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**PROOF** RESEARCH PAPER

# Heavy Metals Contamination of Canned Fish and Related Health Implications in Iran

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**Abstract**

Samples of some popular brands of canned *Thunnus albacares*; *Clupeonella cultriventris caspia*; *Euthynnus affinis*; and *Thunnus tonggol* in the Iranian market were analyzed for determination of Cr, Cu, Fe, Mn and Ni after digestion with 10 ml of 1 N nitric acid by inductively coupled plasma–optical emission spectrometer. The results showed that the concentrations of metals (µg/g wet weight) with an average of 2.66 for Cr, 0.92 for Cu, 54.68 for Fe, 0.33 for Mn and 0.22 for Ni were lower than the Maximum Permissible Limits (MPL) established by WHO. Also, The Health Risk Index (HRI) values were within the safe limits (<1), and there is no potential health risk for adults and children via consumption of canned fish. Despite the fact that our canned fish samples does not significantly contribute to total body burden of analyzed metals, But due to the increased discharge of pollutants into the environment, especially the marine ecosystem, and consider that various species of fish retain substantial amounts of heavy metals during their lifespan, the monitoring of heavy metals concentrations in food is important. Therefore, the study of heavy metal distribution on a temporal basis is recommended.

*Keywords*: Canned fish, health risk index, heavy metals, food safety.

## Introduction

Aquatic environment makes up the major part of our resources and environment, hence, its safety is directly related to human health. Excessive contamination of these ecosystems has raised major environmental and health concerns worldwide (Omar, Zaghloul, Abdel-Khalek & Abo-Hegab, 2013).

Many elements that are present in seafood are essential for human life at low concentrations (Sobhanardakani, Tayebi, Farmany & Cheraghi, 2012; Hosseini, Aflaki, Sobhanardakani, Tayebi, Babakhani Lashkan & Regenstein, 2013a; Sobhanardakani, Hosseini, Kolangi Miandare, Faizbakhsh, Harsij & Regenstein, 2017). However, other elements such as As, Cd, Cr, Hg, and Pb have no known essential function in biological systems and are toxic even at low concentrations (Isibor, 2017; Isibor, Imoobe & Ohiokhioya, 2017). It should be noted that the skin and gills, and also the intake of contaminated food or drinking water as the main resources can cause enter the heavy metals into the fish body. Therefore, accumulation of heavy metals in fish tissues is dependent on the concentration of the metal in the aquatic ecosystem and period of exposure to contaminants in the water (Pourang, Tanabe, Rezvani, & Dennis, 2005; Sobhanardakani, Tayebi & Farmany, 2011; Hosseini *et al.*, 2013a; Hosseini, Sobhanardakani, Batebi Navaei, Kariminasab, Aghilinejad & Regenstein, 2013b)

Fish are healthful for human due to provide a source of dietary protein, low saturated fat, and omega-3(n-3) fatty acids and for this reason they are widely consumed around the world. Also, several studies have documented the long-term cardioprotective and the reproductive benefits of eating fish. However, benefits may be offset by the presence of pollutants, especially heavy metals (Burger, & Gochfeld, 2004; Hosseini *et al.*, 2013a).

The human health risk assessment is the process requires identification, collection, and integration of information on the toxins and chemicals health hazards, exposure of human to the chemical and relationships between exposure, dose and adverse health effects in polluted environmental. On the other hand, a human potential health risk assessment includes hazard identification, dose-response assessment, exposure assessment and risk characterization steps (Sobhanardakani, 2017).

Canned fish is largely eaten in many countries that located on different continents especially Libya, USA, Portugal, the Kingdom of Saudi Arabia,

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Turkey, and Iran (Hosseini *et al.*, 2013a). Because of the concern for the adverse health effects of heavy metals particularly organ failure/damage (e.g., kidney and liver), this study was done to human health risk assessment of Cr, Cu, Fe, Mn and Ni through consumption of different brands commonly consumed canned tuna fish in Iran.

