Presence of arsenic in pet food: a real hazard?

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Summary

In this study, arsenic content in 200 cat- and dog-food samples was estimated by means of electro thermal atomic absorption (Z-ETA-AAS), after using the wet digestion method, that were imported or commercialised in Italy from 2007 to 2012. The maximum value of total arsenic (As) in the samples was 12.5 mg kg^{-1}. Some imported pet food was intercepted as a result of the Rapid Alert System for Food and Feed (RASFF) and rejected at the border or withdrawn from the Italian market, because they exceeded the maximum level of arsenic content imposed in Italy at the time of this study (2002/32/EC). All the samples with a significant arsenic level were fish-based. Recently, the 2013/1275/EC raised the maximum level of As permitted in fish-based pet food. However, the analysis of As species is required (EFSA 2014) in order to identify correctly the different contributions of dietary exposure to inorganic As and to assure pet food quality.

Arsenico nel mangime per animali: è un reale pericolo?

Parole chiave
Arsenico,
Alimenti per cani e gatti,
Spettroscopia in assorbimento atomico.

Riassunto

In questo studio, il contenuto di arsenico è stato determinato mediante la spettroscopia di assorbimento atomico con fornetto di grafite (Z-ETA-AA) dopo digestione umida; il metodo è stato applicato per l'analisi di 200 campioni di cibo per cani e per gatti importato o commercializzato in Italia dal 2007 al 2012. La massima concentrazione di arsenico totale è risultata essere pari a 12.5 mg kg^{-1}. Alcuni dei campioni analizzati hanno dato origine a delle allerte - *Rapid Alert System for Food and Feed* (RASFF), e sono stati respinti alle dogane o ritirati dal mercato italiano, poiché i tenori di arsenico totale eccedevano i limiti massimi al tempo in vigore (2002/32/EC). Verificando i componenti elencati nell'etichetta dei prodotti contenenti le maggiori concentrazioni di arsenico, è emerso che essi erano prevalentemente costituiti da pesci o loro derivati. Il Regolamento 2013/1275/EC nel 2013 ha aumentato il livello massimo di arsenico nei mangimi a base di pesce. Tuttavia, come ha recentemente sottolineato un *Opinion EFSA*, sarà necessario applicare in futuro metodi che consentano di quantificare la percentuale di arsenico inorganico sul totale nei vari costituenti dei mangimi.

Introduction

Metals and other elements enter food from a wide range of environmental sources. Arsenic (As) is a ubiquitous element in the environment. Due to its prevalence in nature and its well-known toxicity, the potential for arsenic contamination of water, air, and soil – from both geological and anthropogenic sources – is a significant environmental health concern. Contamination of aquatic ecosystems (e.g. lakes, rivers, and underground water) with As has received worldwide attention (Bhattacharya *et al.* 2002). Arsenic is present in the environment from natural sources, such as rocks and sediments, and as a result of human activities such as coal burning, copper smelting, and the processing of mineral ores. Arsenic levels are higher in aquatic environments than in soil/land environments, as it is fairly water-soluble and can therefore be washed out of arsenic-bearing rocks.

Arsenic has a considerable tendency to accumulate in bottom sediments (Smedley and Kinniburgh 2002) and has a very complex chemistry in the marine environment. More than 20 chemical forms of arsenic have been identified and characterized, including inorganic forms such as trivalent arsenite [$As(OH)_3$] and pentavalent arsenate [$AsO(OH)_3$], and organic forms: such as monomethylarsonic acid [$CH_3AsO(OH)_2$, MMAV], monomethylarsonous acid [$CH_3As(OH)_2$, MMAIII], dimethylarsinic acid [$(CH_3)_2AsO(OH)_2$, DMAV], dimethylarsonous acid [$(CH_3)_2As(OH)_2$, DMAIII], arsenobetaine [$(CH_3)_3As^+CH_2COO^-$], and arsenocholine [$(CH_3)_3As^+CH_2CH_2OH$]. Arsenate is generally the most abundant form of arsenic in oxygenated and biologically-productive marine waters, while arsenite is the most abundant form at low salinity.

