



Health Risk Assessment of Essential and Toxic Metals in Canned/Pouched Food on Kitten and Adult Cats: an Animal Health Risk Assessment Adaptation Assay

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Abstract

The main aim of this study was to determine the levels of Al, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn in commercial canned and pouched cat foods (salmon, tuna, liver, fish, and other aquatic products) and assess the potential health risks to kitten (≤ 1 years old) and adult cats (≥ 1 years old) associated with the recommended average consumption rate of labels. The study was also aimed to adapt the health risk assessment method to animal health and to support clinical prevention and diagnosis. The detected levels of the metals were below the data from other studies, except the mean Fe in all and Pb levels in salmon and kitten foods. Target hazard quotient (THQ) and total target hazard quotient (TTHQ) values did not exceed 1. That means the studied metals do not pose a health risk for adult cats and kittens. Dietary Hg and Cd should also be considered in the differential diagnosis of cases with clinical or postmortem findings, especially regarding neurological, kidney, and liver tissues. In conclusion, although canned/pouched consumption does not pose a health risk with regard to metals, further studies of health risk assessment for other pollutants by this first adaptation method will be necessary.

Keywords Animal health risk assessment · Heavy metals · Cat food · Target hazard quotient · Total target hazard quotient

Introduction

Anthropogenic or naturally originated chemical pollutants like metals (such as aluminum [Al], cadmium [Cd], chromium [Cr], copper [Cu], iron [Fe], lead [Pb], manganese [Mn], mercury [Hg], nickel [Ni], and zinc [Zn]), metalloids (such as arsenic [As]), pesticides (such as insecticides, herbicides, fungicides, and molluscicides), endocrine disruptors (such as bisphenol A, phthalates, and tributyltin), persistent organic pollutants (such as

polyaromatic hydrocarbons and halogenated compounds), Maillard reaction products (such as acrylamide and furans), human/veterinary pharmaceuticals, personal care products, nano-pollutants, and food/feed additives enter food/feed webs and threaten not only human but also animal health [1, 2]. These potentially toxic pollutants are mainly carcinogens, teratogens, mutagens, and immune, neural, cardiovascular, hepatic, renal, reproductive, and endocrine disruptors [3, 4]. Heavy metals are a broad class of pollutants and toxicologically distinguished from others by intensive uses, non-biodegradability, accumulation, and magnification in the food chain. Some of the most affected/accumulated food chain members are aquatic animals and health risk assessment based on metal levels in the tissues of various fish species is a hot research topic [5–7]. Owners prefer the canned/pouched or homemade diets with fish and liver the most because they are more appetizing to cats. Yet, as demonstrated in previous studies, heavy metals accumulate in higher levels in aquatic organisms [5, 8]. Also, heavy metals accumulate in higher levels in target organs like the liver (metabolism), kidneys (extraction), and bones. As demonstrated in previous studies, some metals accumulate in highest levels in the liver because it

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is the main organ for the metabolism of substances [9–11]. Therefore, diets containing seafood and liver could pose more health risks than others.

Like all living animals, cats require a balanced diet to grow normally and maintain health once they are mature [12]. Commercial feeds formulated in line with the nutritional needs of cats are an effective factor in prolonging the lifespan by supporting health [13]. Energy is necessary for the body's metabolic work, which means cats' daily food intake must provide adequate metabolic energy [12]. Therefore, the daily feed requirement can alter according to the lifestyle (indoor, outdoor, etc.), health status (healthy, sick, obese, or cachectic), or physiological state (sex, pregnant, lactating, geriatric, pediatric, kitten, adult, etc.) of the cat. An ideal diet for a cat is programmed by veterinarians according to its lifestyle, species, breed, and physiological needs. Consequently, the daily consumption rate and quantity should be calculated considering the nutritional and energy requirements of the cat and the nutritional content of the food [14]. On the contrary, pet owners prefer market foods and feeding based on their own free choice primarily and the manufacturer's recommendation secondarily [15]. Cat feeds are classified as dry, wet (canned/pouched), and semi-moist; commercially natural and organic foods; raw feed diets; vegetarian products; and homemade or veterinary (therapeutic) diets according to specific needs [12]. However, animal health specialists suggest consumption of canned feed according to individual nutrient, calorie, or metabolic energy requirements [14, 16], and wet foods form half of the diets or feeding preferences for a large percentage of cats in some countries [13, 17]. The commercially canned adult and kitten cat feed weights range between 70 and 415 g, and the range for pouches is between 80 and 100 g. The average daily consumption recommendations are 300–400 g for a normal active 4–5 kg adult cat and 200–300 g for a normal active 4–10-month-old kitten in two or three portions according to the labels.