## Materials and Methods

### Sample Collection

One hundred and twenty samples of four different brands of canned fish, including 30 samples for each species (yellowfin tuna, *Thunnus albacares*; common Kilka, *Clupeonella cultriventris caspia*; Kawakawa, *Euthynnus affinis*; and longtail tuna, *Thunnus tonggol*; were purchased from different markets in Tehran and used for analysis of concentration of heavy metals (Cr, Cu, Fe, Mn, and Ni).

### Chemical Analyses

After opening, each can content was homogenized thoroughly in a food blender using stainless steel cutters (Boadi, Twumasi, Badu, & Osei, 2011). Then, samples were digested with 10 ml of 1 N $HNO_3$ in closed Teflon vessels in a microwave oven (CEM MARS-5 closed vessel microwave digestion system) according to the method introduced by Sobhanardakani (2017). After digestion, blank solutions were prepared in the laboratory in a similar manner to the field samples. All metal concentrations (µg/g, wet weight) were determined with three replications using inductively coupled plasma–optical emission spectrometer (Optima 2100 DV, Perkin Elmer) (Turkmen, Turkmen, Tepe, Tore & Ates, 2009). Standard solutions were prepared from stock solutions (Merck, multi-element standard). All the instrumental conditions applied for Cr, Cu, Fe, Mn, and Ni concentration determinations were set in accordance with general recommendations (wavelength for Cr, Cu, Fe, Mn and Ni: 267.72 nm, 324.75 nm, 258.59 nm, 257.61 nm, and 231.6 nm, respectively). Also, the Limits of Detection (LOD) and Limits of Quantification (LOQ) were assessed according to the method introduced by Nascimento *et al*. (2008) and both are presented in Table 1.

### Statistical Analysis

The statistical analysis of the obtained results consisted of a first Kolmogorov-Smirnov normality test, followed by the study of the variance homogeneity using an ANOVA parametric test with a DMS post hoc and Duncan multiple range test. The mean levels of heavy metals were compared with international standard using a one-sample test. Probabilities less than 0.05 were considered statistically significant ($P<0.05$). The statistical calculations were done using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) statistical package.

### Potential Health Risk Assessment

For computing potential health risk assessment the average Daily Intake of Metal (DIM) was calculated according to the Equation 1 (Guo, Yue, Li & Yuan, 2016):

$$\mathrm{DIM} = \frac{\mathrm{Cmetal} \times \mathrm{Cfactor} \times \mathrm{Dfood\ intake}}{\mathrm{B}} \quad (1)$$

Where *Cmetal*, *Cfactor*, and *Dfood* intake represent the heavy metal concentrations in canned fish (µg/g), conversion factor, and average daily intake of analyzed canned fish for adult and children (3.5E0-3 kg per person per day) respectively. *B* indicated that average body weight (70.0 kg for adult and 15.0 kg for children). The conversion factor (0.085) is used to convert fresh weight into dry weight (Falco, Ilobet, Bocio, & Domingo, 2006; Omar *et al.*, 2013; Guo *et al.*, 2016; Tang, Cheng, Zhao & Wang, 2015).

The Health Risk Index (HRI) for the local population through the consumption of canned fish was assessed using the Equation 2 (Guo *et al.*, 2016):

$$\mathrm{HRI} = \frac{\mathrm{DIM}}{\mathrm{RfD}} \quad (2)$$

Here, DIM and RfD represent the daily intake of metal and reference dose of metal, respectively. The oral reference doses (mg/kg/day) of metals were 1.50 for Cr, 0.04 for Cu, 0.643 for Fe, 0.014 for Mn and 0.02 for Ni (Harmanescu, Alda, Bordean, Gogoasa, & Gergen, 2011; Xue, Liu, Liu, & Yan, 2012; Omar *et al.*, 2013; Liang, Xue, Wang, Sun, Yang, & Liu, 2015; Zeng, Wang, Wang, Guo, Chen, & Zhuang, 2015; Sobhanardakani, 2016). In this regard, the HRI <1 means the exposed population is assumed to be safe.