As outlined above, there are many different types of As. It has been shown that arsenic toxicity is dependent on arsenic species, and that inorganic arsenic species are more toxic than organic species to living organisms, including humans (Goessler and Kuehnett 2002, Meharg and Hartley-Whitaker 2002). Essentially, fish assimilate metals (including As) by ingestion of particulate material suspended in water, ingestion of food, ion-exchange of dissolved metals across lipophilic membranes (e.g., the gills), and adsorption on tissue and membrane surfaces. Metal distribution between the different tissues depends on the mode of exposure, i.e. dietary and/or aqueous exposure, and can serve as an indicator of pollution (Alam *et al.* 2002). Arsenic is transferred to the marine food chain, especially to marine fish, and the inorganic As accumulated by the marine fish is mainly transformed into the non-toxic organic arsenic arsenobetaine (AsB) (Zhang *et al.* 2011).

Fish tends to be the main contributor of arsenic in the diet of humans and pets. Contaminants in fish predominantly derive from their diet, and bioaccumulation levels of contaminants are greater in fish that occupy higher positions in the food chain. Fish can significantly contribute to the dietary exposure of humans and animals to many contaminants. In this article, we focus on heavy metal contaminants such as As. Products like fishmeal and fish oil have been identified as major sources of contamination with arsenic (EFSA 2009). Some pet food imported in Italy in 2011 and 2012 caused notifications to the Rapid Alert System for Food and Feed (RASFF) and border rejection as they exceeded the limits fixed by Directive 2002/32/EC[1] for total As. Considering the ubiquitous presence of As in marine environments and that certain compound feed materials for pet animals contain a significant proportion of fish and, as a consequence, a high level of total arsenic, the Regulation 2012/744/EC[2] set a maximum level of 10 mg kg^{-1} for As in these compound feed. In fact, the presence of arsenic in pet food is mainly in the form of organic arsenic, which is less toxic. It was therefore deemed appropriate to modify the maximum tolerable level of arsenic in complementary and complete pet food, containing fish, other aquatic animals, and products derived thereof and/or seaweed meal.

The purposes of this study was to report the monitoring of total arsenic concentrations in pet food imported and available in the Italian market during 2007-2012 and to investigate in which cases and by what percentages the sample levels did not meet the legal limits set by the Directives 2002/32/EC and the 2013/1275/EC[2], in order to understand the real risk of pet exposure to arsenic.

Materials and methods

A total of 200 samples of cat- or dog-food were analysed during 2007-2012 (Table I); these comprised 133 samples of complete pet food and 67 samples of complementary pet food.

A total of 137 pet food samples contained fish and fish derivatives as the main ingredients, with the remaining 63 being meat-formulated. Samples were homogenized with an electric mill. De-ionized water (18.2 MXcm), from an Arium611VF system (Sartorius Stedim Italy S.p.A., Antella - Bagno a Ripoli, Florence, Italy), was used to prepare all aqueous solutions. Mineral acids and oxidants (HNO_3, HF, and H_2O_2) were of the highest quality grade (Suprapure). All plastic and glassware were cleaned by soaking overnight in a 25% (v/v) nitric acid solution and then rinsed with ultra-pure water. Accuracy of analysis was examined using standard reference materials DOLT-4 (Dogfish Liver Certified Reference Material for Trace Metals). Samples (1.5-2.0 g wet weight) were mineralized with 7 ml of Suprapure nitric acid (TraceSELECT, SIGMA-ALDRICH), 0.7 ml of hydrofluoridric acid and 1.5 ml of oxygenated water in a closed Teflon PFA vessel using an Ethos 1 (Milestone SrL, Sorisole, Bologna, Itali) microwave digestion system. After cooling, solutions were transferred into 25 ml volumetric flasks and were diluted with ultra-pure water (Sartorius Stedim Italy S.p.A., Antella - Bagno a Ripoli, Florence, Italy).