Even if health risk assessment is not suitable in farm animals (such as cattle, sheep, goats, pigs, fishes, rabbits, poultry, turkey, geese, and ducks) because of their short lifespan, a health risk assessment would need to be considered (a hazard characterization especially including genotoxic effects, developmental neurotoxicology, and behavioral alterations) in companion animals (such as dogs, cats, or horses) which can reach the geriatric stage of life [1].

Health risk assessment is a scientific tool used for the characterization of health risk potential on the target population in various exposure scenarios [18]. Several methods are used in human health risk assessment [5, 19]. Among these, target hazard quotient (THQ) is one of the most commonly used methods to evaluate chronic non-carcinogenic effects on consumers and the most suitable for adaptation in animal health risk assessment.

There are some parameters that should be considered in the adaptation of human health risk assessment to animal health. The average body weight, which is an important parameter for assessment, varies widely in animals compared to humans, relative to breed and gender (male: 2.5–12 kg, female: 2–8 kg). Like body weight, the average life span of cats, 14 years (12–20 years), can vary considerably among breeds [14, 15, 20, 21]. Therefore, calculations should be based on the average body weight or life span by breed and gender. On the other hand, there are some common parameters that facilitate the adaptation of assessment. The oral reference doses (RfD) between these are one of the most important parameters of the risk assessment and calculated according to toxicity studies that have been conducted on laboratory animals for extrapolation to human toxicity [18]. Therefore, considering the variables and parameters used in the method, the risk assessment can be adapted to animal health.

The objectives of this study are (i) to determine heavy metal (Al, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn) contamination levels most associated with environmental pollution in canned and pouched cat foods, (ii) to quantify the potential health risks to cats (male/female and adult/kitten) by recommended average consumption rate of products, and (iii) to adapt the health risk assessment method to animal health science.

Materials and Methods

Sample Collection

A total of 50 commercial (15 different trademarks) canned and pouched cat foods were collected from local markets in Istanbul during 2019 and 2020. The foods were labeled as with salmon ($n = 10$), tuna ($n = 10$), and liver ($n = 10$) for adults, with aquatic products (fish, shrimp, etc.) ($n = 20$) for kittens.

Chemical Analyses

Microwave acid digestion (Cem, Matthews, NC, USA) oven was used to remove the organic parts in the samples. Firstly, all of the canned/pouched cat foods were homogenized with Ultra Turrax homogenizer (IKA T25, Germany), and 0.5 g (± 0.1 mg) was weighed into Teflon vessels. Samples were digested by 8 mL of nitric acid (HNO_3 ; 65%, Merck, Germany) and 2 mL of hydrogen peroxide (H_2O_2 ; 30%, Merck, Germany) in a microwave system (CEM MARS X press, closed Teflon vessel microwave oven) using the 4-step program in accordance with the procedure for 15 min at 85% power and 200 °C. After completing digestion process, the residues were cooled and filtered through a 0.45- μm filter

paper (Whatman, USA). The filtrates were transferred to a volumetric flasks and filled up to 25 mL with deionized water. Finally, the digested solutions were kept at +4 °C until analysis. The Al, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn contamination levels in canned and pouched cat foods were analyzed by inductively coupled plasma optical emission spectrophotometer (ICP-OES-Perkin-Elmer-Norwalk, USA). The following wavelength lines of the ICP-OES analysis were used: Al 396.2 nm, Cd 228.8 nm, Cr 267.7 nm, Cu 324.8 nm, Fe 259.9 nm, Hg 184.95 nm, Mn 257.6 nm, Ni 231.6 nm, Pb 220.4 nm, and Zn 206.2 nm. Prior to metal analysis, standard solutions were prepared from stock solution (ICP multi-element standard solution; Merck, Germany) and used to calibrate the ICP-OES device. The method quality was validated by using certified reference material (DORM-4; National Research Council, Canada), correlation coefficients (r^2) (> 0.99), relative standard deviation (RSD) (< 10), recovery (94.2–103.8%), limit of detection (LOD), and quantification (LOQ) (mg L^{-1} ; Cd, Fe, Hg, Mn, and Zn: 0.0003/0.001; Cr and Cu: 0.0006/0.002; Ni: 0.0008/0.003; Pb: 0.002/0.007; Al: 0.003/0.010). LOD was calculated as 3 SD/m and LOQ was calculated as 10 SD/m (SD: standard deviation, m: the slope of calibration curves). Each sample was analyzed in triplicate and the metal concentrations were expressed as mg kg^{-1} wet weight (ww).

