
		Whole tissue	0.0079	0.0001	0.0001	0.0002	0.0007	0.0022	0.1497	0.3897
Small octopus	0.0184	Muscle	<0.0001	0.0004	0.0001	0.0011	0.0001	0.0010	0.1211	0.5337
		Internal organ	0.0504	0.0025	0.0008	0.0025	0.0081	0.0198	1.7670	4.2447
		Whole tissue	0.0096	0.0009	0.0003	0.0014	0.0014	0.0045	0.3987	1.1564
Webfoot octopus	0.0044	Muscle	<0.0001	0.0001	0.0001	0.0001	<0.0001	0.0003	0.0271	0.2122
		Internal organ	0.0150	0.0004	0.0001	0.0002	0.0022	0.0052	0.2709	0.7923
		Whole tissue	0.0025	0.0001	0.0001	0.0001	0.0004	0.0011	0.0673	0.3119
Crustaceans	0.0232									
Snow crab	0.0027	Muscle	0.0003	<0.0001	0.0002	0.0001	0.0003	0.0002	0.0208	0.0910
		Internal organ	0.0125	0.0001	0.0002	0.0004	0.0006	0.0021	0.0999	0.1269
		Whole tissue	0.0036	<0.0001	0.0002	0.0002	0.0004	0.0007	0.0435	0.1013
Red snow crab	0.0001	Muscle	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0013	0.0045
		Internal organ	0.0007	<0.0001	<0.0001	<0.0001	<0.0001	0.0001	0.0147	0.0062
		Whole tissue	0.0003	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0060	0.0051
Blue crab	0.0203	Muscle	0.0017	<0.0001	0.0008	0.0014	0.0025	0.0016	0.1773	0.7935
		Internal organ	0.0312	0.0002	0.0014	0.0033	0.0078	0.0099	0.4931	1.3280
		Whole tissue	0.0132	0.0001	0.0010	0.0021	0.0044	0.0046	0.2939	0.9729
PTDI (μg/kg/day)[c]			0.83	3.57	0.57	3.0	5.0	20	40	300

[a] ADI, average dietary intake based on Korea health statistics, 2010 *(24)*.

[b] EDI, estimated dietary intake for an adult calculated using the equation EDI = (HC × ADI)/BW, where HC is the mean heavy metal concentration in each part of the cephalopod or crustacean shown in Figure 1, ADI is for cephalopods and crustaceans, and BW is the average body weight of an adult human (62.8 kg) based on the Korean health statistics 2010 *(24)*. Cd, cadmium; Pb, lead; Hg, mercury; Cr, chromium; Ag, silver; Ni, nickel; Cu, copper; Zn, zinc.

[c] PTDI, provisional tolerable daily intake. Values for Pb, Hg, and Cd were based on the provisional tolerable weekly intake (PTWI) data for Pb and Hg and provisional tolerable monthly intake data for Cd from the Joint FAO/WHO Expert Committee on Food Additives *(16–18)*, in which the PTWI of inorganic Hg was used for total Hg. The PTDI of the other metals, i.e., Cr (assuming that total Cr is Cr[VI]), Ag, Ni (assuming that all Ni is Ni soluble salts), Cu, and Zn (assuming that all Zn is Zn and compounds), were based on the oral reference doses established by the U.S. Environmental Protection Agency *(41)*.

the internal organs of red snow crab. For these specimens, the internal organs of both snow crabs and red snow crabs contained more Cd than did the internal organs of blue crabs.

The highest concentration (0.057 ± 0.039 μg/g) of Pb in the internal organs of red snow crab (Fig. 1) were within the regulatory limits for crustaceans (with the exception of blue crab) set by Korea (1.0 μg/g) *(25)* and the EU (0.5 μg/g) *(11)*. The ratio of internal organ to muscle Pb in crabs ranged from 12.0 to 56.7; the highest ratio was in snow crab (Table 1). The internal organs of crabs contained the majority of Pb in all species, from 86.2 to 90.6% of the total body burden.

The maximum concentration of Hg of 0.091 ± 0.008 μg/g (Fig. 1) was found in the internal organs of snow crab and was ca. fivefold lower than the regulatory limit (0.5 μg/g) in the edible portion of crustaceans set by many countries, such as Korea *(25)*, the EU *(12)*, and Australia and New Zealand *(19)*. The ratios of internal organ to muscle Hg in crabs ranged from 1.4 to 1.7; the highest ratio was found in blue crabs (Table 1). The internal organ of crabs contained 43.0 to 58.5% of the Hg, with the highest percentages found in blue crabs.

