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Dietary Intake and Health Risk Assessment of Heavy Metals Consumption of *Anas strepera* and *Anas crecca* in Southeastern Caspian Sea

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Abstract
Every year, many thousands of migratory waterfowls are hunted and consumed by people in the southern Caspian Sea region. Therefore, this study was conducted to estimate the intake and health risks of exposure to cadmium (Cd), chromium (Cr), iron (Fe), lead (Pb), and zinc (Zn) from consumption of Gadwall *Anas strepera* and Common Teal *Anas crecca*, two common waterfowl species being hunted in the region. Observed concentrations (µg g^{-1} wet w) of Cd and Pb in pectoral muscles of Gadwall (Cd: 0.36 ± 0.17, Pb: 0.92 ± 0.19) and Common Teal (Cd: 0.13 ± 0.88, Pb: 0.32 ± 0.21) were above the exposure thresholds defined by the European Commission. The estimated daily and weekly intake of heavy metals were below those recommended by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and oral reference dose suggested by the USEPA. In conclusion, there appears to be little risk of exposure to heavy metals associated with the consumption of the flesh of Gadwall and Common Teal by consumers in this region.

1. Introduction

Contamination of heavy metals, especially in aquatic environments, has been of great concern because of their inherent toxicity, widespread availability and persistence (Wang *et al.* 2013). Anthropogenic sources of heavy metals are derived from mining, smelting, agriculture, the petrochemical industry, printing, aquaculture, the electronic industry and municipal waste. These are ultimately discharged into the aquatic environment where they can be bioaccumulated by aquatic organisms. They are even biomagnified through the food chain, resulting in elevated levels in predatory organisms (Rainbow & Luoma 2011). The human exposure to contaminants such as metals through aquatic food supplements is of global health concern (Lozano *et al.* 2010). Meat is a very good source of nutrients, however, contamination of meat by heavy metals can be a serious health threat (Demirezen & Uruc 2006). When heavy metals appear above the permissible concentrations in aquatic organisms, they have deleterious effects not only on the aquatic organisms but also on the health of the consumers, especially when they are regularly consumed in large quantity (Ihedioha & Okoye 2013). Intake of heavy metals through the food chain by humans has been widely reported throughout the world (Muchuweti *et al.* 2006). Due to the non-

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biodegradable and persistent nature, heavy metals are accumulated in vital organs in the human body such as kidneys, bones, and liver and are associated with a variety of serious health disorders (Duruibe *et al.* 2007).

The aim of the present study was to investigate the concentrations of Cd, Cr, Fe, Pb, and Zn in muscle tissue of the Gadwall and Common Teal to estimate the potential risk for human health derived from ingestion of contaminated Gadwall and Common Teal, because every year many are harvested and consumed by people in southern Caspian Sea. We compared the weekly and daily intake with the provisional tolerable weekly intake (PTWI) recommended by the Joint FAO/WHO Expert Committee on Food Additive online database (JECFA 2013) and with the Reference Dose provided by the Integrated Risk Information System (IRIS) online database (IRIS 2013) as well as other national and international guidelines. We chose to examine concentrations of metals in muscle tissue of the Gadwall and Common Teal because: these species are common; specimens for study are easy to acquire; they are popular game birds in southeastern Caspian Sea where no similar study has been reported; and Gadwall and Common Teal are the largest and the smallest dabbling duck in Iran, respectively. Also we chose ducks as biomonitors of Cd, Cr, Fe, Pb, and Zn in wetlands of the southern Caspian Sea.

2. Methods

2.1. Study area

Gadwall and Common Teal spend autumn and winter seasons in the south Caspian Sea in northern Iran, spending about five months in the region. The ducks examined herein were shot in February 2012, late in their stay, from two sites in southeastern Caspian Sea; Miankaleh International Wetland (36°20′N, 53°43′E, 66,933 ha) and Gomishan International Wetland (54°53'N, 37°9'E, 20,000 ha) under license of the Golestan Provincial Office of the Department of the Environment. These sites are among the important wintering areas in the Middle East (Fig. 1). Over 100 species of waterbirds have been recorded in these wetlands. In general, ducks move to Miankaleh and Gomishan wetlands from late October to early November and stay to the next late March and early April (Sinkakarimi *et al.* 2013).

