

**OPINION OF THE SCIENTIFIC PANEL ON CONTAMINANTS IN THE FOOD CHAIN  
ON A REQUEST FROM THE COMMISSION RELATED TO ARSENIC AS UNDESIRABLE  
SUBSTANCE IN ANIMAL FEED**

**Question N° EFSA-Q-2003-031**

**Adopted on 31 January 2005**

**SUMMARY**

Arsenic is a naturally occurring element, present in soil, ground water and plants. Regions with high geological occurrence of inorganic arsenic have been identified in particular in Asia and other non-European countries. In Europe, environmental arsenic levels are rather low, with the exception of distinct geological or industrial areas. Arsenic is a metalloid, displaying different valences (-3, 0, +3, +5) resulting in a broad variety of arsenic compounds with diverse chemical characteristics. Inorganic and organic forms of arsenic also differ significantly in their toxicity, the organic arsenic compounds exhibiting a very low toxic potential. Consequently, the potential adverse effects of arsenic to animal (and human) health are determined by the inorganic fraction in a given feed (or food) product, and data reporting only total arsenic in food materials are difficult to interpret in terms of the ability to induce adverse effects. Drinking water many contain significant amounts of inorganic arsenic and upper limits have been set in most countries. Seafood and fish have been identified as major source of arsenic in the human diet, and in animal feed materials that contain products derived from fish or other marine organisms. In seafood and fish, arsenic is present predominantly in the organic forms of arsenobetaine and arsenocholine, which are virtually non-toxic. Analytical data from the Member States on total arsenic in feed materials do not indicate arsenic levels of concern in materials others than fish-derived products, for which further data on chemical speciation are needed, to identify the actual levels of inorganic arsenic. As the carry-over of arsenic in its inorganic form into edible tissue of mammals and poultry is low, food derived from terrestrial animals contributes only insignificantly to human exposure.

**KEY WORDS:** Arsenic, speciation analysis, feed materials, toxicity, fish and seafood products

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## BACKGROUND

### 1. General Background

Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed<sup>1</sup> replaces since 1 August 2003 Council Directive 1999/29/EC of 22 April 1999 on the undesirable substances and products in animal nutrition<sup>2</sup>.

The main modifications can be summarised as follows

- extension of the scope of the Directive to include the possibility of establishing maximum limits for undesirable substances in feed additives.
- deletion of the existing possibility to dilute contaminated feed materials instead of decontamination or destruction (introduction of the principle of non-dilution).
- deletion of the possibility for derogation of the maximum limits for particular local reasons.
- introduction the possibility of the establishment of an action threshold triggering an investigation to identify the source of contamination (“early warning system”) and to take measures to reduce or eliminate the contamination (“pro-active approach”).

In particular the introduction of the principle of non-dilution is an important and far-reaching measure. In order to protect public and animal health, it is important that the overall contamination of the food and feed chain is reduced to a level as low as reasonably achievable providing a high level of public health and animal health protection. The deletion of the possibility of dilution is a powerful mean to stimulate all operators throughout the chain to apply the necessary prevention measures to avoid contamination as much as possible. The prohibition of dilution accompanied with the necessary control measures will effectively contribute to safer feed.

During the discussions in view of the adoption of Directive 2002/32/EC the Commission made the commitment to review the provisions laid down in Annex I on the basis of updated scientific risk assessments and taking into account the prohibition of any dilution of contaminated non-complying products intended for animal feed. The Commission has therefore requested the Scientific Committee on Animal Nutrition (SCAN) in March 2001 to provide these updated scientific risk assessments in order to enable the Commission to finalise this review as soon as possible (Question 121 on undesirable substances in feed)<sup>3</sup>.

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<sup>1</sup> OJ L140, 30.5.2002, p. 10

<sup>2</sup> OJ L 115, 4.5.1999, p. 32

<sup>3</sup> Summary record of the 135<sup>th</sup> SCAN Plenary meeting, Brussels, 21-22 March 2001, point 8 –New questions ([http://europa.eu.int/comm/food/fs/sc/scan/out61\\_en.pdf](http://europa.eu.int/comm/food/fs/sc/scan/out61_en.pdf))

It should be noted that Council Directive 1999/29/EC is a legal consolidation of Council Directive 74/63/EEC of 17 December 1973 on the undesirable substances in animal nutrition<sup>4</sup>, which has been frequently and substantially amended. Consequently, several of the provisions of the Annex to Directive 2002/32/EC date back from 1973.

The opinion on undesirable substances in feed, adopted by SCAN on 20 February 2003 and updated on 25 April 2003<sup>5</sup> provides a comprehensive overview on the possible risks for animal and public health as the consequence of the presence of undesirable substances in animal feed.

On the basis of this opinion, some provisional amendments are proposed to the Annex of Directive 2002/32/EC in order to guarantee the supply of some essential, valuable feed materials as the level of an undesirable substance in some feed materials, due to normal background contamination, is in the range of or exceeds the maximum level laid down in the Annex I of Directive 2002/32/EC. Also some inconsistencies in the provisions of the Annex have been observed.

It was nevertheless acknowledged by SCAN itself for several undesirable substances and by the Standing Committee on the Food Chain and Animal Health that additional detailed risks assessments are necessary to enable a complete review of the provisions in the Annex.

## **2. Specific Background**

SCAN concluded<sup>6</sup> that the ions and elements, including arsenic, listed in Council Directive 1999/29/EC are commonly encountered substances with known toxicity. In each case, the contribution of food products of animal origin to the human exposure is limited and the listing of these elements as undesirable substance in feed, although concomitantly contributing to an overall reduction of human exposure to toxic forms, is mainly justified by reasons of animal health.

A detailed risk assessment of the presence of arsenic in animal feed and the possible effects for animal health and public health is urgently needed as it appears that arsenic in its organic forms has a limited toxicity, therefore the determination of total arsenic may not accurately reflect the risk posed by the inorganic forms.

However, the distinction between the organic and inorganic forms of arsenic can only be performed by a complex analysis, not readily applicable for analysis in the framework of official control. Therefore the maximum levels in legislation refer to total arsenic.

More than 95 % of the arsenic present in feed materials of marine origin is in the less toxic organic forms. Recent developments in formulating fish feed incorporating higher ratios of

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<sup>4</sup> OJ L 38, 11.2.1974, p. 31

<sup>5</sup> Opinion of the Scientific Committee on Animal Nutrition on Undesirable Substances in Feed, adopted on 20 February 2003, updated on 25 April 2003 ([http://europa.eu.int/comm/food/fs/sc/scan/out126\\_bis\\_en.pdf](http://europa.eu.int/comm/food/fs/sc/scan/out126_bis_en.pdf))

<sup>6</sup> Opinion of the Scientific Committee on Animal Nutrition on Undesirable Substances in Feed, point 6.11. Conclusions and recommendations.

fish oil and fishmeal have to be taken into account. Occurrence data indicate that current maximum levels in legislation do not reflect the normal background contamination of some feed materials, mainly of marine origin. Therefore legislation needs urgently to be amended. SCAN indicated that a detailed risk assessment should address the risks related to the inorganic forms of arsenic. In view of the uncertainty of the availability of a method of analysis for the determination of inorganic arsenic separately and which is readily applicable in the framework of official control, an assessment of the typical ratio between arsenic in organic form and arsenic in inorganic form should be provided for the different (groups of) feed materials.

## **TERMS OF REFERENCE**

The European Commission requests the EFSA to provide a detailed scientific opinion on the presence of arsenic in animal feed.