Animal Health Risk Assessment Adaptation

The potential health risk was assessed regarding metal contamination levels according to recommended consumption rates of commercial canned and pouched (salmon, tuna, liver, and aquatic products) cat foods. The THQ method [19] was adapted to assess the health risks of heavy metal contamination in samples according to the formula given below:

$$\text{THQ} = \frac{E_F \times E_D \times F_{IR} \times C}{\text{RfD} \times W_{AB} \times T_A} \times 10^{-3}$$

where E_F is exposure frequency (365 days year⁻¹), E_D is exposure corresponding to average lifespan (14 years for adult and 1 year for kitten) [12, 21], F_{IR} is the consumption rate (recommended average consumption rate of products is 400 g day⁻¹ for adults and 300 g day⁻¹ for kittens), C is the metal concentration in feed (mg kg^{-1} ww), RfD is the oral reference doses ($\text{mg kg}^{-1}/\text{day}$) as given in Table 1 [22–24], W_{AB} is the average body weight (bw) (5.0 kg for male and 4.2 for female adults and 2.4 for male and 1.4 for female 24–26 week old kittens) [15, 25], and T_A is the average non-carcinogenic exposure duration ($E_F \times E_D$) [5].

The values less than 1 of THQ indicate that the consumption of the selected canned/pouched feeds in terms of heavy metals does not pose a risk to the specified individuals according to the consumption scenario.

Table 1 RfD values ($\text{mg kg}^{-1}/\text{day}$) for studied metals

Metal	RfD values and references		
	USEPA.gov	MDEQ.gov	USORNL.gov
Al	1.0E+0 ^a	1.0E+0	NC
Cd	1E-3	1.0E-4	1E-3
Cr ^b	1.5E+0	1.5E+0	1.0E+0
Cu	NE	1.0E-3	NC
Fe	7E-1 ^a	7.0E-1	NL
Hg	NE	1.0E-4 ^c	3E-4
Mn	1.4E-1	4.7E-2	1.4E-1
Ni	2E-2 ^d	6.0E-3	2E-2
Pb	QD	NA	NA
Zn ^e	3E-1	3.0E-1	3.0E-01 ^f

NE not evaluated, NA not available, NC has not been calculated, NL not listed, QD qualitative discussion

^aProvisional RfD

^bChromium III

^cMercury, organic

^dNickel, soluble salts

^eZinc and compounds

^fZinc, metallic

The total THQs (TTHQs) were calculated by summing up the THQ values of each metal for each wet cat food as formulated below:

$$\text{TTHQ} = \text{THQ}_{\text{Al}} + \text{THQ}_{\text{Cd}} + \text{THQ}_{\dots} + \text{THQ}_{\text{Zn}}$$

Statistical Analysis

After normality and homogeneity tests, the one-way analysis of variance (ANOVA) with post hoc Tukey's HSD was performed to evaluate results statistically. The differences at level $p < 0.05$ were considered significant.

Results

The sampled international trademarked cat food product countries were Austria, Czech Republic, England, France, Germany, Italy, Malaysia, the USA, and Turkey. The determined mean metal concentrations of samples are shown in Table 2. The highest mean Cd and Pb concentrations were determined in canned/pouched salmon; the highest mean Cu, Fe, Hg, and Ni concentrations were determined in canned/pouched tuna; the highest mean Al, Mn, and Zn concentrations were determined in canned/pouched liver for adults; and the highest mean Cr and Hg concentrations were determined in canned/pouched aquatic products for kittens.

Table 2 Metal concentrations (mg kg⁻¹) in salmon, tuna, and liver canned/pouched cat foods