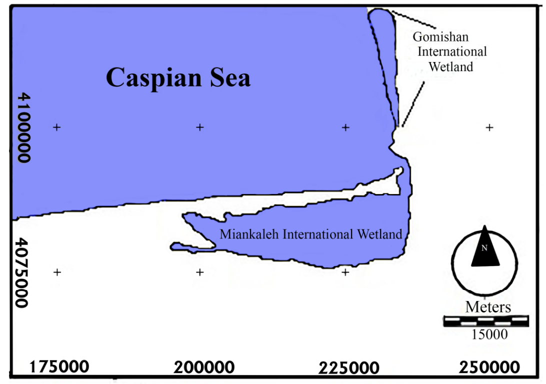

Fig. 1. Sampling sites in the southeastern Caspian Sea, Iran.

2.2. Preparation of specimens

Upon collection, specimens were immediately transported to the laboratory where pectoral muscles were excised and samples were frozen for analysis. Approximately, 5 g of the wet tissue of muscle was placed in a crucible porcelain and dried at 135°C for 2 hours. Samples were then transferred to a cool muffle furnace and the temperature slowly raised to 450–500°C overnight. After cooling, 2 ml of HNO3 was added and the sample was then heated on a hot plate until dried. The sample was returned to the cooled furnace and the temperature was raised to 450–500°C for an hour. After cooling, 10 ml of 1N HCl was added and heated on the hot plate to dissolve the ash. Digested samples were filtered and diluted to 25 ml by 1N HCl (AOAC 2000). Metal concentrations were determined using an atomic absorption spectrophotometer (GFS97, Thermo Electron, Cambridge, UK). Accuracy of the analysis was checked by measuring certified reference material tissue. Recoveries ranged from 95 to 105 percent. The precision was calculated as the percentage of relative standard deviation (%RSD) of three replicated samples of the prepared standard, and was found to be less than 5%. Detection limits (µg g^{-1} wet weight) were 0.004 for Cd, 0.03 for Cr, 0.05 for Fe, 0.001 for Pb and 0.005 for Zn. The metal concentration in the samples was expressed as microgram per gram wet weight (µg g^{-1} ww).

2.3 Estimated Daily Intake (EDI) and Estimated Weekly Intake (EWI)

The EDI and EWI depend on both the metal concentration in food and the daily and weekly food consumption. In addition, body weight influences the tolerance to heavy metals and other contaminants exposure. The EDI and EWI were calculated as follow:

$$\text{EDI } (\mu g\ g^{-1}\ daily^{-1}) = (C \times FIR_D) / BW \quad (1)$$

$$\text{EWI } (\mu g\ g^{-1}\ week^{-1}) = (C \times FIR_W) / BW \quad (2)$$

Where C is the concentration of the studied metals in food; FIR_D (g day^{-1}) and FIR_W (g $week^{-1}$) are food ingestion rate in the study cohort; and BW represents the body weight. Sinka Karimi *et al.* (2013) indicated that a hunter's family members consume about 95g day^{-1} of waterfowl in Golestan province. A human body weight of 70 kg was used in this study (USEPA 1989).

3. Results and Discussion

The concentration of metals in the pectoral muscle of Gadwall and Common Teal are shown in Table 1. The concentration of metals follows the sequence: Fe>Zn>Pb>Cd>Cr.