This detailed scientific opinion should comprise the

- determination of the toxic exposure levels (daily exposure) of inorganic arsenic and, if relevant, of organic arsenic for the different animal species (difference in sensitivity between animal species) above which
  - signs of toxicity can be observed (animal health / impact on animal health) or
  - the level of transfer/carry over of inorganic arsenic and organic arsenic from the feed to the products of animal origin results in unacceptable levels of inorganic arsenic and, if relevant, of organic arsenic in the products of animal origin in view of providing a high level of public health protection.
- identification of feed materials which could be considered as sources of contamination by arsenic and the characterisation, insofar as possible, of the distribution of levels of contamination, in particular the typical ratio between arsenic in inorganic forms and arsenic in organic forms for the different (groups of) feed materials.
- assessment of the contribution of the different identified feed materials as sources of contamination by inorganic arsenic and if relevant of organic arsenic
  - to the overall exposure of the different relevant animal species to inorganic arsenic and organic arsenic,
  - to the impact on animal health,
  - to the contamination of food of animal origin (the impact on public health), taking into account the ration between arsenic in inorganic forms and arsenic in organic forms, the dietary variations and variable carry over rates (bio-availability)

depending on the nature of the different feed materials and the form in which arsenic is present<sup>7</sup>.

- identification of eventual gaps in the available data which need to be filled in order to complete the evaluation.

## ASSESSMENT

### 1. Introduction

Arsenic is a metalloid found in water, soil and air from natural as well as anthropogenic sources. Arsenic occurs in inorganic and numerous organic forms that differ not only in their physical and chemical properties but also in their occurrence and toxicity.

Organic arsenic compounds have been used as feed additives for disease control and improvement of weight gain in swine and poultry since the mid 1940s and are still used in various countries. These compounds are phenylarsonic acid, arsanilic acid, 3-nitro-4-hydroxyphenylarsonic acid, 4-nitrophenylarsonic acid and 4-ureidophenylarsonic acid and their salts.

#### 1.1. Physico-chemical properties

Arsenic (As) [CAS nr. 7440-38-2] is allocated to group 15 (formerly named subgroup VA) of the Periodic Table of Elements, according to the IUPAC classification. Arsenic has the atomic number 33, and a mass of 74.92160, whereas the mass of its isotopes ranges from 68 to 80, but <sup>75</sup>As is the only stable isotope. As a metalloid, arsenic has both, metallic and non-metallic properties and resembles many characteristics of phosphorus, which explains also a number of its toxic properties. Arsenic displays different valences (-3, 0, +3, +5) and occurs in cationic and anionic forms. At present, more than 25 different arsenic compounds have been identified in the environment and in biota (see Table 1 for examples). As a result, the chemistry, biology and toxicology of this element are complex. Arsenic trihydride (arsine AsH<sub>3</sub>) is gaseous and a strong reducing agent and used in the production of various arsenides, but does not occur in feed materials and will thus be neglected in the further discussions. Arsenic trioxide (As<sub>2</sub>O<sub>3</sub> – white arsenic) is a tasteless and odourless compound and represents the common commercial form of the element that serves as basic material for various synthetic products. Arsenic pentoxide (As<sub>2</sub>O<sub>5</sub>) represents the +5 oxidation state of the element.

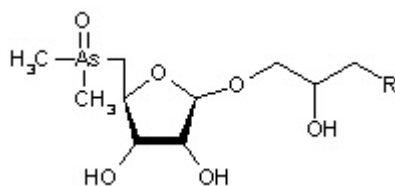
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<sup>7</sup> Importance of the human exposure to total arsenic from foods of animal origin compared to overall human dietary total arsenic exposure can be assessed making use of the information contained in the report on a task on human exposure assessment to total arsenic which has been recently performed at EU level within the framework of co-operation by Member States in the scientific examination of questions related to food (SCOOP – Task 3.2.11)(EC, 2004).

Table 1. Examples of common naturally occurring arsenic compounds (WHO, 2001, Francesconi and Kuehnelt, 2002)

	Arsenic species (IUPAC)	Formula
As (III)	As trioxide, arsenous oxide	As <sub>2</sub> O <sub>3</sub>
As (III)	Arsenous acid (arsenites)	OH-As(OH) <sub>2</sub>
As (V)	As pentoxide	As <sub>2</sub> O <sub>5</sub>
As(V)	Arsenic acid (arsenate)	O=As-(OH) <sub>3</sub>
MMA (V)	Monomethylarsonic acid	CH <sub>3</sub> AsO(OH) <sub>2</sub>
DMA (V)	Dimethylarsinic acid (Cadodylic acid)	(CH <sub>3</sub> ) <sub>2</sub> AsO(OH)
AsB(III)	Arsenobetaine	(CH <sub>3</sub> ) <sub>3</sub> As <sup>+</sup> CH <sub>2</sub> -CH <sub>2</sub> -COO <sup>-</sup>
TMAO(V)	Trimethylarsine oxide	(CH <sub>3</sub> ) <sub>3</sub> AsO
AsC(III)	Arsenocholine (ion)	(CH <sub>3</sub> ) <sub>3</sub> As <sup>+</sup> CH <sub>2</sub> -CH <sub>2</sub> -OH
TMA (III)	Trimethylarsine	(CH <sub>3</sub> ) <sub>3</sub> As
MeAs <sup>+</sup> (III)	Tetramethylarsonium ion	(CH <sub>3</sub> ) <sub>4</sub> As <sup>+</sup>
TMAP(III)	Trimethylarsoniumpropionate	(CH <sub>3</sub> ) <sub>3</sub> As <sup>+</sup> CH <sub>2</sub> -CH <sub>2</sub> -COOH
PAA(V)	Phenylarsonic acid	C <sub>6</sub> H <sub>5</sub> AsO(OH) <sub>2</sub>

Arsenosugar(V)s



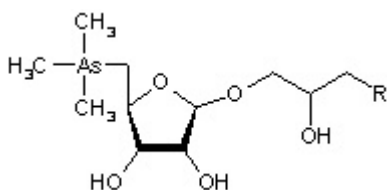
Dimethylarsinoylribosides

R = -OH or

R = - OP(O)(OH)OCH<sub>2</sub>CH<sub>2</sub>CH(OH)CH<sub>2</sub>OH  
or

R = -SO<sub>3</sub>H or

R = -OSO<sub>3</sub>H



Trimethylarsonioribosides

R = OH or

R = -OSO<sub>3</sub>H

## 1.2. Occurrence of inorganic arsenic compounds

Arsenic is present in igneous and sedimentary rocks and ores. Weathering, volcanism and dissolution in water lead to the distribution of arsenic in the environment. The upper Earth's crust contains 1.5 – 2 mg/kg arsenic, coal between 0.5 and 93 mg/kg and brown coal up to 15,000 mg/kg. Geological arsenic concentrations in ground water used as drinking water are of public health concern in various countries, particularly in Bangladesh. Arsenic oxide is also a common by-product of copper, lead and nickel smelting considered as the major source of airborne anthropogenic arsenic together with burning of fossil fuels as well as steel production and non-ferrous alloying (Matschullat, 2000). Other sources of volatile arsenics are waste deposits where these compounds can be formed by anaerobic bacterial activity (Wickenheiser *et al.*, 1998). Terrestrial plants may accumulate arsenic following uptake from the soil and groundwater via the roots and by absorption of airborne arsenic deposited on the leaves. In the past, arsenic insecticides, pesticides, fungicides and rodenticides (mainly as lead arsenate) have been used worldwide in agricultural production and wood preservation. The annual production of arsenic trioxide has, however, undergone major changes since the end of the 1980s. A peak production was recorded in the mid 1980s with a production of about 62,000 metric tons that has declined to approximately 35,000 tons in 2002, the main producers remaining China, Chile, Mexico and Peru (Reese, 2003). The most prominent application of arsenic trioxide remains its use in wood preservation with chromated copper arsenate (CCA) as most widely applied compound (Stillwell *et al.*, 2003; Townsend *et al.*, 2003). The use of arsenic trioxide in agricultural chemicals declined from 13,500 tons in 1979 to 1,500 tons in 1998 and to virtually zero in 2001 (Grund and Hanusch, 2002).