	Salmon Mean ± SE Min.–Max	Tuna Mean ± SE Min.–Max	Liver Mean ± SE Min.–Max	Aquatic products Mean ± SE Min.– Max
Al	11.65 ± 0.97 ^a (7.63–16.94)	13.50 ± 1.77 ^a (7.27–22.13)	30.95 ± 3.66 ^b (19.03–58.14)	12.83 ± 1.47 ^a (7.79–21.13)
Cd	0.78 ± 0.06 (0.47–1.18)	<LOQ	<LOQ	<LOQ
Cr	0.16 ± 0.04 (<LOQ–0.39)	0.17 ± 0.06 (<LOQ–0.74)	0.02 ± 0.004 (<LOQ–0.03)	0.20 ± 0.07 (<LOQ–0.74)
Cu	2.18 ± 0.39 (0.37–3.25)	2.95 ± 0.61 (1.18–6.36)	2.04 ± 0.53 (0.84–4.48)	2.66 ± 0.52 (0.37–6.36)
Fe	106.3 ± 24.73 (29.60–230.00)	132.4 ± 30.27 (24.29–240.10)	71.4 ± 17.03 (32.04–148.90)	121.2 ± 27.40 (29.60–240.10)
Hg	0.03 ± 0.004 (0.01–0.05)	0.04 ± 0.006 (<LOQ–0.07)	0.03 ± 0.006 (<LOQ–0.06)	0.04 ± 0.007 (<LOQ–0.07)
Mn	2.46 ± 0.20 ^a (1.31–3.06)	2.80 ± 0.39 ^{ab} (1.17–3.96)	3.64 ± 0.31 ^b (2.04–4.68)	2.64 ± 0.31 ^{ab} (1.17–3.96)
Ni	0.08 ± 0.03 (<LOQ–0.26)	0.11 ± 0.02 (0.01–0.19)	0.08 ± 0.01 (0.03–0.12)	0.10 ± 0.02 (<LOQ–0.26)
Pb	3.10 ± 1.11 (1.25–12.97)	0.82 ± 0.12 (0.39–1.31)	0.64 ± 0.09 (0.28–0.93)	2.05 ± 1.21 (0.39–12.97)
Zn	20.81 ± 1.40 (13.87–26.05)	20.25 ± 2.03 (6.80–28.29)	21.39 ± 0.73 (19.09–24.56)	21.10 ± 1.42 (13.87–28.29)

The differences between means with different letters in the same column were statistically significant ($P < 0.05$)

LOQ: limit of quantification

The differences in Al levels between liver and others, in Cd levels between salmon and others, and in Mn levels between salmon and liver were statistically significant ($p < 0.05$).

Authorities have not set a maximum limit for essential metals (Cu, Fe, Mn, Se, and Zn), but some sectoral federations recommended maximum (Cu: 28, Fe: 1420, Mn: 170, Se: 568, and Zn: 284) and minimum (for kitten, Cu: 10, Fe: 80, Mn: 10, Se: 300, Zn: 75, and for adults, Cu: 5, Fe: 80, Mn: 5, Se: 300, and Zn: 75) limits for essential metals in cat foods [26]. Authorities have set maximum limits (mg kg⁻¹) for some non-essential toxic metals such as As, Cd, Hg, and Pb. The limits in the EC were Cd: 2 for “feed materials of animal origin,” Hg: 0.5 for “feedingstuffs produced by the processing of fish or other marine animals,” 0.4 for “complete feedingstuffs for dogs and cats,” 0.2 for “complementary feedingstuffs for dogs and cats,” and Pb: 5 for “complete feedingstuffs” [27]. The US Food and Drug Administration set maximum limits as Cd: 10, Cr: 10, Hg: 0.267, Ni: 50, and Pb: 10, while the Association of American Feed Control Officials has only set a maximum limit for Se in dog and cat food [28, 29].

Although none of the contamination levels in the study exceeded the legal limits of metals and commercial

animal feed/foods labeled these metals between recommended essential and toxic levels as contents, it is not possible to reveal toxic potential without a species-specific risk assessment of a dietary toxicants intake and eating habits.

The target hazard quotient values calculated according to mean metal concentrations, shown in Table 3, were all below 1 for both male/female adult cats and kittens. The highest TTHQs for female and male adults were 2.9E-01 and 3.5E-01, respectively, by a tuna consumption scenario, and for female and male kittens were 7.2E-01 and 4.2E-01 by an aquatic products consumption scenario in producer-recommended consumption rates. The TTHQ values were ordered as female kitten > male kitten > female adults > male adults from highest to lowest. Total target hazard quotient values did not exceed 1 in recommended average consumption rates of products, which means that the studied metals do not pose a health risk for adult cats and kittens. However, consumption of canned/pouched fish over 418 g per day may pose a risk for 24–26 week old female kittens with a body weight of 1.4 kg or less.

There are critical points in the THQ calculation method for non-adult animals that may lead to incorrect results. It should be noted that the E_D , bw , and F_{IR} values can alter for kitten and in different age-staged animals.