Detection of As was performed using ETA-AAS (Electro Thermal Atomic Absorption), via a Perkin-Elmer AAnalyst 800 atomic absorption spectrophotometer

[1] European Union (EU) 2013. Commission Regulation (EU) of 6 December 2013 amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council, regarding maximum levels for arsenic, cadmium, lead, nitrites, volatile mustard oil and harmful botanical impurities (UE/1275/2013). *Off J*, **L 328**, 07/12/2013, 86-92.

[2] European Union (EU) 2012. Commission Regulation (EU) of 16 August 2012 amending Annexes I and II to Directive 2002/32/EC of the European Parliament and of the Council, regarding maximum levels for arsenic, fluorine, lead, mercury, endosulfan, dioxins, *Ambrosia* spp., diclazuril and lasalocid A sodium and action thresholds for dioxins (UE/744/2012). *Off J*, **L 219**, 17/08/2012, 5-12.

Table I. *Number of fish or meat-based pet food sampled from 2007 to 2012.*

Year	2007		2008		2011		2012		Total
Pet food	**Fish**	**Meat**	**Fish**	**Meat**	**Fish**	**Meat**	**Fish**	**Meat**	
Complete cat /dog food	32	17	40	24	2	1	16	1	133
Complementary cat /dog food	6	0	3	0	11	4	27	16	67
Total for year Fish or meat based	38	17	43	24	13	5	43	17	200
	55		**67**		**18**		**60**		**200**

Table II. *Arsenic detection: ET-AAS instrumental conditions.*

		Heating program: temperature °C, ramp time (s), hold time (s)
Argon flow (ml/min)	250	110 (10, 10)
Sample volume (μl)	10	130 (15, 25)
Modifier* (μl)	10	950 (30, 30)
		1200 (15, 20)
Slit (nm)	0.7	2300 (0, 5)
Wave length (nm)	193.7	2500 (1, 5)
		20 (5, 5)

*matrix modifier: 0.008 mg Ni

equipped with a transversely heated graphite atomizer (THGA) system, with Zeeman-effect for background correction and integrated auto-sampler. Instrumental conditions are shown in Table II. Graphite tubes with a pyrolytic graphite coating and platforms made of pyrolytic graphite were used throughout this study . Argon of 99.998% purity was used as an inert gas.

Calibration was carried out by adding standards to the matrix solution. The limit of quantification (LOQ) was 0.85 mg kg^{-1}. All analyses were conducted in duplicate. To check the purity of the reagents, "blanks" were analysed for each calibration run, using the same procedure.

Statistical analysis: results reported to be < LOQ (0.85 mg kg^{-1}) (left-censored data) were handled by substitution and the value was imputed as 1/2 of the limit of quantification (LOQ/2). Descriptive statistics (mean, standard deviation, range) were performed.

Table III. *Arsenic content (mg kg^{-1}) in fish-based pet food analyzed from 2007 to 2012.*

	2007	2008	2011	2012
N. samples analyzed	55	67	18	60
Mean (mg kg^{-1})	2.20	1.50	3.30	2.10
Range	1.20 - 12.50	0.88 - 5.50	1.80 - 11.0	0.86 - 6.20
Total n. samples	200			
Minimum As content	0.86			
Maximum As content	12.50			

Results

In 4 years of official controls in animal feed, we found that 47 pet food samples (24% of the analysed samples) contained total arsenic concentrations that exceeded the legal limit set by Directive 2002/32/EC (2 mg kg^{-1} in complete feed and 4 mg kg^{-1} in complementary feed). After the 1275/2013 UE was imposed, all samples were found to be compliant with the maximum limit of 10 mg kg^{-1}.

Our data showed that only pet food containing fish or fish derivatives contained a detectable level of arsenic (concentrations of total arsenic in pet food are shown in Table III). In fact, no meat-based pet food was found positive for arsenic content. The percentage of pet food containing fish that exceeded the EU limits for arsenic content ranged from 20% to 36%, when the Directive 2002/32/EC was in force. In 2011-2012 period, our laboratory carried out 7 notifications to the RASFF, signalling the presence of As in imported pet food that exceeded the EU maximum limits. Some of these products were already distributed in the Italian market and were withdrawn after notification; others were intercepted at border controls and were re-dispatched to the country of origin after border rejection (Table IV).