## 1.3. Occurrence of organic arsenic compounds

Organic arsenic compounds such as arsenobetaine, arsenocholine, tetramethylarsonium salts, arsenosugars and arsenic-containing lipids are mainly found in marine organisms, and subsequently seafood and fish has been identified as major source of human food exposure (Ballin *et al.*, 1994). In the marine biosphere the production of arsenosugars (arsenoribosides) is prevalent and it is assumed that in marine microalgae more than 85 % of the total arsenic consists of arsenosugar (Wei *et al.*, 2003). Depending on the species, in larger algae arsenosugars and DMA<sup>V</sup> will be formed which can during bioaccumulation be re-converted into inorganic arsenic (Gao and Burau, 1997, Castlehouse *et al.*, 2003).

In bivalves, arsenobetaine is the most prevalent arsenic compound, and in marine (teleost) fish arsenobetaine and arsenocholine represent 99 % of the total measurable arsenic. Bioaccumulation factors in marine organisms are generally higher than those observed in freshwater invertebrates and fish.

Previously, distinct bio-geological cycles of arsenic have been described for the terrestrial and marine environments (Edmonds *et al.*, 1977; WHO, 2001), based on the hypothesis that As<sup>III</sup> is converted into arsenosugars and arsenocholine, and subsequently into arsenobetaine that was considered to occur only in the marine fauna. Recent investigations, however, question

this strong hierarchic concept (Yoshida *et al.*, 2001, Hughes *et al.*, 2003), whilst confirming that in marine organisms organic arsenic species prevail and inorganic arsenic can be found only in small quantities.

#### 1.4. Acute and chronic toxicity of inorganic and organic arsenic compounds

The toxicity of arsenic compounds depends on the chemical form and the valence: inorganic forms are much more toxic than organic As, and trivalent arsenic is more toxic than pentavalent arsenic (Hindmarsh and McCurdy, 1986).

These pronounced differences become evident when the acute toxicity of inorganic arsenic is compared with methylated metabolites or complex organic arsenic compounds. For instance, the oral LD<sub>50</sub> for rodents for arsenic trioxide varies between 15 and 26 mg/kg b.w., whereas the oral LD<sub>50</sub> for MMA and DMA are 916 mg and 648 mg/kg b.w., respectively. The acute toxicity of biogenic, organic arsenics, such as for example arsenobetaine, is even lower with an LD<sub>50</sub> of 5,500 mg/kg b.w. (Kaise and Fukui, 1992; Shiomi, 1994; Donohue and Abernathy, 1999). Signs of acute toxicity include vomiting, oesophageal and abdominal pain and bloody (rice-water) diarrhoea.

Chronic exposure to arsenic (via drinking water) results in skin lesions, hypo- and hyperpigmentation (Blackfoot Disease) and vaso-occlusive diseases with gangrenous changes. Other symptoms associated with chronic arsenic exposure are peripheral neuropathy, encephalopathy, altered heme metabolism, hepatomegaly, bone marrow depression, diabetes and renal function impairment (papillary and cortical necrosis) (NRC, 1999, Ng *et al.*, 2003a).

At the molecular level, pentavalent arsenic (arsenate) replaces phosphate in many biochemical reactions, as for examples in the form of glucose-6-arsenate that is formed following exposure to arsenates. These arsenates ultimately uncouple the formation of adenosine-5'-triphosphate, again replacing phosphate and hence inhibiting oxidative phosphorylation in various cell types (so called arsenolysis) (Crane and Lipmann, 1953; Dixon, 1997).

Trivalent arsenics react particularly with thiol-containing molecules, such as GSH and cysteine, which in turn results in the inhibition of various enzymes, such as pyruvate dehydrogenase (Peters, 1955, Hu *et al.*, 1998). The finding that MMA(III) is an even more potent inhibitor of pyruvate dehydrogenase challenges the paradigm that methylation is solely a detoxification process (Thomas *et al.*, 2001). MMA is also a potent inhibitor of GSH-reductase (Styblo *et al.*, 1995) and thioredoxin reductase (Lin *et al.*, 1999), resulting in an increased susceptibility of cells to oxidative stress.

Arsenic (in the form of inorganic arsenic) was the first element to be identified as a human carcinogen. Based on the induction of primary skin cancer, as well as the induction of lung, urinary bladder and kidney cancer, the IARC allocated inorganic arsenic to Group 1 (carcinogenic to humans) (IARC, 1987). Moreover, arsenite has been found to induce sister chromatid exchange, chromosome aberrations, and gene amplification in a variety of *in vitro*

systems (Liu and Huang, 1997). Arsenic toxicity in terms of the ability to cause chromosomal aberrations can be ranked as follows: arsenite > arsenate > DMA > MMA > TMAO. Arsenic mediated DNA-protein interactions may play a major role in arsenic carcinogenesis, and the induced protein-associated DNA-strand breaks could provide an explanation for chromosome aberrations and sister-chromatid exchanges induced by arsenic *in vivo* and *in vitro* (Dong and Luo, 1993, Das *et al.*, 1993). Exposure to inorganic arsenic can also result in the development of reactive oxygen species, which cause lipid peroxidation and cellular damage (Schlenk *et al.*, 1997). Bioassays in rodents did not unequivocally confirm the carcinogenicity of arsenicals, and suggested that these compounds act as tumour promoters rather than as tumour inducers, which would be in agreement with various epidemiological studies (Tsuda *et al.*, 1995, Bates *et al.*, 1995, Pott *et al.*, 2001, Hughes, 2002). However, in one 2-year mouse study involving the strain C57BL/6J that displays a very low incidence of spontaneous tumours, a daily dose of approximately 67 µg As/kg b.w., resulted in carcinogenesis in adult animals, with tumors in lung, intestinal tract and skin (Ng *et al.*, 1999).

In contrast to inorganic arsenic, organic forms including arsenocholine, arsenobetaine and TMA are only toxic at very high doses (OyaOhta *et al.*, 1996). Kaise and Fukui (1992) examined the acute toxicity of arsenobetaine, and minor arsenicals found in marine organisms (trimethylarsine oxide, arsenocholine and tetramethylarsonium salt) to mice. Following administration of arsenobetaine to mice at concentrations that may occur in the diet, no specific toxic symptoms were observed, and arsenobetaine was excreted in urine in its non-metabolised form. Neff (1997) confirmed these data, and recent investigation on the immunotoxicity and genotoxicity of these compounds provided no evidence for long-term adverse effects (Sakurai *et al.*, 2004, Guillamet *et al.*, 2004). Hence the WHO Joint Expert Group concluded that organic arsenic species abundant in seafood and fish comprise no significant health hazard (WHO, 2001, as amended in 2004).

