

Mercury as undesirable substance in animal feed¹

Scientific opinion of the Panel on Contaminants in the Food Chain

Question N° EFSA-Q-2005-288

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¹ For citation purposes: Opinion of the Scientific Panel on Contaminants in the Food chain on a request from the European Commission on mercury as undesirable substance in feed, *The EFSA Journal* (2008) 654, 1-76.

² In chapter 8 on page 50 the CONTAM Panel clarified the derivation of a no-observed-adverse effect level for cats and the possible health effects for these animals in relation to the current EU maximum levels. This clarification now takes into account a 12% water content of the feed material and consequently the respective figure in the conclusion was revised. The changes do not affect the overall conclusions of the opinion. To avoid confusion, the original version of the opinion has been removed from the website, but is available on request as is a version showing all the changes made.

SUMMARY

Mercury exists in the environment as elemental mercury (metallic), inorganic mercury and organic mercury (primarily methylmercury). Elemental and inorganic mercury released into the air from mining, smelting, industrial activities, combustion of fossil fuels, is deposited to soil, water and thereby to sediments where the mercury is transformed into methylmercury. Methylmercury bioaccumulates and biomagnifies along the food chain, particularly in the aquatic food chain; longlived carnivorous fish and marine mammals exhibiting the highest contents. The toxicity and toxicokinetics of mercury in animals and humans depends on its chemical form. Elemental mercury is volatile and mainly absorbed through the respiratory tract, whereas its absorption through the gastrointestinal tract is negligible. Gastrointestinal absorption of inorganic mercury is in the 10-30% range. Following absorption, inorganic mercury distributes mainly to the kidneys and, to a lesser extent, to the liver. The critical effect of inorganic mercury is renal damage. In animals, as in humans, methylmercury and its salts are readily absorbed in the gastrointestinal tract (>80%). Absorbed methylmercury is widely distributed to all tissues, although the largest deposition occurs in the kidney. Excretion of unchanged methylmercury occurs predominantly in the faeces through biliary excretion. The enterohepatic cycle results in a long half-life for this compound compared to inorganic mercury. Methylmercury is able to cross the blood-brain and the placental barriers. As a consequence, the nervous system is the primary site of toxicity in animals and humans. In humans, effects on neurological development have been observed in children of mothers orally exposed to methylmercury. Animal studies confirmed these neurodevelopmental effects in foetus of dams exposed to methylmercury in the diet.

A substantial number of feed materials have been analysed for total mercury in recent years within the EU Member States, and for the large majority, the concentrations were below the maximum level specified in the feedingstuffs legislation. The most common source of mercury in feed materials is fishmeal, however, in this category, no sample exceeded the maximum level of 0.5 mg/kg. In contrast, approximately 8% of the complete feedingstuffs for fish exceeded the maximum level of 0.1 mg/kg. The relatively few data available on the speciation of mercury in fishmeals indicate that it is mainly present as methylmercury. The most sensitive domestic animal species to methylmercury toxicity are cats and mink. Based on the available data on the occurrence of total mercury in feed materials and complete feedingstuffs, it is unlikely that these species will be exposed to toxic levels.

The maximum concentration reported in farmed salmonids is approximately five times lower than the EU maximum level for mercury in fish for human consumption (500 µg/kg for salmonids). This mercury concentration in salmonids would allow weekly consumption of two fish meals, as recommended by nutritionists, without appreciable health risk. The maximum level for fish feed is sufficient to ensure that contamination levels in farmed salmonids pose no appreciable risk to consumers, but the validity of the maximum level need to be ascertained for other farmed fish.

KEYWORDS: Mercury, methylmercury, organic mercury, inorganic mercury, animal feed, occurrence, toxicity, analysis, bioaccumulation, carry over, animal health, human health, human exposure.

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BACKGROUND AS PROVIDED BY THE REQUESTOR

1. General background

Directive No (EC) 2002/32 of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed³ has, since 1 August 2003, replaced Council Directive No (EC) 1999/29 of 22 April 1999 on the undesirable substances and products in animal nutrition⁴.

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 of 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, thereby providing a high level of public health and animal health protection. The deletion of the possibility of dilution is a powerful means of stimulating 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 prior to the adoption of Directive No (EC) 2002/32 the Commission made the commitment to review the provisions laid down in Annex I on the basis of updated scientific risk assessments, taking into account the prohibition of any dilution of contaminated non-complying products intended for animal feed. The Commission therefore requested the Scientific Committee on Animal Nutrition (SCAN) in March 2001 to provide these updated

³ OJ L140, 30.5.2002, p. 10

⁴ OJ L 115, 4.5.1999, p. 32

scientific risk assessments in order to enable the Commission to finalise this review as soon as possible (Question 121 on undesirable substances in feed)⁵.