Table 3 THQs/TTHQs estimates for individual metals caused by the consumption of canned/pouched foods for the inhabitants cats

THQs	Female					
	Male			Kitten		
	Adult Tuna	Liver	Aquatic products	Adult Tuna	Liver	Aquatic products
Al	9.3E-04	2.5E-03	1.6E-03	1.3E-03	2.9E-03	2.7E-03
Cd	6.2E-02	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
Cr	8.5E-06	1.1E-06	1.7E-05	1.1E-05	1.3E-06	2.9E-05
Cu	1.7E-01	1.6E-01	3.3E-01	2.8E-01	1.9E-01	5.7E-01
Fe	1.2E-02	8.2E-03	2.2E-02	1.8E-02	9.7E-03	3.7E-02
Hg	2.4E-02	2.4E-02	5.0E-02	3.8E-02	2.9E-02	8.6E-02
Mn	1.4E-03	2.1E-03	2.4E-03	1.9E-03	2.5E-03	4.0E-03
Ni	3.2E-04	3.2E-04	6.3E-04	5.2E-04	3.8E-04	1.1E-03
Pb	NC	NC	NC	NC	NC	NC
Zn	5.5E-03	5.7E-03	8.8E-03	6.4E-03	6.8E-03	1.5E-02
TTHQ	2.8E-01	2.1E-01	4.2E-01	3.3E-01	2.5E-01	7.2E-01

NC has not been calculated, THQ target hazard quotient, TTHQ total target hazard quotient

Discussion

Although potential health risk assessment of contaminants in foods through consumption rates is frequently conducted for human, they have yet to be adapted to animal health. It is also important that these foods do not pose a health risk for animals, especially for those feeds in the same food group used daily and for long periods, as with cats [30]. Contaminants accumulate in fish tissues based on the trophic level, life span, and feeding habits as well as the chemical characteristics of the contaminant. This accumulation biomagnifies up through the food chain and reflects a risk for the consumer's (both human and animal) health [2, 5, 31]. Similar to human health risk assessments, in the study, the scenario of if the wet foods (randomly purchased and feed with the recommended consumption amounts in their labels) pose a health risk to adult and kitten cats, in terms of food safety-related toxic heavy metals was created. The reason for the selection of foods with fish and liver is that they are the most preferred ones by cats. Metals are potentially toxic above tolerable limits whether they are essential (Cu, Fe, Mn, Zn, etc.) or not (Cd, Hg, Pb, etc.) for the biological structure and functions of organisms [5, 32–35]. Although the determination of metal levels in biological samples (tissue, blood, urine, feces, and hair) of animal species and feed or foods can play a key role in diagnosis, there are no reference values (for biological samples) or maximum tolerable limits (MTL) for cats. For example, it has been reported that the concentrations of > 6 mg kg⁻¹ in blood and 4 mg kg⁻¹ in the feed are points to the diagnosis of Hg poisoning in most animal species [34].

Comparison of the selected metal concentrations with other study results analyzed by ICP-OES and set or recommended limits by international authorities or federation is shown in Table 4 [26, 27, 29, 44–54]. The contamination levels of the metals included in the study were well below the legal or recommended limits. Among these metals, the closest level to the limit was determined as Pb. There is no legal limit or recommended maximum level for Al. Average Al levels were in the ranges determined by other studies on seafood (Table 4).

Al is neurotoxic and leads to behavioral changes such as memory loss, anemia, bone abnormalities, osteoarthritis, and myocardial infarction [3]. The average Al concentration of tuna measured in the study is higher than measured by Ababneh and Al-Momani [51]. The average Al concentrations of all aquatic products in the study are lower than measured in fishes by Ahmad Al-Subeihi [44], and within the ranges measured in fishes by Sunjog et al. and Subotić et al. [48, 50]. There is no legal or recommended maximum contamination level for Al in the feed or food of animals. Although no MTL value has been specified for cats, it has

Table 4 Fish, aquatic products, and liver metal concentrations (ppm) in other studies and setted or recommended limits

Metal	Level	Product	Legal/recommended limits	Authority/federation	Reference	
Al	11.65	Salmon			Present study	
	13.50	Tuna				
	12.83	Aquatic products				
	30.95	Liver	-	-		
	86.30	Fish				[44]
	0.08–146.76	Fish				[48]
	3.86–31.66	Fish				[50]
	0.26	Tuna				[51]
Cd	0.78	Salmon	2	EC	Present study	
	<LOQ	Tuna	10	[27]		
	<LOQ	Aquatic products		FDA		
	<LOQ	Liver		[29]		
	0.01–0.81	Fish				[45]
	0.15–0.60	Aquatic products				
	0.02–0.06	Fish				[46]
	ND-0.91	Fish				[47]
	<LOQ	Fish				[49]
	0.005–0.28	Fish				[50]
	0.06	Tuna				[51]
	ND-3.3	Fish				[52]
	0.10	Fish				[53]
	0.02	Liver				[54]
Cr	0.16	Salmon	10	FDA	Present study	
	0.17	Tuna		[29]		
	0.20	Aquatic products				
	0.02	Liver				
	0.01–0.06	Fish				[46]
	0.21–2.12	Fish				[47]
	ND-0.23	Fish				[48]
	0.005–0.11	Fish				[49]
Cu	0.71	Liver			[54]	
	2.18	Salmon	28	FEDIAF [26]	Present study	
	2.95	Tuna				
	2.66	Aquatic products				
	2.04	Liver				
	0.75	Fish				[44]
	1.16–45.51	Fish				[47]
	0.75–31.17	Fish				[48]
	0.275	Fish				[49]
	0.75–33.49	Fish				[50]
2.7–7.7	Fish			[52]		
3.30	Liver			[54]		