The Institute of Turkish Standards for Food (ITSF) and European Commission (EC) considered 0.5 and 0.1 $\mu g.g^{-1}$ w.w, respectively as the permissible threshold of Pb in food (ITS 2000; EC 2001). The flesh of both duck species clearly exceeded the EC threshold and the flesh of Gadwall exceeded ITSF threshold, and thus pose health risks to humans consuming their meat. In contrast, the maximum permitted concentration of Pb in food proposed by Australian National Health and Medical Research Council (ANHMRC), Spanish legislation, MAFF, Chile, Italy, Finland, Poland, New Zealand, United Kingdom and Venezuela are 2.0 $\mu g\ g^{-1}$ as wet weight basis (Plaskett & Potter 1979; Nauen 1983; BOE 1991). Also, the United Kingdom has legislated the permissible level of Pb in food at 1.0 $\mu g\ g^{-1}$ (UKLFR 1979). In this study, the concentrations of Pb in Gadwall and Common Teal flesh were below these thresholds (Table 1). There are few studies for comparison of xenobiotic metals like Pb in muscles of ducks or waterfowl; most previous studies focused on liver and kidneys. The concentration of metals in muscle tissues of hunted waterfowls, especially ducks, is important from the standpoint of consumption. In a similar study in the southeastern Caspian Sea, Sinkakarimi *et al.* (2013) examined Pb levels in muscles of Pochard *Aythya ferina* and Mallard *Anas platyrhynchos*, and concluded that their flesh was fit for human consumption. The concentration of Pb ($\mu g.g^{-1}$ w.w) in this study was greater than, Canada Geese *Branta canadensis* (0.003±0.002) from USA (Tsipoura 2011), Mallard (0.23), Tufted Duck *Aythya fuligula* (0.15) from Warmia and Mazury, Poland (Szymczyk & Zalewski 2003) and Mallard (0.04) and Coot (0.06) from Zator, in southern Poland (Binkowski 2012).

The mean Cd concentrations we observed in Gadwall and Common Teal did not exceed the maximum permitted concentration of the ANHMRC (2 $\mu g\ g^{-1}$), Western Australian authorities (5.5 $\mu g\ g^{-1}$) and Spanish legislation (1 $\mu g\ g^{-1}$) (Plaskett & Potter 1979; BOE 1991). In contrast, observed concentrations of Cd in the flesh of Gadwall and Common Teal were greater than the EC threshold (0.05 $\mu g.g^{-1}$ ww) for Cd in foods (Plaskett & Potter 1979; UKLFR 1979; EC 2001). In similar study, Binkowski (2012) reported that muscles of Mallard and Coot *Fulica atra* from Poland were safe based on EC threshold for human consumption. The mean concentration of Cd in our study was greater than Coot (0.13±0.1), from western Iran (Mansouri & Majnooni 2014), Canada Geese (0.007±0.002) from USA (Tsipoura *et al.* 2011), Mallard (0.01) and Coot (0.02) from Zator, in southern Poland (Binkowski 2012), Scaup *Aythya marila* (0.06±0.02) from Szczecin Lagoon, Poland (Kalisińska & Salicki 2010), Spot-billed Duck *Anas poecilorhyncha* and Mallard (0.03±0.03) and Coot (0.07±0.1) from Slovakia (Gasparik *et al.* 2010).

The concentration of Cr observed was lower than the Western Australian Food and Drug regulations, stated concentration of 5.5 $\mu g\ g^{-1}$ for Cr in seafood consumption (Plaskett & Potter 1979). The concentration of Zn we observed in Gadwall and Common Teal muscles was far below the maximum acceptable limit (1000 $\mu g\ g^{-1}$) set by ANHMRC in foods (Plaskett & Potter 1979), and WHO (Cliton *et al.* 2008).

The mean concentration of the essential elements (Fe and Zn) in this study was within the ranges normally found in other ducks in other regions (Szymczyk & Zalewski 2003; Kalisińska *et al.* 2004; Bojar & Bojar 2009).