Despite its obvious toxicity, arsenic has been also considered an essential element as indicated by various studies with rats, hamsters, miniature pigs, goats and chickens. Anke (1986) reported that animal feeds containing less than 0.05 mg arsenic per kg may be deficient. Nielsen (1996) suggested however that adverse effects of arsenic deficiency occur only when at the same time the diet is imbalanced with respect to other nutrients. As arsenic does not meet all criteria set for essential elements, its status in the human diet is still controversially discussed (US-EPA, 1988, Uthus, 1992, White and Sabboni, 1998).

Arsenic and arsenic compounds have been reviewed by various bodies, including the WHO (2001), and the US Department of Health and Human Services (ATSDR, 2000). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established a provisional tolerable weekly intake (PTWI) of 0.015 mg As/kg b.w. (FAO/WHO, 1989). The WHO guideline on Drinking Water Quality (1993) recommended as provisional guideline value for arsenic in drinking water a concentration of 0.010 mg/L. In the same fact sheet it is stated that this concentration represents the realistic limit for control measurements. However, based on health criteria a lower level would have to be recommended (WHO, 2001). Countries, in which this guideline value is frequently violated under normal conditions are Argentina,

Australia, Bangladesh, Chile, China, Hungary, India, Mexico, Peru, Thailand and the United States. Countries, reporting adverse health effects associated with high concentrations of arsenic in drinking water include Bangladesh, China, India (West Bengal) and the USA. In other regions, arsenic concentrations in drinking water vary between 0.001 - 0.010 mg/L (Mandal and Suzuki, 2002), but mineral waters may contain significant higher amounts (Savory and Wills, 1984).

## **2. Methods of analysis and statutory limits in feed materials**

### **2.1. Methods of analysis**

The most common analytical methods used for the determination of total arsenic in feed and foods are based on atomic absorption spectrometry; either electrothermal atomic absorption spectrometry (ETAAS) or hydride generation atomic absorption spectrometry (HGAAS).

The principle of the ETAAS method is based on digestion of the test samples in closed vessels in a microwave oven in a mixture of nitric acid and hydrogen peroxide. The resulting solution is diluted with water, and the total arsenic contents are determined by ETAAS using matrix modifiers (e.g. Pd and Mg or Ni) and standard addition procedure (Julshamn *et al.*, 1996; Julshamn *et al.*, 2000). The precision and trueness of the method were established by the Nordic Committee on Food Analysis (NMKL) in an inter-laboratory test in 2000 (NMKL, 2000). The method is a published standard CEN/TC 275 (PrEN14332, CEN, 2002a) and recommended by AOAC in USA (Julshamn *et al.*, 2000).

The principle of the HGAAS method is based on the destruction of the test samples either by dry-ashing or pressure digestion prior to the determination of the element in the test solution by hydride generation atomic absorption spectrometry. Prior to the final determination step, the arsenic ions are reduced to arsine by sodium borohydride in acidic solution. This method is a published standard CEN/TC 275 (EN 14332; PrEN14627, CEN, 2003, PrEN14546, CEN, 2002b). The precision and trueness of the methods were established and verified in inter-laboratory tests evaluated in accordance with ISO 5725-2:1994. In addition, inductively-coupled plasma atomic emission spectrometry (ICP-AES) and ICP-mass spectrometry (ICP-MS) are increasingly common techniques for the analysis of arsenic; both methods can generally provide lower detection limits than absorbance detection methods. Hydride generation combined with atomic fluorescence spectroscopy (HG/AFS) is another new technique with a low limit of detection and a wide linearity range (Vilano and Rubio, 2001).

### **2.2. Speciation analysis of arsenic**

To date, more than 25 different naturally occurring arsenic compounds (organoarsenicals) have been identified in the environment, mainly in samples of marine origin (see Table 1). The most commonly employed technique for the determination of the different arsenic compounds (speciation analysis) is HPLC-ICP-MS. Different chromatographic approaches have been applied to separate the different arsenic compounds. The organoarsenicals are most

often separated using cation exchange chromatography (Kirby and Maher, 2002; Sloth *et al.*, 2003). Since the inorganic arsenic is not retained on a cation exchange column, anion exchange (Brisbin *et al.*, 2002, Sloth *et al.*, 2005) or ion-pairing chromatography (Kohlmeyer *et al.*, 2002; Wrobel *et al.*, 2002) are used for the analysis of the latter.

Selective distillation (Oygard *et al.*, 1999) or extraction (Munoz *et al.*, 1999) of inorganic arsenic using HCl and chloroform followed by detection by HGAAS has been described. However, this method does not allow to discriminate between the different arsenic species, which are thus reported as a sum. Furthermore, monomethylarsenous acid (MMA) and trimethylarsine oxide may be extracted too and thereby resulting in an overestimation of the concentration of inorganic arsenic.

### 2.3. Statutory limits for arsenic in feed materials

The current EU maximum levels for arsenic in feed materials are given in Table 2. It should be noted that these data refer to total arsenic, and do not differentiate between the different forms of arsenic.

Table 2. Statutory limits for total Arsenic in feedingstuffs, mg/kg, at a moisture content of 12 %<sup>8</sup>

Feed materials with the exception of:	2
— meal made from grass, from dried lucerne and from dried clover, and dried sugar beet pulp and dried molasses sugar beet pulp	4
— palm kernel expeller	4
— phosphates and calcareous marine algae	10*
— calcium carbonate	15
— magnesium oxide	20
— feedingstuffs obtained from the processing of fish or other marine animals	15*
— seaweed meal and feed materials derived from seaweed	40*
Complete feedingstuffs with the exception of:	2
— complete feedingstuffs for fish and complete feedingstuffs for fur animals	6*
Complementary feedingstuffs with the exception of:	4
— mineral feedingstuffs	12

\* Upon request of the competent authorities, the responsible operator must perform an analysis to demonstrate that the content of inorganic arsenic is lower than 2 mg/kg. This analysis is of particular importance for the seaweed species *Hizikia fusiforme*.

<sup>8</sup> Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed – L 140, 30.5.2002 as last amended by Commissions Directive 2003/100 EC of 31 October 2003, L 285, 1.11.2003.

### 3. Occurrence of arsenic in feed materials and animal exposure

Data of the occurrence of arsenic in feed materials have been provided by a number of individual EU Member States or taken from reports published within the EU. Some of these data are difficult to evaluate because of limited information on the nature of the samples (e.g. compound feed without any detailed information on designated species) or inadequate sample description. The data that can be reliably categorised are summarised for feed materials in Table 3, and for commercially manufactured compound or complementary feeds in Table 5<sup>9</sup>. It should be noted that all these data refer to total arsenic.

Table 3. Total arsenic concentrations (mg/kg dry matter) in certain feed materials.

	<b>Mean</b>	<b>SD*</b>	<b>Median</b>	<b>Min</b>	<b>Max</b>	<b>n</b>
Fish meal	4.7	3.17	4.21	0.11	16.3	95
Fish oil	7.6	1.15	8.14	6.3	8.9	7
Oils seed meals	0.09	0.08	0.04	0.012	0.2	17
Maize grain and maize by-products	0.26	0.15	0.20	0.005	0.51	7
Other cereals and cereal by-products	0.06	0.20	0.01	0.001	1.08	47
Minerals and mineral supplements (unspecified)	6.8	3.44	3.05	0.01	15.7	42

\* Standard deviation

The highest levels of arsenic are found in fishmeal and fish oils, and this is reflected in the levels of arsenic in compound feeds for fish (see below), for which these are major ingredients. The situation appears not to have changed significantly over the last 40 years; a survey in 1973 reported fish meals containing 2 - 20 mg As/kg dry matter (Ammerman *et al.*, 1973, cited by Underwood and Suttle, 1999). Feedingstuffs Regulations (Table 2) permit a maximum of 15 mg As/kg dry matter in feeds obtained from the processing of fish, and none of the samples analysed exceeded this. While the data for other feed materials are derived from a relatively low number of samples, there is no evidence that maximum permitted levels are being exceeded.