been reported as 200–1000 mg kg⁻¹ for other species [35]. In this respect, according to the THQ result of the study, Al contamination does not pose a risk in any product.

Cd is nephrotoxic, mutagen, carcinogen, teratogen, endocrine disruptor and regulators have set legal maximum contamination limits for pet foods [3, 4, 27, 29, 37]. The average Cd concentration of Salmon measured in the study

is higher than measured in fishes by Nikolić et al., Subotić et al., Ababneh and Al-Momani, and Sobhanardakani [46, 50, 51, 53], and measured in the liver by Naseri et al. [54]. The average Cd concentrations of all aquatic products measured in the study are within the ranges measured in fishes by Milenkovic et al., Rašković et al., and Ural et al. [45, 47, 52]. The average Cd concentrations of all aquatic

Table 4 (continued)

Metal	Level	Product	Legal/recommended limits	Authority/federation	Reference	
Fe	106.3	Salmon	1420	FEDIAF [26]	Present study	
	132.4	Tuna				
	121.2	Aquatic products				
	71.4	Liver				
	41.50–1356.79	Fish				[47]
	7.06–495.39	Fish				[48]
	1.051	Fish				[51]
	13.64–241.07	Fish				[50]
	30.3–197.0	Fish				[52]
Hg	0.03	Salmon	0.2–0.5 0.267	EC [27] FDA [29]	Present study	
	0.04	Tuna				
	0.04	Aquatic products				
	0.03	Liver				
	0.07	Fish				[44]
	0.01–1.47	Aquatic products				[45]
	0.17–0.32	Fish				[46]
	ND-1.95	Fish				[48]
	0.014	Fish				[49]
	1.12–1.90	Fish				[50]
	0.21	Tuna				[51]
	ND	Fish				[52]
	0.13	Fish				[53]
Mn	2.46	Salmon	170	FEDIAF [26]	Present study	
	2.80	Tuna				
	2.64	Aquatic products				
	3.64	Liver				
	1.12–29.82	Fish				[47]
	0.3–40	Fish				[48]
	0.44–16.27	Fish				[50]
	4.1–23.4	Fish				[52]
Ni	0.08	Salmon	50	FDA [29]	Present study	
	0.11	Tuna				
	0.10	Aquatic products				
	0.08	Liver				
	0.03–1.16	Fish				[47]
	0.14	Fish				[49]
	0.51	Tuna				[51]
	1.9–3.7	Fish				[52]
	0.10	Liver				[54]

products measured in the study are lower than both the legal (2.5-fold lower) and recommended (12-fold lower) maximum level. Although no MTL value has been specified for cats, it has been reported as 10 mg kg⁻¹ for other species [35]. Also according to the THQ result of the study, Cd contamination does not pose a risk in any product.

Hypoactivity, mydriasis, lacrimation, and diarrhea are the signs of acute Cr toxicosis and regulators have set a legal maximum contamination limit for pet foods [29, 35]. The average Cr concentrations of all aquatic products measured in the study are higher than measured in fishes by Nikolić et al. and Heshmati et al. [46, 49], lower than measured in

Table 4 (continued)

Metal	Level	Product	Legal/recommended limits	Authority/federation	Reference
Pb	3.10	Salmon	5 10	EC [27]	Present study
	0.82	Tuna			
	2.05	Aquatic products		FDA [29]	
	0.64	Liver			
	0.10–6.56	Fish			[45]
	0.01–0.29	Fish			[46]
	ND-2.70	Fish			[47]
	0.056	Fish			[48]
	0.09	Tuna			[51]
	0.75	Fish			[53]
Zn	0.04	Liver			[54]
	20.81	Salmon	284	FEDIAF [26]	Present study
	20.25	Tuna			
	21.10	Aquatic products			
	21.39	Liver			
	16.01	Fish			[44]
	4.25–7.49	Fish			[46]
	43.66–387.77	Fish			[47]
	16.62–365.58	Fish			[48]
	0.635	Fish			[49]
	15.14–1186.37	Fish			[50]
	26.4–190.0	Fish			[52]
	12.61	Fish			[53]

ND not detected, FEDIAF European Pet Food Industry, EC European Commission; FDA The US Food and Drug Administration

fishes by Rašković et al. [47], and within the ranges measured in fishes by Sunjog et al. [48]. The average Cr concentration of liver measured in the study is lower than measured by Naseri et al. [54]. The average Cr concentrations of all products measured in the study are 50-fold lower than the legal maximum level. Although no MTL value has been specified for cats, it has been reported as 100–500 mg kg⁻¹ for other species [35]. Also according to the THQ result of the study, Cr contamination does not pose a risk in any product.