An important aspect in assessing risk to human health from potentially harmful chemicals is the dietary intake of foods containing those substances, so that exposure can remain within the established safety margins (i.e. PTWI and PTDI suggested by FAO/WHO Expert Committee on Food Additives). Thus, the daily and weekly intake of Cd, Cr, Fe, Pb and Zn were estimated and compared with the recommended values to assess whether the concentrations of heavy metals found in pectoral muscle of Gadwall and Common Teal from southeastern Caspian Sea were safe for human consumption (Table 2). In the present study we have shown that the EDI and EWI of Pb, Cd, Zn, and Fe by the consumption of Gadwall and Common Teal meat by adults are lower than the tolerable EDI and EWI recommended by the JECFA online database (JECFA 2013). With respect to Cr, JECFA (2013) does not set a provisional tolerable intake. However, comparing the estimated intake with the oral reference dose suggested by the USEPA (IRIS 2013) (equal to 3 µg g^{-1} day^{-1} for Cr VI and 1500 µg g^{-1} day^{-1} for Cr III), our result indicate that daily intake was lower than this reference dose (Table 2). Also, our results for Cd, Fe and Zn were lower than oral reference dose suggested by the USEPA (Cd: 1 µg g^{-1} day^{-1}, Fe: 700 µg g^{-1} day^{-1} and Zn: 300 µg g^{-1} day^{-1}) (IRIS 2013).

Although the observed concentrations of Cd and Pb in the flesh of Gadwall and Common Teal were above the thresholds set by the European Commission, there was not a health risk from standpoint of EWI and EDI, because consumption risk is a function of both food content and consumption rate. However, the risk of metal exposure from the consumption of duck flesh must be considered in the context of the total daily intake of food and drink containing the elements of concern. For example, other products such as rice are also known to contain Cd and Pb. Therefore, further studies of heavy metals in dietary components are needed.

4. Conclusions

Each year, thousands of waterfowl are harvested in the wetlands of southern Caspian Sea, Iran. To date, little information is present on the risk of consumption of the flesh of waterfowl. The results of this study indicate that the estimated PTWI and PTDI values for Pb, Cd, Cr, Zn and Fe in the pectoral muscle of Gadwall and Common Teal were far below the established values by various authorities except for more restrictive European Commission guideline.

Table 1. Metal concentrations (µg g^{-1} ww; Mean±SD) in the pectoral muscle of Gadwall and Common Teal collected from wetlands in southeastern Caspian Sea, Iran.

Species	n	Pb	Cd	Cr	Zn	Fe
Gadwall	20	0.92±0.19	0.36±0.17	0.18±0.10	8.06±2.74	65.72±22.94
Common teal	20	0.32±0.21	0.13±0.88	0.08±0.03	8.55±2.71	39.84±21.55

Table 2. The estimated daily and weekly intakes for Gadwall and Common Teal flesh consumed by adult people in southeastern Caspian Sea, Iran.

Metal	PTWI[*]	PTWI[b]	PTDI[c]	Gadwall EWI[d] (EDI)[e]	Common Teal EWI (EDI)
Pb	25[a]	1750	250	8.7 (1.3)	3.0 (0.4)
Cd	6[a]	420	60	3.4 (0.5)	1.2 (0.2)
Cr	-	-	-	1.71 (0.2)	0.8 (0.1)
Zn	7000[a]	490,000	70,000	76.6 (10.9)	8.2 (11.6)
Fe	5600[a]	392,000	56,000	624.3 (89.2)	378.5 (54.0)

[*] Provisional Permissible Tolerable Weekly Intake (PTWI) in µg/week/kg body weight.
[**] Mean Daily waterfowl consumption in Iran is 0.95 kg per person.
[a] (JECFA 2013)
[b] PTWI for 70 kg adult person (µg/week/70 kg body weight).
[c] PTDI, permissible tolerable daily intake (µg/day/70 kg body weight).
[d] EWI, estimated weekly intake in µg/week/70 kg body weight.

[e] EDI, estimated daily intake in µg/day/70 kg body weight.

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