In contrast to other undesirable substances, mineral supplements are not generally considered to be a major source of arsenic. Only one the 42 samples of minerals for beef cattle with a

<sup>9</sup> Where data have been reported as being below the level of detection, e.g. < 0.1 mg/kg, a value of half of the level of detection has been used in calculating the mean and standard deviation.

concentration of 15.7 mg/kg dry matter exceeded the maximum permitted limit for mineral feedingstuffs.

Concentrations of arsenic in soils are usually low. Richardson (1980) suggested a typical soil concentration of 6 mg/kg, with a range of 0.1 to 40 mg/kg. Archer and Hodgson (1987) reported a median concentration of 10.4 mg, although values of up to 140 mg/kg (DM) were reported in soils in close proximity to mining or industrial processes. Grazing animals may ingest a considerable amount of soil, and thus for heavy metals soil has been considered as additional factor potentially contributing to total exposure of livestock. The oral bioavailability of arsenic from the soil is considerably lower than from water or food, being influenced not only by the solubility of the individual arsenic compounds but also by soil chemistry (Ellickson *et al.*, 2001, Ng *et al.*, 2003b). Hence it can be assumed that soil ingestion contributes little to total exposure, with the exception of distinct highly contaminated soils in the proximity of industrial areas.

Arsenic uptake from the soil by plants depends on various factors, including the amount of soluble arsenic species in the soil, soil properties, redox and pH conditions, and microbiological activity, as well the plants species. Moreover, uptake by plants competes with phosphate and vanadate and hence depends on the soil levels of these elements (Merian *et al.*, 2004). Limited information is available regarding the speciation and metabolism of arsenic in terrestrial plants. Data suggest that mainly As<sup>III</sup> and As<sup>V</sup> and lower amounts of MMA and DMA occur in plant materials, but also arsenosugars and arsenobetaine have been detected in plants, mosses and mushrooms (Byrne *et al.*, 1995; Pizzaro *et al.*, 2003).

Despite the fact that the uptake of arsenic by plants is variable, particularly on clay soils, the concentrations in forages and crops grown on non-contaminated soils remain usually < 0.5 mg/kg dry matter (Underwood and Suttle, 1999). Richardson (1980) reported forage arsenic concentrations of between 0.07 and 0.65 mg/kg dry matter, but these were not in proportion to soil arsenic concentrations. Higher concentrations in fresh grass (0.32 mg/kg dry matter; Veen and Vreman, 1985) and maize silage (0.5 mg/kg dry matter; Vreman *et al.*, 1988) have been reported. These values may reflect soil contamination of the sample. Concentrations of up to 73 mg/kg dry matter were reported in grass sampled 2.5 km from an industrial smelting plant (ADAS, 1991)<sup>10</sup>. It would appear that arsenic levels in forages fed to livestock are generally well below the maximum for feed materials (2.27 mg As/kg dry matter), although values may be increased by contamination in the proximity of industrial areas.

For arsenic in forages, only few data have been reported by the Member States. However, limited analyses of forages from the UK (Nicholson *et al.*, 1999) suggest that levels of arsenic in forages are relatively low (Table 4), and do not exceed the maximum permitted level (2.27 mg As/kg dry matter) specified in Commission Directive 2002/32/EC<sup>11</sup> as amended by Directive 2003/100/EC<sup>12</sup>.

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<sup>10</sup> Data from reports in which contamination is known to have occurred have been excluded from Table 5

<sup>11</sup> OJ L 140, 30.5.2002, p. 10

<sup>12</sup> OJ L 285, 1.11.2003, p. 33

Table 4. Concentrations (mg/kg dry matter) of total arsenic in forages (Nicholson *et al.*, 1999).

Forage	Mean	Max	n
Grass silage	0.12	0.44	28
Hay	0.05	0.10	2
Maize silage	0.05	0.10	2
Straw	0.05	0.19	4

In estimating dietary exposure to arsenic, two approaches were considered. The first was to describe typical inclusion rates for feed materials used in the manufacture of compound feeds, and to calculate the final arsenic concentrations. However, this approach had to be rejected, primarily because information on many individual raw materials was scarce or not available. The alternative approach was to use data for manufactured compound feeds. This is the approach that was used previously by SCAN in its reviews of zinc and copper, and it has been adopted in this report. Information on the arsenic concentrations in complete feedingstuffs and complementary feedingstuffs, obtained as part of routine surveillance in a number of member states, are summarised in Table 5.

Table 5. Concentrations of total arsenic (mg/kg dry matter) in commercial compound feeds for farm livestock and fish (data reported by EU member states)<sup>13,14</sup>

	Mean	SD	Median	Min	Max	n
Poultry – Layers	0.20	0.13	0.25	0.05	0.29	3
Poultry - Broilers	0.34	0.19	0.25	0.15	0.60	5
Poultry (unspecified)	1.83	2.56	3.00	0.05	6.70	6
Fish	4.25	1.50	4.13	1.38	15.40	421
Pigs < 17 weeks	0.72	0.49	0.52	0.26	2.10	19
Pigs (growers/finishers)	0.31	0.06	0.06	0.26	0.39	4
Pigs (Sows)	0.85	1.44	0.69	0.20	5.68	15
Pigs (unspecified)	0.62	1.18	11.36	0.11	5.00	19
Ruminants (unspecified)	0.27	0.10	0.26	0.18	0.38	4
Ruminants (beef)	0.36	0.15	0.37	0.10	0.60	10
Ruminants (dairy)	0.24	0.09	0.20	0.20	0.49	12

<sup>13</sup> Data obtained as part of routine surveillance of feed materials, and provided by Member States.

<sup>14</sup> No data on horse or rabbit feeds have been provided.

Median concentrations, although sometimes based on a few individual values only, provide no evidence that any of the categories of complete feedingstuffs exceeded the maximum permitted levels on a systematic basis. Four samples (two each for pigs and poultry) exceeded the maximum permitted levels as set out in Directive 100/2003/EC. Moreover, for fish 20 of the 421 feed samples analysed had total arsenic levels > 6.8 mg/kg dry matter. Recent data for arsenic in compound fish feeds showed that inorganic arsenic concentrations remained in the range of 10 - 61 µg/kg as As (n = 13), and inorganic arsenic in these samples represented less than 1.2 % of total arsenic (Sloth *et al.*, 2005). Fishmeal samples were also analysed for inorganic arsenic and all samples had concentrations below the detection limit (*i.e.* < 7 µg/kg, Sloth *et al.*, 2005). These concentrations are far below the permitted maximum content of inorganic arsenic of 2000 µg/kg (2 ppm) as indicated in Commission Directive 2003/100/EC.

For the majority of non-ruminant livestock (pigs and poultry as well as farmed fish) in the EU, feed is provided as compounded feed, consisting of a mixture of individual feed components, to which additives and/or mineral supplements are added. Intake of arsenic may therefore be estimated by multiplying the concentrations given in Table 5 by the estimated intake of the compound for the particular class of livestock. Estimating intake by ruminants is less straightforward. For cattle, goats and sheep, the daily ration usually consists of forage (or mixture of forages), either fresh or conserved, together with complementary feeds or individual feed materials as necessary to achieve the required level of production (growth rate, milk yield). The ratio of concentrate feeds to forage in the diet is also influenced by the digestibility of the forage. However, combining data for forages (Table 4) and compound feeds for ruminants (Table 5) would suggest that typical dietary concentrations are < 1.0 mg/kg dry matter, and hence remain well below the maximum permitted level specified for feed materials in Directive 2003/100/EC.