The excessive dietary intake of Cu can cause liver damage primarily in animals and a maximum contamination limit has been recommended for pet foods [26, 35, 36]. The average Cu concentrations of all aquatic products measured in the study are higher than measured in fishes by Ahmad Al-Subeihi and Heshmati et al. [44, 49], and within the ranges measured in fishes by Rašković et al., Sunjog et al., and Subotić et al. [47, 48, 50]. The average Cu concentration of liver measured in the study is lower than measured by Naseri et al. [54]. The average Cu concentrations of all

products measured in the study are more than tenfold lower than the recommended maximum level. Although no MTL value has been specified for cats, it has been reported as 15–500 mg kg⁻¹ for other species [35]. Also according to the THQ result of the study, Cu contamination does not pose a risk in any product.

Fe damage the cardiovascular and digestive systems, liver, and brain, and a maximum contamination limit has been recommended for pet foods [26, 35, 36]. The average Fe concentrations of all aquatic products measured in the study are lower than measured in fishes by Ababneh and Al-Momani [51], and within the ranges measured in fishes by Rašković et al., Sunjog et al., Subotić et al., and Ural et al. [47, 48, 50, 52]. The average Fe concentrations of all aquatic products measured in the study are more than tenfold lower than the recommended maximum level. Although no MTL value has been specified for cats, it has been reported as 200–1000 mg kg⁻¹ for other species [35]. Also according to the THQ result of the study, Fe contamination does not pose a risk in any product.

Mercury causes neurotoxic effects like ataxia, imbalance, and seizures and can lead to death, and regulators have set legal maximum contamination limits for pet foods [3, 27, 29, 30, 38]. The average Hg concentrations of tuna and other aquatic products measured in the study are higher than measured in fishes by Nikolić et al., Heshmati et al., Ababneh and Al-Momani, and Ural et al. [46, 49, 51, 52], lower than measured in fishes by Ahmad Al-Subeihi and Subotić et al. [44, 50], and within the ranges measured in fishes by Milenkovic et al. and Sunjog et al. [45, 48]. The average Hg concentrations of all aquatic products measured in the study are fivefold lower than legal maximum levels for animal food and feeds. Although no MTL value has been specified for cats, it has been reported as 500–3000 mg kg⁻¹ for other species [35]. Also according to the THQ result of the study, Hg contamination does not pose a risk in any product.

Decrease in Fe level and hematological changes are the signs of chronic Mn toxicosis and a maximum contamination limit has been recommended for pet foods [26, 35]. The average Mn concentrations of fishes measured in the study are lower than measured in fishes by Ababneh and Al-Momani and Ural et al. [51, 52], and within the ranges measured in fishes by Rašković et al., Sunjog et al., and Subotić et al. [47, 48, 50]. The average Mn concentrations of all aquatic products measured in the study are 56-fold lower than recommended maximum levels for animal food and feeds. Although no MTL value has been specified for cats, it has been reported as 200–1000 mg kg⁻¹ for other species [35]. Also according to the THQ result of the study, Mn contamination does not pose a risk in any product.

Decrease in feed intake, growth, and reproductive performance, kidney damage, and hematological changes are the common signs of chronic Mn toxicosis [35]. Regulators have set a legal maximum contamination limit for pet foods [29]. The average Ni concentrations of fishes measured in the study are lower than measured in fishes by Heshmati et al. and Ababneh and Al-Momani [49, 51], and within the ranges measured in all products by Rašković et al. and Naseri et al. [47, 54]. The average Ni concentrations of all products measured in the study are 500-fold lower than legal maximum levels for animal food and feeds. Although no MTL value has been specified for cats, it has been reported as 400–2000 mg kg⁻¹ for other species [35]. Also according to the THQ result of the study, Ni contamination does not pose a risk in any product.