Heavy metals accumulated to significantly higher concentrations ($P < 0.05$) in internal organs than in muscles of cephalopods and crustaceans. The Cd concentrations in all internal organ samples were the highest of the three hazardous metals (Cd, Hg, and Pb). The mean Cd concentrations in all analyzed internal organ samples exceeded the regulatory limits set by Korea (with the exception of blue crab) and the EU. In particular, the Cd concentrations in all tested whole tissues of squid and octopus (relatively large cephalopods), red snow crab, and snow crab exceeded the regulatory limit of the EU. However, the concentrations of Cd in the muscle samples of all species were far below the limits set by Korea and the EU.

Dietary intake estimation and risk assessment of heavy metals effects from consumption of cephalopods and crustaceans. The EDIs of heavy metals represent the daily intake of heavy metals through the consumption of

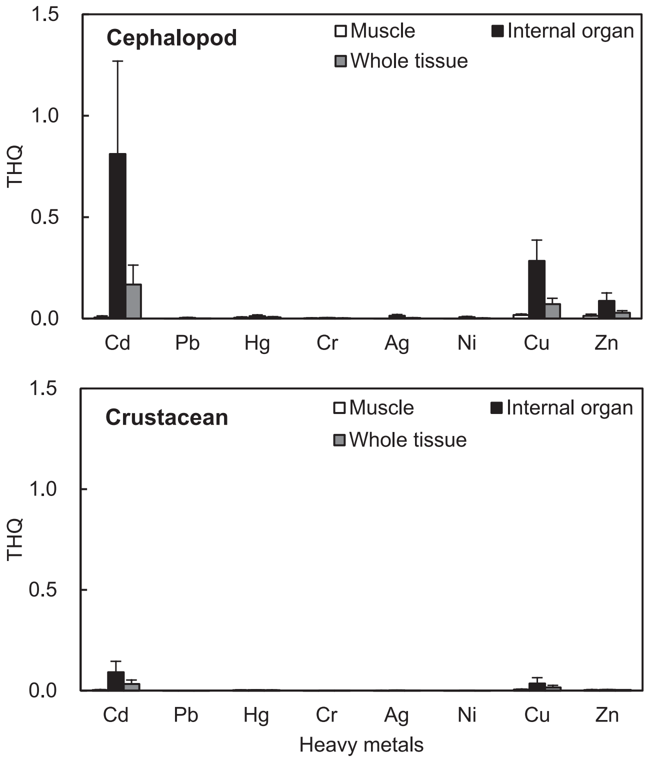

FIGURE 2. *Target hazard quotients (THQs) for heavy metals ingested from consumption of cephalopods and crustaceans in Korea. Scale bar represents one standard deviation. Cd, cadmium; Pb, lead; Hg, mercury; Cr, chromium; Ag, silver; Ni, nickel; Cu, copper; Zn, zinc. THQs were based on the U.S. Environmental Protection Agency Human Health Risk Assessment approach (41). The THQ was calculated using the equation THQ* $= (EF \times ED \times DI \times HC)/(RfD \times BW \times ET)$, *where EF is the exposure frequency (350 days per year); ED is the exposure duration (81 years), equivalent to the average lifetime in Korea (39); DI is the average daily intake of cephalopods and crustaceans (grams per day) shown in Table 2; HC is the heavy metal concentration in each part of the cephalopods and crustaceans (micrograms per gram) shown in Figure 1; RfD is the oral reference dose (micrograms per kilogram per day); BW is the average body weight of an adult human in Korea (62.8 kg) (24); and ET is the average exposure time for noncarcinogens (ED* $\times$ *365 days per year). The RfDs of Pb, Hg, Cr, Ag, Ni, Cu, and Zn used the provisional tolerable daily intake shown in Table 2. The RfD of Cd was set at 1.0 as established by the EPA (41).*

cephalopods and crustaceans for an adult human. In this study, the daily intake of internal organs or of whole tissue assumes that consumers ingest only the internal organs or ingest the whole tissue including internal organs, respectively. The EDI was compared with the PTDI proposed by the JECFA *(16–18)* or the EPA *(41)*. The provisional tolerable weekly intake for Hg and Pb *(16, 17)* and the provisional tolerable monthly intake for Cd *(18)* were established by the JECFA because of the risks of even trace concentrations of these metals to human health.

Among the hazardous heavy metals (Cd, Pb, and Hg), the EDI values of Cd for muscles, internal organs, and whole tissues of both cephalopods and crustaceans ranged from <0.0001 to 0.0122, 0.0007 to 0.9316, and 0.0003 to 0.2293 μg/kg/day, which accounted for <0.01 to 1.47%, 0.08 to 112.24%, and 0.03 to 27.63% of the PTDI, respectively; the highest values were found in each part of squid (Table 2). The sum of the Cd EDIs for muscle, internal organ, and whole tissue samples of all species were 1.73, 130.57, and 32.11%, respectively, of the PTDI. The very high Cd EDIs in internal organs and whole tissues were due to both the relatively large daily intake of squid (Table 2) and the particularly high Cd concentration in the internal organs of squid (Fig. 1).