In conclusion, present data suggest that total exposure of livestock, including fish, does not exceed the limits imposed by Directive 2003/100/EEC in any systematic way or to any significant extent.

#### **4. Adverse effects in livestock**

In peracute cases of intoxications animals may die in cardiovascular collapse. The most prominent clinical signs in acute cases are intense abdominal pain, vomiting where possible, salivation, watery diarrhoea, exhaustion, collapse and death (Humphreys, 1988, Selby *et al.*, 1977). The lethal oral dose for arsenic trioxide varies between 0.5 to 1 g (total exposure) in pigs to 10 - 45 g per animal in horses. Sodium arsenite appears to be toxic at 0.05 - 0.25 g per animal for pigs, and 1 - 3 g per animal for horses. These data are difficult to compare, as the actual body weight of the animals are not given, but indicate that lethal dosages vary between 5 - 100 mg/kg b.w. for arsenic trioxide and 0.5 - 10 mg/kg b.w. for sodium arsenite (Clarke *et al.*, 1981; Hapke, 1975).

Additional clinical signs observed in subacute cases are depression, loss of appetite, staggering gait, paralysis, trembling, stupor and convulsions. In chronic cases signs of indigestion, thirst and wasting can be observed (Selby *et al.*, 1977, Bahri and Romdane, 1991).

#### **4.1. Cattle and small ruminants**

The principal clinical sign associated with arsenic poisoning in cattle is an acute hemorrhagic diarrhea attributable to hemorrhagic gastroenteritis. Loss of appetite, muscular weakness, ataxia, emaciation and recurrent epileptiform convulsions may also occur (Riviere *et al.*, 1981, Thatcher *et al.*, 1985). Cattle fed during 5 days either 1.6 or 3.2 mg of arsenic per kg body weight in the form of arsonic or arsanilic acid, exhibited no sign of toxicity (Calvert and Smith, 1972).

In contrast, the LD<sub>50</sub> of sodium arsenite in goats and was found to be 125 mg/kg b.w. (Biswas *et al.*, 2000). Animals treated with 75 and 100 mg/kg b.w. of sodium arsenite showed gastrointestinal and renal signs of toxicity during 12 hours post-treatment onwards, without any mortality.

Thatcher *et al.* (1985) described an episode of arsenic poisoning in cattle after the ingestion of ashes of wood treated with arsenic preservative (arsenic pentoxide, 780 mg/kg ash) in which several animals died and had levels of 13.9, 23.7 and 25.8 mg As /kg in liver, kidney and rumen contents, respectively.

#### **4.2. Horses**

The major clinical observed in affected horses were profuse diarrhoea, extreme dehydration, extensive subserous haemorrhages, cyanotic mucous membranes and marked hyperaemia of the gastric and small intestinal mucosa (Seddon 1951; Seawright *et al.*, 1983). At moderate dosages, clinical signs in horses include watery diarrhoea, excessive salivation, muscle tremors, ataxia and depression (Pace *et al.*, 1997).

#### **4.3. Pigs**

The main clinical signs caused by arsenic poisoning in pigs include transient diarrhoea, hyperaesthesia and incoordination followed by posterior paresis, progressive blindness, tremor of the head and ataxia. In addition, dermatitis can occur after ingestion of arsenic (Bahri and Romdane, 1991). In contrast to inorganic arsenic, pigs given 100 mg arsanilic acid/kg diet for 6 weeks showed only a reduced feed intake (Morrison and Chavez, 1983) whereas 1 g arsanilic acid/kg feed leads to clinical signs of intoxication (Hapke, 1988).

#### 4.4. Poultry

Clinical signs observed in poultry exposed to arsenic are a decrease in feed consumption and weight gain and neurological symptoms. Laying hens showed a decreased feed intake (- 24 %) and egg production (- 20 %) when fed a diet containing 44 mg arsenite/kg feed (Chiou *et al.*, 1997). Egg mass was reduced when the concentration in feed exceeded 15 mg arsenic oxide/kg (Holeman *et al.*, 2001). Palmer (1972) studied the toxicity of organic arsenicals including monosodium methanearsonate, disodium methanearsonate and hydroxydimethylarsine oxide. Chickens had reduced weight gains after ten daily doses of 100 mg hydroxydimethylarsine oxide/kg b.w., but did not exhibit toxic symptoms following the exposure to the other arsenic compounds up to a daily dose of 250 mg/kg b.w. Adult Japanese quails tolerated up to 30 mg arsenite/kg diet (El Begearmi *et al.*, 1982).

#### 4.5. Rabbits

Rabbits show weight loss or reduced weight gain in response to arsenic. In severe cases of intoxication, diarrhoea, terminal convulsions and death can occur. Nemeč *et al.* (1998) investigated the developmental toxicity of arsenic acid in rabbits. Animals were given (by gavage) 0, 0.19, 0.75 or 3.0 mg/kg b.w./day on gestation days 6 through 18 and examined at sacrifice (gestation day 29) for evidence of toxicity. At the high dose, 30 % animals died and prenatal mortality was increased; survivors had signs of toxicity including decreased body weight. In the study a NOAEL of 0.75 mg/kg/day for both maternal and developmental toxicity was established.

#### 4.6. Dogs

Dogs tolerated a single oral doses of 1.2 mg/kg b.w./day of As(III) or As(V) without exhibiting major clinical symptoms (Byron *et al.*, 1967). However, repetitive doses of 3 mg/kg/day, the only other dose tested caused mortality within a few days, reaching 100 % in dogs exposed to As(III), a significant growth retardation was observed in dogs exposed to As(V).

#### 4.7. Fish

Toxic effects in fish following dietary exposure to inorganic arsenic include elevated hepatic metallothionein levels, histopathological alterations in liver and gall bladder, and decreased growth rate (Cockell and Hilton, 1988; Cockell *et al.*, 1992; Pedlar *et al.*, 2002a; Pedlar *et al.*, 2002b). Rainbow trout exposed to inorganic arsenic (180 mg arsenic trioxide/kg diet and 137 mg disodium arsenate heptahydrate/kg diet) for 8 weeks showed similar toxic responses, including altered feeding behaviour and reduced growth (Cockell and Hilton, 1988). In contrast, no toxic effects were observed in fish exposed to ten fold higher dietary

concentrations (1500 mg/kg diet) of the organic arsenic forms dimethylarsinic acid or arsinilic acid for 8 weeks (Cockell and Hilton, 1988).

## 5. Toxicokinetics and tissue disposition

### 5.1. Absorption

Gastrointestinal absorption of inorganic arsenic compounds correlates with their water solubility, water-soluble compounds being more readily absorbed (Vahter, 1983). In most (laboratory) animal species the rate of absorption is high, with approximately 90 % of the administered dose being absorbed from the gastrointestinal tract (Pott *et al.*, 2001). In rats receiving a single oral dose of arsenic at 5 mg/kg b.w., bioavailability could be ranked as follows: sodium arsenite > calcium arsenite > sodium arsenate (Ng *et al.*, 2003b). In contrast, oral bioavailability is lower in ruminant species as demonstrated after an application of <sup>73</sup>As to fattening lambs. In this species the calculated rate of absorption was 46 %, which is lower than that in monogastric animals, and most likely related to pre-systemic methylation of arsenic in the rumen. Significant interspecies differences have been reported with respect to the bioavailability of organic arsenic compounds such as dimethylarsinic acid that are absorbed to a significant extent (> 40 % of ingested dose) from the gastrointestinal tract, while the trivalent organic compounds are generally poorly absorbed (Vahter, 1994).