The opinion on undesirable substances in feed, adopted by SCAN on 20 February 2003 and updated on 25 April 2003⁶ 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.

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

2. Specific background

Mercury in the natural environment is present in both inorganic and organic forms. The inorganic forms are less toxic, but can be converted into organic form by the micro-flora and micro-fauna in the environment. Among organic forms, the most toxic is methylmercury. Chromatographic techniques to separate organic mercury from inorganic mercury are available and validated. However they are not used routinely because of their complexity and cost. As a consequence, only total mercury content is routinely determined, mostly by atomic absorption spectrometry.

Directive No (EC) 2002/32 of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed establishes maximum levels for total mercury in feed materials and compound feed.

SCAN concluded⁷ that the ions and elements, including mercury, listed in Council Directive No (EC) 2002/32 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 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.

SCAN concluded furthermore that a detailed risk assessment of the presence of mercury in animal feed and the possible effects for animal health and public health is necessary and that this detailed assessment should address the risks related to the organic forms of mercury.

⁵ Summary record of the 135th SCAN Plenary meeting, Brussels, 21-22 March 2001, point 8 – New questions (http://europa.eu.int/comm/food/fs/sc/scan/out61_en.pdf)

⁶ 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)

⁷ Opinion of the Scientific Committee on Animal Nutrition on Undesirable Substances in Feed, point 6.11. Conclusions and recommendations.

Indeed, methylmercury is recognised as significantly more toxic than inorganic mercury and therefore the determination of total mercury in feed may not always accurately reflect the risk posed by the organic forms.

TERMS OF REFERENCE AS PROVIDED BY THE REQUESTOR

In accordance with Article 29 (1) of Regulation (EC) No 178/2002 the European Commission asks the European Food Safety Authority requests to provide a scientific opinion on the presence of mercury in animal feed.

This detailed scientific opinion should comprise the

- determination of the toxic exposure levels (daily exposure) of organic forms of mercury (methylmercury) and, if relevant, of inorganic mercury 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 organic forms of mercury (methylmercury) and inorganic mercury from the feed to the products of animal origin results in unacceptable levels of organic forms of mercury (methylmercury) and, if relevant, of inorganic mercury in the products of animal origin in view of providing a high level of public health protection⁸.
- identification of feed materials which could be considered as sources of contamination by mercury and the characterisation, insofar as is possible, of the distribution of levels of contamination, in particular the typical ratio between mercury in organic forms and mercury in inorganic forms for the different (groups of) feed materials.
- assessment of the contribution of the different identified feed materials as sources of contamination by organic forms of mercury (methylmercury) and if relevant of inorganic mercury
 - to the overall exposure of the different relevant animal species to organic forms of mercury (methylmercury) and inorganic mercury,
 - to the impact on animal health,

⁸ The possible risks to human health from the consumption of foods contaminated with mercury and methyl mercury has been assessed by EFSA – Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to mercury and methyl mercury in food (Request N° EFSA-Q-2003-030, opinion adopted on 24 February 2004), EFSA Journal (2004) 34, 1-14
http://www.efsa.eu.int/science/contam/contam_opinions/259/opinion_contam_01_en1.pdf

- to the contamination of food of animal origin (the impact on public health), taking into account the ratio between mercury in organic forms and mercury in inorganic 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 mercury is present⁹.
- identification of eventual gaps in the available data which need to be filled in order to complete the evaluation.

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ASSESSMENT

1. Introduction

Mercury (Hg) and its compounds are ubiquitous and persistent in the environment. Mercury is a naturally occurring element that is released from a variety of sources including human activities. Once released into the environment, mercury undergoes a series of complex chemical and physical transformations as it cycles between atmosphere, land, and water. Humans, plants, and animals are routinely exposed to mercury and accumulate it during this cycle, potentially resulting in a variety of health impacts.

Mercury may exist in elemental, inorganic or organic forms.

Elemental (or metallic) mercury is a liquid at normal ambient temperatures and pressures; it partitions strongly to air in the environment. Most of the mercury encountered in the atmosphere is elemental mercury gas, whereas in all other environmental compartments inorganic mercury salts and organomercurials predominate.