The Total HRI (THRI) of heavy metals for the canned fish was calculated according to the Equation 3 (Guo *et al.*, 2016):

$$\mathrm{THRI} = \mathrm{HRI\ (toxicant\ 1)} + \mathrm{HRI\ (toxicant\ 2)} + \ + \mathrm{HRI\ (toxicant\ n)} \quad (3)$$

## Results

The concentrations of Cr, Cu, Fe, Mn and Ni in the analyzed canned fish samples are presented in Table 1. Among the analyzed canned fish samples, Cr was detected in amounts ranging from 1.65 µg/g to 3.24 µg/g, Cu ranged from 0.73 µg/g to 1.18 µg/g, Fe ranged from 34.02 µg/g to 77.53 µg/g, Mn ranged from 0.04 µg/g to 1.01 µg/g and Ni ranged from 0.14

**Table 1.** Concentrations and analysis of LOD and LOQ of metals in canned fish samples (µg/g, wet weight)

| Metal | Min. | Max. | Mean | Std. | LOD | LOQ |
|---|---|---|---|---|---|---|
| Cr | 1.65 | 3.24 | 2.66d* | 0.73 | 0.048 | 0.150 |
| Cu | 0.73 | 1.18 | 0.92c | 0.19 | 0.037 | 0.115 |
| Fe | 34.02 | 77.53 | 54.68e | 18.89 | 0.072 | 0.222 |
| Mn | 0.04 | 1.01 | 0.33b | 0.46 | 0.075 | 0.220 |
| Ni | 0.14 | 0.37 | 0.22a | 0.10 | 0.029 | 0.085 |

* The letters (a, b, c, d, e) represent the statistical significant differences ($P<0.05$) in the contents of analyzed metals between canned fish samples.

µg/g to 0.37 µg/g, respectively.

Comparing the heavy metal concentrations in studied canned fish with the maximum permissible limits (µg/g) (8.0 for Cr, 30.0 for Cu, 100.0 for Fe, 1.0 for Mn, and 1.0 for Ni) established by Agency for Toxic Substances and Disease Registry (ATSDR), Food and Agricultural Organization/World Health Organization (FAO/WHO), Food and Drug Administration (FDA), European Union (EU), and WHO (WHO, 1989; FDA, 2001; ATSDR, 2004;2005,2012a,b; EU, 2005; FAO/WHO, 2009), indicated that the concentration of all analyzed metals were lower than MPL. Therefore, canned fish's contribution to the total body burden of Cr, Cu, Fe, and Ni can be considered as negligibly small.

In addition, all the calculated HRI values of heavy metals were within the safe limits (HRI<1) (Table 2). Furthermore, the THRI values, which varied from— 6.98E-05 to 2.06E-04 for adults and from 3.26E-04 to 9.63E-04 for children— and were also within the safe limit (THRI<1) in this study.

## Discussion

Some factors, such as water chemistry, duration of exposure of fish to contaminants in water, concentrations of contaminants in water column, feeding habit of fish, contamination of fish during handling and processing, quality of canned fish and shelf life of canned fish can affect in the level of contaminants in fish. However, the metal levels in canned fishes is influenced by the pH of the canned product, oxygen concentration in the headspace, the quality of the lacquer coatings of canned products, quality of coating and also storage place (Tahán, Sanchez, Granadillo, Cubillan & Romero, 1995; Hosseini *et al.*, 2013a). The metals accumulation varies greatly between both fish species and/or fish tissues. Generally, fish could translocate the large quantities of toxic heavy metals in the liver, gill, and also muscle tissues (Sobhanardakani, Tayebi, Farmany & Cheraghi, 2012). Contaminants in fish can pose a health risk to the fish themselves, to their predators, and to humans who consume them (Burger, & Gochfeld, 2005).