### 5.2. Distribution

Following absorption, arsenic is distributed between the plasma and the erythrocytes, in which it is bound to the globin moiety of haemoglobin. The relative distribution between blood plasma and erythrocytes depends on the valence and dose of arsenic administered, as well as on the animal species<sup>15</sup>. In most species, initially the highest tissue concentrations are found in liver, kidney, spleen and lung. In mice and rats, for example, orally administered <sup>74</sup>As-labelled DMA accumulated in kidneys > lungs > intestinal mucosa > stomach > testes within 6 hours after dosing. Although the concentration of DMA decreased rapidly in most tissues, the longest retention times were observed for lungs, thyroid and intestinal mucosa (Vahter *et al.*, 1984). However, several weeks later, arsenic still is detectable in hair, nails and skin because of the high concentration of sulfur-containing (keratinous) proteins in these tissues. In experiments with mice dosed orally with <sup>74</sup>As-labelled arsenite or arsenate three times a week for a period of 12 weeks, the tissue concentrations at the end of the experiment could be ranked as follows: hair > skin > liver > kidneys > lung > intestinal mucosa (Vahter, 1983).

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<sup>15</sup> Several authors consider that rats are not a good model to use for arsenic metabolic studies since the blood arsenic concentration in this species may be 300 times higher than that in mice exposed to arsenic under similar conditions

Trivalent and pentavalent inorganic arsenic is reported to cross the placenta in laboratory animals (WHO, 2001). Human studies have shown that the blood arsenic concentration of the mother and the cord blood concentration was similar (Kagey *et al.*, 1977; Concha *et al.*, 1998) suggesting that arsenic readily crosses the placenta. Arsenic may also be excreted with (mothers) milk.

### 5.3. Metabolism

Following absorption, arsenate is reduced to arsenite, either pre-systemically or in blood. Arsenite undergoes oxidative methylation in the liver by addition of a carbonium ion from S-adenosylmethionine, resulting in the formation of MMA(V). The pentavalent arsenic is then reduced to the trivalent form MMA(III). Formation of MMA(III) facilitates the addition of a second carbonium ion via oxidative methylation to yield DMA(V) that is generally considered as endpoint of arsenic biotransformation (Pott *et al.*, 2001). Glutathione and probably other thiols serve as reducing agents for As(V) and MMA(V). MMA(V) and DMA(V) were detected in all tissues after repeated oral administration of arsenate to mice (Hughes *et al.*, 2003). The trivalent intermediates (MMA III) seem to contribute to As toxicity (Carter *et al.*, 2003), and thus methylation can not be defined unequivocally as detoxification mechanism (Thomas *et al.*, 2001). Nevertheless, the remarkable inter-species and inter-individual differences in arsenic methyltransferase activity seem to account for the wide variability in sensitivity of humans and animals to arsenic toxicity (Goering *et al.*, 1999, Vahter *et al.*, 2002). Ingested organoarsenicals are less extensively metabolised and are rapidly excreted (Vahter, 1999).

### 5.4. Excretion

Arsenic and its metabolites are readily excreted in urine and bile. In rats and hamsters intraperitoneally injected with arsenate, approximately 50 % of the administered dose was excreted in bile and urine within 2 hours, whereas rabbits eliminated 20 % of dose during the same period. In all investigated species, the major part of the arsenate dose was eliminated in urine (Csanaky and Gregus, 2002). In contrast, animals receiving arsenite under similar conditions excreted more arsenic into bile than urine (except rabbits). The urinary excretion profiles of arsenic and its methylated metabolites are highly variable among species (Vahter, 1994; Csanaky and Gregus, 2002).

## 6. Carry over and tissue residues

Experimental data available in the literature indicate that arsenic compounds are deposited in tissues of livestock proportionally to dietary intake and rate of absorption (NRC, 1980; Eisler, 1994). Following ingestion, tissue concentrations are initially elevated in the liver, kidney, spleen and lung, as mentioned before. Within a few hours, re-distribution to ectodermal tissues (hair, nails) starts. Hence, residues of arsenic in edible tissues (liver, kidney and

muscle) of cattle (Vreman *et al.*, 1986, 1988; Thatcher *et al.*, 1985) sheep (Woolson, 1975; Veen and Vreman, 1985) and poultry (Proudfoot *et al.*, 1991) fed standard or control diets (< 2 mg/kg dry matter) are usually less than 0.01 mg/kg wet weight. Similar arsenic residues are found in monitoring studies in animal products from various agricultural regions (Kramer *et al.*, 1983; Salisbury *et al.*, 1991; Kluge-Berge *et al.*, 1992; Jorhem *et al.*, 1991; Vos *et al.*, 1987; López Alonso *et al.*, 2000).

With increasing dietary arsenic exposure, both in experimental studies and in animals from naturally contaminated areas, or those in the proximity of industrial areas, arsenic residues significantly increase in all the tissue analysed compared with control animals. However, the absolute arsenic residue levels vary significantly depending on animal species, arsenic compounds and duration of exposure (Vreman *et al.*, 1986; Eisler, 1994)

For example, cows fed 33 mg arsenate/animal/day for 3 months, had slightly elevated tissue levels in muscle (20 µg/kg wet weight vs. 5 µg/kg in controls) and liver (30 µg/kg wet weight vs. 12 µg/kg in controls) but normal levels in milk and kidney. Following the exposure to arsenite (33 mg daily per animal for 15 to 28 months) the animals had tissue levels of 2 µg/kg in milk (vs. < 1 µg/kg for controls), 30 µg/kg in muscle tissue (vs. 5 µg/kg in controls), 100 µg/kg in the liver (vs. 12 µg/kg in controls) and 160 µg/kg mg/kg in the kidneys (vs. 53 µg/kg controls) (Vreman *et al.*, 1986). Bulls receiving a diet containing 2.7 mg/kg feed as As<sub>2</sub>O<sub>5</sub> for the last five months of the fattening period until slaughter had 170, 100 and 46 µg/kg weight in kidneys, liver and muscle tissue, respectively (versus 60, 22 and 11 µg/kg, respectively, measured in controls animals) (Vreman *et al.*, 1988).

López Alonso *et al.* (2000) measured arsenic residues in calves (males and females between 6 and 12 months old) and cows (2 - 16 years old). The geometric mean concentrations in calves and cows were 10.8 and 10.2 µg/kg in liver, 11.3 and 15.2 µg/kg in kidney, 3.75 and 4.25 µg/kg in muscle and 3.23 and 2.92 µg/kg in blood. There were no differences between male and females in any of the tissues analysed. The concentrations of arsenic in the liver and kidney that are associated with toxicity in cattle are approximately 14 mg/kg wet weight (Humphreys, 1990). In lactating dairy cows fed arsenilic acid, (1.6 or 3.2 mg/kg b.w./day) for 5 days, an increase over background in the level of arsenic residues in milk (from 0.08 to 0.21 µg/g dried milk) was observed during the exposure period, but only in the animals receiving the higher dose (Calvert and Smith, 1980). One week after arsenic feeding was stopped, milk arsenic level returned to the values observed at the beginning of the experiment. Investigations in arsenic rich regions in Mexico showed a carry-over factor for arsenic in feed to dairy milk of up to  $6 \times 10^{-4}$  based on pharmacokinetic data analyses (Rosas *et al.*, 1999).