Clinical symptoms of Pb toxicosis in cats include neurologic (behavior changes and intermittent seizures) and gastrointestinal (abdominal discomfort, anorexia, vomiting, diarrhea, lethargy, and weight loss) signs [36]. Regulators have set legal maximum contamination limits for pet foods [27, 29]. The average Pb concentration of fishes and liver measured in the study is higher than measured in fishes by Nikolić et al., Sunjog et al., Ababneh and Al-Momani, and Sobhanardakani [46, 48, 51, 53], and within the ranges

measured in aquatic products by Rašković et al. and Milenkovic et al. [45, 47]. The average Pb concentrations of all products measured in the study are 1.6–eightfold lower than legal maximum levels for animal food and feeds. Although no MTL value has been specified for cats, it has been reported as 10–100 mg kg⁻¹ for other species [35]. Also according to the THQ result of the study, Pb contamination does not pose a risk in any product.

Zn toxicity limits the absorption of Fe, Cu, and calcium and causes clinical symptoms in cats and a maximum contamination limit has been recommended for pet foods [26, 35, 36]. The average Zn concentrations of all aquatic products measured in the study are higher than measured by Ahmad Al-Subeihi, Nikolić et al., Sunjog et al., Heshmati et al., and Sobhanardakani [44, 46, 48, 49, 53], lower than measured by Ural et al. [52], and within the ranges measured in all products by Rašković et al., Sunjog et al., and Subotić et al. [47, 48, 50]. The average Zn concentrations of all products measured in the study are 13.5-fold lower than recommended maximum levels for animal food and feeds. Although no MTL value has been specified for cats, it has been reported as 250–1000 mg kg⁻¹ for other species [35]. Also according to the THQ result of the study, Zn contamination does not pose a risk in any product.

Compared to the mean metal concentrations (analyzed with atomic absorption spectrometer) from a study of canned fish cat foods in Turkey, Cd, Cr, Cu, Mn, and Pb in the present study were low, while the Fe and Ni means were high [39]. Since that study did not specify if the sampled food was for adult cats or kittens and since no risk assessment was evaluated, it is thought that the results suggested cat foods pose a higher health risk than that indicated in our study, especially for Cu. Compared to another study conducted in Egypt, the levels of Cd and Pb (in tuna for adults and in aquatic products for kittens and analyzed with atomic absorption spectrometer) were high, while Cr and Pb levels (in salmon) were low in the present study [40]. In a study conducted in Brazil (analyzed with ICP-MS), estimated daily intakes (EDI) of cats were calculated over the metal levels of six canned adult cat foods which contained 83.5% fish and 16.5% beef and/or liver [41]. According to the results, the studied metal levels (Cr, Cu, Fe, Mn, and Zn) were 7–30 times higher than those determined in the present study. The concern about the presence of Hg and Cd, which exceeded the MTLs in canned foods, was especially emphasized in the study [41]. In a study conducted in chicken liver, while Cd, Cu, and Pb levels were higher than our results, the Zn level was found to be lower than ours [42]. Compared to a study conducted in Pakistan, in chicken, lamb, and beef livers, Cd was not detected as in our study, Ni level was higher while Cu, Fe, Pb, and Zn levels were low than ours [43]. According to the THQ and TTHQ results of the study,

contamination of each metal does not pose a risk in any product alone or together.

Conclusions

This study illuminated that THQ and TTHQ values of selected metals did not exceed 1 and may mean that consumption of canned or pouched food at the recommended rate does not pose a health risk for adult cats and kittens. Even though the levels of the selected metals are below the legal or recommended limits, in the ranges indicated in other studies, possible intake of other dietary contaminants (unevaluated metals and other pollutants) and consumption over the considered rate in the study may increase the risk. Whereas heavy metal concentration levels in human diets and health risk assessment are a popular and important field of study, it is an omitted subject in animal health science. Species-specific animal risk assessment adaptation studies should be conducted based on dietary habit surveys with high participation rates and should include consideration of other contaminants. The consumption rates of canned foods, especially in breeds predisposed to neurological, liver, and kidney diseases and animals in risk groups (with chronic disease, pediatric, geriatric, etc.) must be well evaluated. Preventing metal contamination in the production stages of canned foods and keeping levels below the legal limits are important for animal food safety. However, it will be a more accurate approach to create consumption suggestions as a result of the risk assessment to be made over the consumption scenarios and potential consumer groups. Extensive studies are needed to identify potential risks and clinical impacts associated with heavy metal exposure due to the canned/pouched food consumption of animals of different breeds, life stages, health status, feeding habits, and physical and physiological conditions.

Author Contribution Not applicable.

Data Availability Not applicable.

Code Availability Not applicable.

Declarations

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Conflict of Interest The authors declare no competing interests.

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