Chromium is an essential mineral in humans that helps the body use protein, lipid, and carbohydrate metabolism (Cefalu, & Hu, 2004). Also, Cr is widely distributed in human tissues in extremely low and variable concentrations (Hosseini, Sobhanardakani, Tahergorabi & Delfieh, 2013c). Chronic exposure to Cr causes damage to the liver, kidney, circulatory and nerve disorders, as well as skin irritation (Kabata-Pendias, 2010). In this regard, the US National Research Council recommended daily amount of Cr about 60 µg/day for a 70 kg person (NRC, 1989). The results showed that the mean concentrations of Cr in canned fish with an average of 2.66 ± 0.73 µg/g were much lower than the MPL. The maximum value of Cr in canned fish samples (3.24 µg/g wet weight) we obtained was higher than values obtained for canned fish (0.06 µg/g w.wt) in the USA (Ikem & Egiebor, 2005), and in canned sardines in Brazil (1.11 µg/g w.wt) (Tarley, Coltro, Matsushita & de Souza, 2001). In another study, Hussein and Khaled (2014) reported that the mean concentrations of Cr in muscle of three tuna species from Alexandria, Egypt were 0.82 ± 0.07 µg/g w.wt. Also, Islam, Bang, Kim, Ahmed, & Jannat, (2010) reported that the mean concentration of Cr (µg/g, DW) in canned longtail tuna which was imported from the USA, canned bluefin tuna which was imported from the Thailand and canned bluefin tuna (produced in Korea) were 0.58 ± 0.06, 0.32 ± 0.002 and 0.25 ± 0.02, respectively and similar to this study lower than MPL (Islam, Bang, Kim, Ahmed, & Jannat, 2010). A comparison of the Cr concentrations in the canned fish samples marketed in Iran and other selected regions are presented in Table 3.

Copper is an essential element for good health and seafood is known as a good source of dietary copper, but it is very toxic when consumed excessively (Mol, 2011), and for that reason, a maximum limit intake of Cu was set at 30 mg/day by WHO (WHO, 1996). Therefore, a high intake of Cu has been recognized to cause adverse health problems such as kidney and liver damage for human (Hussein, & Khaled, 2014; Stancheva, Makedonski & Peycheva, 2014). According to the results of the present study, Cu in canned fish samples with an average of 0.92 ± 0.19 µg/g wet weight was much lower than the MPL. The results of other study showed that the mean concentrations of Cu in the muscle of three tuna species from Alexandria, Egypt were 1.25 ± 0.43 µg/g w.wt (Hussein & Khaled, 2014). Also, Ikem and Egiebor (2005) reported that the mean concentrations of Cu (µg/kg w.wt) in canned fish from Georgia and Alabama were 0.32 for

**Table 2.** Daily intakes of metals (DIM, mg) and health risk index (HRI) for individual heavy metal caused by the canned tuna fish

| | Cr | Cu | Fe | Mn | Ni |
|---|---|---|---|---|---|
| Adults | | | | | |
| DIM | 1.13E-05 | 3.91E-06 | 2.32E-04 | 1.40E-06 | 9.35E-07 |
| STD | 3.10E-06 | 8.07E-07 | 8.03E-05 | 1.96E-06 | 4.25E-07 |
| Min | 7.01E-06 | 3.10E-06 | 1.45E-04 | 1.70E-07 | 5.95E-07 |
| Max | 1.38E-05 | 5.01E-06 | 3.29E-04 | 4.29E-06 | 1.57E-06 |
| HRI | 7.54E-06 | 9.77E-05 | 3.61E-04 | 1.00E-04 | 4.68E-05 |
| STD | 2.07E-06 | 2.02E-05 | 1.25E-04 | 1.40E-04 | 2.13E-05 |
| Min | 4.68E-06 | 7.76E-05 | 2.25E-04 | 1.21E-05 | 2.98E-05 |
| Max | 9.18E-06 | 1.25E-04 | 5.12E-04 | 3.07E-04 | 7.86E-05 |
| Children | | | | | |
| DIM | 5.28E-05 | 1.82E-05 | 1.08E-03 | 6.54E-06 | 4.36E-06 |
| STD | 1.45E-05 | 3.77E-06 | 3.75E-04 | 9.12E-06 | 1.98E-06 |
| Min | 3.27E-05 | 1.45E-05 | 6.75E-04 | 7.93E-07 | 2.78E-06 |
| Max | 6.43E-05 | 2.34E-05 | 1.54E-03 | 2.00E-05 | 7.34E-06 |
| HRI | 3.52E-05 | 4.56E-04 | 1.69E-03 | 4.68E-04 | 2.18E-04 |
| STD | 9.65E-06 | 9.42E-05 | 5.83E-04 | 6.52E-04 | 9.92E-05 |
| Min | 2.18E-05 | 3.62E-04 | 1.05E-03 | 5.67E-05 | 1.39E-04 |
| Max | 4.28E-05 | 5.85E-04 | 2.39E-03 | 1.43E-03 | 3.67E-04 |