## 7. Human dietary exposure

Various food-basket studies indicated that in most countries the major source of arsenic in the human diet is seafood (with the exception of areas with an endemic high drinking water contamination). In Japan, a country with a traditional high consumption of seafood, a mean total daily As intake of  $195 \pm 235$  µg (15.8 - 1039 µg) was deduced from a food basket study

(Yamaouchi *et al.*, 1992). In Canada, a comprehensive diet study reported an average As intake of 38.1 µg/day (14.9 – 59.2 µg), of which 64 % could be attributed to the consumption of fish and shellfish (Dabeka *et al.*, 1993). In a recent study from Catalonia, the mean intake by adult inhabitants was found to be 223.6 µg/day, and it was calculated that fish and seafood accounted for 91 % of the exposure (Llobet *et al.*, 2003). Ysart *et al.* (1999) estimated in a UK total diet study conducted in 1994, a daily total As intake of 63 µg of which 89 % could be attributed to seafood. The SCOOP report (EC, 2004) concluded on the basis of the available data from Member States that fish and other seafood are the main source of As in the diet of the mean adult population. The origin of fish, i.e. the type of water in which it was caught is of great importance. Fish from marine waters have arsenic-levels up to ten times higher than fish from brackish waters, which in turn have levels up to ten times higher than fish from freshwater lakes and rivers. Hence an intake estimation remains complicated and uncertain. However, based on the available information from Member States, it was calculated that the daily intake of As from fish and other seafood is below 0.350 mg/day in all Member States. Children have a lower intake than adults, but since they have a lower body weight, their body burden/kg may be higher than that of adults.

It is worthwhile to recall that in fish and seafood often more than 90 % of the total arsenic substances represent the non-toxic arsenobetaine and arsenocholine (Buchet and Lauwerys, 1994). Many of previously published surveys on the occurrence of As in different fish and seafood species, however, reported only total As concentrations, and thus there is still a need for more comprehensive data with analytical techniques clearly discriminating between inorganic and organic arsenic compounds.

## CONCLUSIONS

- Arsenic is a naturally occurring element. In Europe the levels of inorganic arsenic in surface and ground waters and in soil are generally low, and do not seem to pose a risk to animal health. Only a few distinct geological and contaminated industrial areas with high levels of arsenic have been identified. The arsenic found in the marine biota is mainly present as organic arsenic compounds.
- From the toxicological data available for farm animals, it can be concluded that all animal species are susceptible to the toxic effects of inorganic arsenic. Organic arsenic is less toxic than inorganic arsenic.
- An assessment of the levels of exposure of farm animals to individual arsenic compounds is not possible because the majority of data on feed materials are reported as total arsenic.
- Analytical methods allowing a speciation of arsenic compounds have become available for food recently. Application of these methods in the analysis of feed materials would facilitate quantitative exposure assessment.

- Marine organisms accumulate arsenic, predominantly as non-toxic arsenobetaine and arsenocholine. Products like fishmeal and fish oil have been identified as major sources of feed contamination with arsenic, and it is likely that the measured arsenic represents predominantly these organic compounds. The limited data available suggest that the non-toxic organic arsenic in feed materials do not pose a significant health risk to animals. Other components of feedstuffs contribute only little to animal exposure to arsenic.
- In mammalian species (and poultry), inorganic arsenic is converted into methylated metabolites, which are rapidly excreted comparable to other organic arsenic compounds. Hence the carry-over of arsenic compounds from feeds to edible tissues of mammalian species and poultry is very low.

## **RECOMMENDATIONS**

- While analytical methods are available for food speciation methods of arsenic analysis need to be validated for common feed materials, including products from marine organisms used in animal nutrition.
- For products from marine organisms the concentration of inorganic arsenic needs to be determined as a prerequisite for a comprehensive assessment of the potential animal health risks.
- More information on release of inorganic arsenic from organoarsenic compounds such as arsenosugars is needed.

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### **SCIENTIFIC PANEL MEMBERS**

Jan Alexander, Herman Autrup, Denis Bard, Angelo Carere, Lucio Guido Costa, Jean-Pierre Cravedi, Alessandro Di Domenico, Roberto Fanelli, Johanna Fink-Gremmels, John Gilbert, Philippe Grandjean, Niklas Johansson, Agneta Oskarsson, Andrew Renwick, Jirí Ruprich, Josef Schlatter, Greet Schoeters, Dieter Schrenk, Rolaf van Leeuwen, Philippe Verger.

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## ANNEX

Table 1. Sublethal effects and bioaccumulation of various arsenic compounds in fish.

Concentration (mg/kg)	Exposure (days)	NOEL (mg/kg)	LOEL (mg/kg)	Tissue (mg/kg wet weight)	Species	Description of effects	Reference
1, 180, 360, 732 and 1477 mg arsenic trioxide (AT)/kg	56	1	180	Whole body burden increased from 0.9 to 21.6 mg/kg (control and highest treatment)	Rainbow trout ( <i>Oncorhynchus mykiss</i> )	Regurgitation of food and reduced growth.	Cockell and Hilton, 1988
1, 137, 262, 500 and 1053 mg disodium arsenate heptahydrate (DSA)	56	1	137	Whole body burden increased from 0.9 to 14.5 mg/kg (control and highest treatment)	Rainbow trout ( <i>Oncorhynchus mykiss</i> )	Regurgitation of food and reduced growth.	Cockell and Hilton, 1988
1, 163, 362, 793 and 1497 mg dimethyl-arsinic acid (DMA)	56	1497		Whole body burden increased from 0.5 to 11.4 mg/kg (control and highest treatment)	Rainbow trout ( <i>Oncorhynchus mykiss</i> )	No effects.	Cockell and Hilton, 1988
1, 193, 405, 735, 1503 mg arsanilic acid (AA)	56	1503		Whole body burden increased from 0.5 to 6.1 mg/kg (control and highest treatment)	Rainbow trout ( <i>Oncorhynchus mykiss</i> )	No effects.	Cockell and Hilton, 1988

Concentration (mg/kg)	Exposure (days)	NOEL (mg/kg)	LOEL (mg/kg)	Tissue (mg/kg wet weight)	Species	Description of effects	Reference
1, 32 and 60 mg DSA/kg	84	1	32		Rainbow trout ( <i>Oncorhynchus mykiss</i> )	Decreased feed consumption and body weight gain at highest concentration. Decreased lipid digestibility in fish fed 32 mg As/kg. Increased prevalence of lesions in the gallbladder. Mild to moderate anemia.	Cockell <i>et al.</i> , 1992 (experiment 1)
1 and 55 mg DSA/kg	84	1	55	Accumulation in plasma, bile, gallbladder and liver.	Rainbow trout ( <i>Oncorhynchus mykiss</i> )	Increased prevalence of lesions in the gallbladder. Mild to moderate anemia.	Cockell <i>et al.</i> , 1992 (experiment 2)
0.9, 160 and 1300 mg arsenate/kg	20	0.9	160	No accumulation in muscle tissue (concentration range for control groups 0.4-0.5 mg/kg)	Lake whitefish ( <i>Coregonus clupeaformis</i> ) and lake trout ( <i>Salvelinus namaycush</i> )	Decreased growth in lake trout at the highest exposure concentration. Histopathological alterations in gallbladder of exposed fish.	Pedlar <i>et al.</i> , 2002a
0, 1, 10 and 100 mg arsenate/kg DW	64	0	1		Lake whitefish ( <i>Coregonus clupeaformis</i> )	Increased hepatic metallothionein level. Histopathological alterations in liver and gallbladder.	Pedlar <i>et al.</i> , 2002b