Discussion

Arsenic is a widely-occurring contaminant, resulting from both natural sources and human activity. Accumulation of heavy metals in fish organs varies significantly. A non-essential element, such as As, is expected to be present in a wide range of concentrations, reflecting the environmental exposure levels and the feeding behaviour. The highest content of As in fish was usually found in muscle. In a previous study, we found a maximum value of 2.04 mg kg^{-1} and a mean level of 0.06 mg kg^{-1} of As in muscle tissues of a predator fish (European catfish); while in other tissues, As levels ranged

Table IV. *Italians notifications in RASFF Portal* about As content in pet food during the years 2011 and 2012.*

	RASFF reference	Subject	Product category	Meat
Border rejection	07/03/2012	2012.AOQ	Arsenic (6.03 mg/kg - ppm) in canned cat food from Thailand	Pet food
Border rejection	05/12/2011	2011.COO	Arsenic (11 mg/kg - ppm) in pet food from Thailand	Pet food
Information for attention	27/01/2012	2012.0146	Arsenic (5.79 mg/kg - ppm) in feed for cats from Thailand	Pet food
Border rejection	05/12/2011	2011.COJ	Arsenic (6.7 mg/kg - ppm) in feed for cats from Thailand	Pet food
Border rejection	05/12/2011	2011.COK	Arsenic (8.5 mg/kg - ppm) in feed for dogs from Thailand	Pet food
Alert	13/10/2011	2011.1419	Arsenic (7.0 mg/kg - ppm) in tuna with rice feed for cats from Thailand	Pet food
Information for attention	29/11/2011	2011.1731	Arsenic (7.6 mg/kg - ppm) in rice with tuna from Thailand	Pet food

from 0.01 mg kg^{-1} to 0.08 mg kg^{-1} (Squadrone *et al.* 2012). Other authors (Delgado-Andrade *et al.* 2003) found total As in muscles ranging from 0.39 mg kg^{-1} to 12.58 mg kg^{-1}. De Rosemond and colleagues (De Rosemond *et al.* 2008) analysed 5 freshwater fish species from Back Bay near Yellowknife (Northwest Territories, Canada) and reported total arsenic levels in muscles ranging from 0.57 mg kg^{-1} to 1.15 mg kg^{-1}. Jankong and colleagues (Jankong *et al.* 2007) analysed the arsenic content of freshwater fish in Thailand, and reported high arsenic concentrations in the edible muscle tissue (0.05-0.81 mg kg^{-1}). In addition, Ruangwises and colleagues (Ruangwises *et al.* 2011) examined marine biota collected in the Gulf of Thailand and found levels of total As ranging from 0.40 mg kg^{-1} to 7.03 mg kg^{-1}. The Ministry of Public Health of Thailand set a maximum limit of 2 mg kg^{-1} of total arsenic in fish destined for consumption. If a sample exceed the limit, it must be analysed for inorganic arsenic content: the maximum inorganic arsenic limit is 2 mg kg^{-1} (Kerdthep *et al.* 2009). The maximum level of total arsenic in fish feed reported in a Turkish survey was 6 mg kg^{-1}, while the maximum level of inorganic arsenic was 2 mg kg^{-1} (Budiati 2010).

In October 2009, the EFSA CONTAM Panel adopted an opinion on arsenic in food. This opinion mainly focused on inorganic arsenic, which is the more toxic form of arsenic. The Panel stressed the need for more species data (i.e. levels of organic and inorganic arsenic in different food and feed commodities), as well as for more data on the relationship between arsenic intake levels and possible health effects. In this respect, it is worth noticing that the current EU maximum levels for arsenic in feed materials and compound feed refer to total arsenic, and do not differentiate between the different forms of arsenic.