**Table 3.** Comparison of the heavy metal concentrations (µg/g) in canned fish marketed in Iran and other selected regions

| Location | Metal | | | | | Reference |
|---|---|---|---|---|---|---|
| | Cr | Cu | Fe | Mn | Ni | |
| Iran | 2.66 | 0.92 | 54.68 | 0.33 | 0.22 | Present study |
| Iran | - | 0.17-8.0 | 0.009-14.21 | - | - | (Zarei *et al.*, 2010) |
| Ghana | - | - | 1.06-21.45 | 0.006-0.036 | | (Boadi *et al.*, 2011) |
| Saudi Arabia | 0.38 | 0.27 | - | - | | (Ashraf, 2006) |
| Egypt | - | - | 139.0-191.4 | - | | (Hussein, & Khaled, 2014) |
| USA | - | - | 0.003-0.016 | - | 0.00-0.21 | (Ikem, & Egiebor, 2005) |
| USA | - | 0.93 | 107.17 | 0.58 | 0.36 | (Islam *et al.*, 2010) |
| Thailand | - | 1.86 | 35.66 | 0.32 | 0.26 | (Islam *et al.*, 2010) |
| Korea | - | 3.13 | 31.07 | 0.25 | 0.12 | (Islam *et al.*, 2010) |
| Iran | 0.38 | 1.18 | - | 0.23-1.37 | - | (Taghipour, & Aziz, 2010) |
| Nigeria | 0.02 | 0.01 | 8.04-48.18 | - | 0.04-3.26 | (Iwegbue *et al.*, 2009) |

pink salmon, 0.47 for red salmon, 0.25 for tuna, 0.81 for mackerel, 0.83 for sardines and 0.60 for herring. Table 3 shows a comparison of metal concentration in the canned fish from Iran with some other regions reported in the literature.

Iron is an essential mineral and is the most abundant transition element, and probably the most well-known metal in biologic systems especially plays an important role in the human physiology. Iron deficiency causes anemia, reducing cognitive function and also physical work capacity. Whereas, high intake of this element may be the cause of organ failure (Mol, 2011; Hussein, & Khaled, 2014; Stancheva *et al.*, 2014; Wheal, DeCourcy-Ireland, Bogard, Thilsted, & Stangoulis, 2016). Vertebrates (mammals, birds and fish) meat is known good sources of Fe for their high total Fe concentration, as well as presence of haem Fe. It has been proved that haem Fe has greater bioavailability than non-haem Fe due to the different physiological mechanisms of transport across intestinal membranes (Wheal *et al.*, 2016). Accordingly, fish is a major source of Fe for adults and children. The US National Academy of Medicine (NAM) recommends a Recommended Dietary Allowance (RDA) for Fe in elderly men and women-10000 µg/day (National Academy of Medicine, 2001). The results of the present study showed that the highest mean concentration of Fe with an average of 77.53 µg/g wet weight was found in Kawakawa samples. Iron concentration in the literature was reported between 8.04 µg/g w.wt to 48.18 µg/g w.wt for canned sardines consumed in Nigeria (Iwegbue, Nwajei, Arimoro & Eguavoen, 2009). Table 3 shows a comparison of Fe concentration in the canned fish from Iran with some other regions reported in the literature.

