Presence of arsenic in pet food: a real hazard?

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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pet food was intercepted as a result of the Rapid Alert System for Food and Feed (RASFF) and rejected at the border or withdrawn from the Italian market, because they exceeded the maximum level of arsenic content imposed in Italy at the time of this study (2002/32/EC).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.

Keywords
Arsenic, Cat and dog food, Electro thermal atomic absorption.

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pet food was intercepted as a result of the Rapid Alert System for Food and Feed (RASFF) and rejected at the border or withdrawn from the Italian market, because they exceeded the maximum level of arsenic content imposed in Italy at the time of this study (2002/32/EC).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-AAS) 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 $[\text{As(OH)}_3^-]$ and pentavalent arsenate $[\text{AsO}_4^{3-}]$, and organic forms: such as monomethylarsonic acid $[\text{CH}_3\text{As(OH)}_2^-]$ MMAV, monomethylarsonous acid $[\text{CH}_3\text{As(OH)}_3]$ MMAIII, dimethylarsonic acid $[\text{CH}_3\text{OAs(OH)}_2^-]$ DMAV, dimethylarsinous acid $[\text{CH}_3\text{As(OH)}_2]$ DMAIII], arsenobetaine $[\text{CH}_3\text{As}^+\text{CH}_2\text{COO}^-]$, and arsenocholine $[\text{CH}_3\text{As}^+\text{CH}_2\text{CH}_2\text{OH}]$. Arsenic 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 Kuehnelt 2002, Meagher 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 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 generally higher 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 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 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, 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 MΩcm), from an Arium611VF system (Sartorius Stedim Italy S.p.A., Antella - Bagno a Ripoli, Florence, Italy), was used to prepare all aqueous solutions. All mineral acids and oxidants (HNO$_3$, HF, and H$_2$O$_2$) 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 hydrofluoric acid and 1.5 ml of oxygenated water in a closed Teflon PFA vessel using an Ethos 1 (Milestone Srl, Sorisole, Bologna, Italy) 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.
Table I. Number of fish or meat-based pet food sampled from 2007 to 2012.

<table>
<thead>
<tr>
<th>Year</th>
<th>2007</th>
<th>2008</th>
<th>2011</th>
<th>2012</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pet food</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete cat /dog food</td>
<td>32</td>
<td>17</td>
<td>40</td>
<td>24</td>
<td>133</td>
</tr>
<tr>
<td>Complementary cat /dog food</td>
<td>6</td>
<td>0</td>
<td>3</td>
<td>11</td>
<td>27</td>
</tr>
<tr>
<td>Total for year Fish or meat based</td>
<td>38</td>
<td>17</td>
<td>43</td>
<td>24</td>
<td>200</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Heating program: temperature °C, ramp time (s), hold time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argon flow (ml/min)</td>
</tr>
<tr>
<td>Sample volume (μl)</td>
</tr>
<tr>
<td>Modifier* (μl)</td>
</tr>
<tr>
<td>Slit (nm)</td>
</tr>
<tr>
<td>Wave length (nm)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>2007</th>
<th>2008</th>
<th>2011</th>
<th>2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. samples analyzed</td>
<td>55</td>
<td>67</td>
<td>18</td>
<td>60</td>
</tr>
<tr>
<td>Mean (mg kg⁻¹)</td>
<td>2.20</td>
<td>1.50</td>
<td>3.30</td>
<td>2.10</td>
</tr>
<tr>
<td>Range</td>
<td>1.20 - 12.50</td>
<td>0.88 - 5.50</td>
<td>1.80 - 11.0</td>
<td>0.86 - 6.20</td>
</tr>
<tr>
<td>Total n. samples</td>
<td>200</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimum As content</td>
<td>0.86</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum As content</td>
<td>12.50</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

Substitution and the value was imputed as 1/2 of the limit of quantification (LOQ/2). Descriptive statistics (mean, standard deviation, range) were performed.
from 0.01 mg kg\(^{-1}\) to 0.08 mg kg\(^{-1}\) (Squadrone \textit{et al.} 2012). Other authors (Delgado-Andrade \textit{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 \textit{et al.} 2008) analysed 5 freshwater fish species from Back Bay near Yellowknife (Northwest Territories, Canada) and reported high arsenic concentrations in the edible muscle tissue (0.05-0.81 mg kg\(^{-1}\)). In addition, Ruangwises and colleagues (Ruangwises \textit{et al.} 2007) examined marine biota collected in the Gulf of Thailand and found levels of total As ranging from 0.57 mg kg\(^{-1}\) to 1.15 mg kg\(^{-1}\). Jankong and colleagues (Jankong \textit{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 \textit{et al.} 2011) examined marine biota collected in the Gulf of Thailand and found levels of total As ranging from 0.57 mg kg\(^{-1}\) to 1.15 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 \textit{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.
References