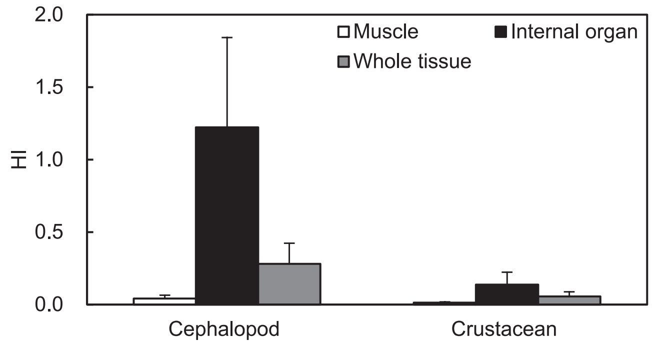

FIGURE 3. *Hazard index (HI) for heavy metals from the consumption of cephalopods and crustaceans in Korea. Scale bars represent one standard deviation. HI was calculated by summing the target hazard quotients of individual heavy metals shown in Figure 2.*

The dietary intake of Pb was the lowest among the hazardous metals in all tested samples; the highest EDI value was again in the internal organs of squid but represented only 0.28% of the PTDI. The highest Hg EDI values were also found in the internal organs of squid and accounted for 1.17% of the PTDI. The sums of the Hg EDIs for muscles, internal organs, and whole tissues in all analyzed species were only 0.93, 1.67, and 1.12%, respectively, of the PTDI.

The EDIs of other metals (Cr, Ag, Ni, Cu, and Zn) were compared with the PTDI values based on the reference doses established by the EPA *(41)*. In the internal organs, which had the highest metal concentrations in each species, the highest EDIs for Cr, Ag, Ni, Cu, and Zn were 0.21, 1.15, 0.30, 18.59, and 5.29%, respectively, of the PTDI, and were found in squid.

The THQ and HI were used to assess the risk to an adult human of ingestion of heavy metals via consumption of cephalopods and crustaceans. The THQ was estimated by comparing the ingested amount of a heavy metal with a standard reference dose (Fig. 2); the HI is the sum of the various THQs (Fig. 3). The THQ and HI values proposed by the EPA are integrated risk indexes and are used widely in the risk assessment of various contaminants in foods *(40)*. Both THQ and HI are useful parameters for evaluating the risk of heavy metal ingestion associated with the consumption of contaminated foods *(1, 29)*. The mean THQ of heavy metals in muscles, internal organs, and whole tissues of cephalopods ranged from <0.001 to 0.017, 0.003 to 0.811, and 0.001 to 0.168, respectively; the highest THQ was for Cd in internal organs. In contrast, the mean THQ of heavy metals was low in each part of each type of crab, ranging from <0.001 to 0.092. The THQ was relatively high for Cd and Cu in all samples analyzed but low for Pb, Hg, Cr, Ag, and Ni.

An HI exceeding 1.0 indicates that the contaminant is toxic and represents a hazard to human health *(1, 28, 29)*. The heavy metal HI was higher for cephalopods than for crustaceans (Fig. 3) because of the high THQ of Cd in internal organs of cephalopods (Fig. 2). The mean HIs in muscles, internal organs, and whole tissues of cephalopods were 0.042, 1.223, and 0.281, respectively; the highest HI was in internal organs (>1.0). The maximum HI for whole tissue of cephalopod species was also quite high (0.424) but was lower for muscle (0.065). In contrast, the mean HI was low in each part of each type of crab, ranging from 0.014 to 0.138; the maximum was found for internal organs and was substantially less than 1.0.

In conclusion, we found that heavy metals accumulated to significantly higher levels ($P < 0.05$) in internal organs than in muscles of cephalopods and crustaceans. The mean concentrations of Cd in the internal organ samples of all species exceeded the regulatory limits for Cd in the edible portion of these animals as set by Korea (with the exception of the blue crab) and the EU. The Cd concentrations in all tested whole tissues of squid and octopus (relatively large cephalopods), red snow crab, and snow crab exceeded the regulatory limit set by the EU. However, the Cd concentrations in the muscle samples of all species were far below the limits set by Korea and the EU. The sums of the Cd EDIs for muscles, internal organs, and whole tissues of all species tested were 1.73, 130.57, and 32.11%, respectively, of the PTDI. The HI was higher for cephalopods than for crustaceans because of the high THQ of Cd in the internal organs of cephalopods, particularly squid. The mean HIs in the muscle, internal organ, and whole tissue samples of cephalopods were 0.042, 1.223, and 0.281, respectively; the highest HI for internal organs was greater than 1.0. The highest HI for whole tissue samples of cephalopods was also quite high (0.424). These results suggest that the internal organs of relatively large cephalopods (squid and octopus) and crabs (with the exception of the blue crab) should be removed prior to consumption to reduce exposure to Cd.

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