Moreover, the EFSA (EFSA 2014) highlights that it is not straightforward trying to derive the amount of inorganic As from total As in fish and seafood. The available data in the literature show that there is not a consistent relationship between the total As content (mainly due to arsenobetaine) and the inorganic As content in these samples (Francesconi 2010).

Considering our results, the highest value found for total As (in 1 out of the 200 analysed samples of pet food) was 12.5 mg kg^{-1}. Toxicological studies concerning pet food have shown that adverse effects were associated with long-term ingestion of inorganic arsenic at doses of at least 2.4 mg kg^{-1} body weight per day (EFSA 2009). We should also take into account that the main contributors to dietary exposure to inorganic As were, besides fish, cereals, i.e. rice (EFSA 2014), which are often constituents of pet food. Therefore, as underlined by the EFSA, species analysis are required in order to identify correctly the different contributions of the dietary exposure to inorganic As, in order to assure pet food quality and to perform a correct risk assessment.

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References

Alam M.G., Allinson G., Stagnitti F., Tanaka A. & Westbrooke M. 2002. Arsenic contamination in Bangladesh groundwater: a major environmental and social disaster. *Int J Environ Health Res*, **12**, 235-253.

Bhattacharya P., Jacks G., Ahmed K.M., Routh J. & Khan A.A. 2002. Arsenic in groundwater of the Bengal delta plain aquifers in Bangladesh. *Bull Environ Contam Toxicol*, **69**, 538-545.

Budiati T. 2010. The presence of arsenic as heavy metal contaminant on salmon: a risk assessment. *Int J Basic & Appl Sci*, **10** (5), 4-7.

de Rosemond S., Xie Q. & Liber K. 2008. Arsenic concentration and speciation in five freshwater fish species from Back Bay near Yellowknife, NT, Canada. *Environ Monit Assess*, **147**, 199-210.

Delgado-Andrade C., Navarro M., Lopez H. & Lopez M.C. 2003. Determination of total arsenic levels by hydride generation atomic absorption spectrometry in foods from south-east Spain: estimation of daily dietary intake. *Food Addit Contam*, **20**, 923-932.

EFSA. 2014. Dietary exposure to inorganic arsenic in the European population. *EFSA Journal*, **12**, 3597.

EFSA. 2009. Scientific Opinion on Arsenic in Food. *EFSA Journal*, **7**, 13-51.

Francesconi K.A. 2010. Arsenic species in seafood: origin and human health implications. *Pure Appl Chem*, **82**, 373-381.

Goessler W. & Kuehnett D. 2002. Analytical methods for the determination of arsenic and arsenic compounds in the environment. *In* Environmental Chemistry of Arsenic. Marcel Dekker, New York, 27-50.

Jankong P., Visoottiviseth P. & Khokiattiwong S. 2007. Enhanced phytoremediation of arsenic contaminated land. *Chemosphere*, **68**, 1906-1912.

Kerdthep P., Tongyonk L. & Rojanapantip L. 2009. Concentrations of cadmium and arsenic in seafood from Muang district, Rayong province. *J Health Res*, **23** (4), 179-184.

Meharg A.A. & Hartley-Whitaker J. 2002. Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species. *New Phytologist*, **154**, 29-43.

Ruangwises S. & Ruangwises N. 2011. Concentrations of total and inorganic arsenic in fresh fish, mollusks, and crustaceans from the Gulf of Thailand. *J Food Prot*, **74**, 450-455.

Smedley P.L. & Kinniburgh D.G. 2002. A review of the source, behaviour and distribution of arsenic in natural waters. *Applied Geochemistry*, **17**, 517-568.

Squadrone S., Prearo M., Brizio P., Gavinelli S., Pellegrino M., Scanzio T., Guarise S., Benedetto A. & Abete M.C. 2012. Heavy metals distribution in muscle, liver, kidney and gill of European catfish (*Silurus glanis*) from Italian Rivers. *Chemosphere*, **90**, 358-365.

Zhang W., Huang L. & Wang W-X. 2011. Arsenic bioaccumulation in a marine juvenile fish Terapon jarbua. *Aquatic Toxicology*, **105**, 582-588.