The deficiency of manganese, as an essential element, is very striking ranging from severe convulsions, asthma, skeletal and reproductive abnormalities (Finley & Davis, 1999; Samsel & Seneff, 2015). Daily intake of small amounts of Mn is needed for the good health and growth of children.

Children, as well as adults, who lose the ability to remove excess Mn from their bodies, develop nervous system problems. According to the ATSDR, there is no information on the carcinogenicity of this element (ATSDR, 2012b). The results showed that the mean concentrations of Mn in canned fish samples were 0.33 ± 0.46 µg/g. Manganese concentrations in the literature have been reported in the range of 0.51 µg/g w.wt to 1.66 µg/g w.wt in muscle of three tuna species collected from the Alexandria, Egypt (Hussein, & Khaled, 2014), and also 0.64 µg/g w.wt to 1.71 µg/g w.wt for canned sardines consumed in Nigeria (Iwegbue *et al.*, 2009). A comparison of our findings with other studies is shown in Table 3.

Trace amounts of Ni act as activator of some enzyme systems but its toxicity at higher levels can cause respiratory problems (bronchial failure) and also is carcinogenic. Its acute toxicity arises from competitive interaction with Ca, Co, Cu, Fe and Zn. Literature review mentioned that the upper tolerable intake level of Ni for children (1-3 years old) and males/females (19-70 years old) is 7000 µg/day and 40000 µg/day, respectively (National Academy of Medicine, 2001). Also, the WHO recommends 100-300 µg of this element for daily intake (WHO, 1996). The Ni concentration detected in the present study with an average of 0.22 ± 0.10 µg/g was much lower than the MPL. Also, there was found no significant variability ($P<0.05$) in the range of Ni when canned fish samples were compared. Nickel concentrations in the literature have been reported in the range of 0.37 ± 0.0 µg/g w.wt in muscle of tuna species samples that collected from the Alexandria, Egypt (Hussein, & Khaled, 2014). Similarly, Malakootian, Tahergorabi, Daneshpajooh, & Amirtaheri (2011) reported the concentration of Ni in canned fish consumed in Iran was detected in amounts ranging from 0.10 µg/g w.wt to 0.16 µg/g w.wt (Malakootian *et al.*, 2011), and Pourjafar Ghasemnejad, & Noori, (2014) reported Ni concentrations in the range of 0.113 µg/g w.wt to 0.589 w.wt µg/g (Pourjafar *et al*., 2014). The results of present study are within the ranges for those reported earlier 0.09-0.48 µg/g w.wt (Ashraf, 2006). A comparison of our findings with other studies is shown in Table 3.

As shown in Table 2, the average HRI values for adults and children were 1.23E-04 and 5.73E-04 respectively, and therefore, the non-carcinogenic risks for children are greater than adults. In this regard, Hussein and Khaled (2014) reported that the from the human health point of view, Cr, Cu, and Mn, THQ values were less than 1 and show a situation of no risk for the consumer of the investigated tuna species collected from the Alexandria, Egypt. Also, Ordiano-Flores, Galván-Magaña & Rosiles-Martínez (2011) reported that the estimated THQ values of Hg were <1 in each population group (children and adults) due to consumption of yellowfin tuna collected from the Eastern Pacific Ocean (Ordiano-Flores *et al*., 2011).

## Conclusion

Based on the results, canned yellowfin tuna, common Kilka, Kawakawa and longtail tuna have concentrations within permissible limits of Cr, Cu, Fe, Mn and Ni. Also, HRI values of all metals for children and adults were less than safe limits. Therefore, it can be concluded that target population might have no potential significant health risk through only consuming canned tuna fish from the Iran.

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