⁹ Importance of the human exposure to mercury from foods of animal origin compared to overall human dietary mercury exposure can be assessed making use of the information contained in the report on a task on human exposure assessment to mercury which has been recently performed at EU level within the framework of cooperation by Member States in the scientific examination of questions related to food (SCOOP – Task 3.2.11) http://europa.eu.int/comm/food/food/chemicalsafety/contaminants/scoop_3-2-11_heavy_metals_report_en.pdf

Inorganic mercury compounds are salts and are used in numerous industrial processes. They have been extensively used in batteries and included in products such as fungicides, antiseptics or disinfectants.

There are several organic mercury compounds; however, by far the most common in the environment and in the food chain is methylmercury. Organic mercury compounds have been used as fungicides and as pharmaceutical agents (Mercurochrome as topical antiseptics; Thiomersal as a preservative in vaccines). Phenylmercury salts were used as fungicides and in pharmaceutical and cosmetic preparations to control growth of microbial organisms while the primary use of phenylmercury acetate was in latex paint as a preservative. Like the inorganic mercury compounds, methylmercury, ethylmercury and phenylmercury exist as salts such as chloride or acetate.

Although inhalation of gaseous mercury in ambient air, ingestion of drinking water contaminated with mercury, and exposure to mercury through medical treatments can contribute to the exposure to this contaminant in animals and in humans, dietary intake is considered as the most important source of non accidental and non occupational exposures to mercury (ATSDR, 1999).

1.1. Chemistry

Mercury occurs in three valence states: elemental mercury (also known as metallic mercury, Hg^0), monovalent-mercurous (Hg_2^{++}), and the divalent mercuric (Hg^{++}); the Hg^0 and Hg^{++} being the most important in nature. Elemental mercury is the most stable form and does not react readily with oxygen, although thermodynamically favoured, or water (Cotton and Wilkinson, 1988). Generally, mercuric and mercurous mercury are thermally unstable and readily decompose to elemental mercury during heat treatment, exposure to light and reducing agents. Hg^0 is only slightly water-soluble (Table 1), and is more soluble in non-polar organic solvents than water. Hg^0 is relatively volatile and vapours of elemental mercury can occur at room temperature presenting a hazard if spillages occur.

The most common and abundant mineral of mercury is the red cinnabar (mercuric sulfide), HgS . HgS precipitating in for example sediments is black, metacinnabar. HgS is water insoluble and Hg^{++} has generally high affinity for sulfur and mercaptans; even elemental mercury reacts with elemental sulfur and hydrogen sulfide (but not mercaptans) (Nowak and Singer, 2000; Wilhelm *et al.*, 2006). Hg^{++} has affinity for Group VIb elements in the order: $\text{O} \ll \text{S} < \text{Se} \approx \text{Te}$, and the affinity of Hg^{++} decreases in the order $\text{RS}^- > \text{SH}^- > \text{OH}^- > \text{Cl}^-$ which is of general importance for speciation of Hg^{++} . Organic matter, especially humic substances, abundant in soil, water and sediments, forms very stable complexes with Hg^{++} which are relatively insensitive to pH (Jackson, 1998; Skyllberg *et al.* 2006). Mercuric chloride (HgCl_2) is a linear molecule in the solid state and exists almost entirely as discrete covalent and linear molecules in aqueous solutions and organic solvents (Greenwood and Earnshaw, 1997).

HgCl₂ is soluble in water, Table 1, but also in some organic solvents (Nowak and Singer, 2000).

Mercurous chloride (Hg₂Cl₂) contains the diatomic cation Hg₂⁺⁺ and is very unstable in most natural environments; it forms no stable aqueous complexes and disassociates spontaneously to elemental mercury and complexed Hg⁺⁺ in the presence of ligands that bind Hg⁺⁺ (Jackson, 1998) or at pH > 3-4 (Lindqvist *et al.*, 1991).

Methylmercury chloride and other halides of methylmercury, together with dimethylmercury are linear molecules like HgCl₂. As the Hg-C bond is highly covalent, organometallic Hg⁺⁺ compounds are resistant to oxidation and hydrolysis and are kinetically stable (but not thermodynamically) in water and O₂ (Jackson, 1998). Dimethylmercury is much more lipophilic than methylmercury and devoid of dipole moment with stable, largely covalent bonds that do not dissociate in water at pH > 5.6 (Fagerström and Jernelöv, 1972). Below pH 5, dimethylmercury is thermodynamically unstable in water and is spontaneously converted to methylmercury (Fagerström and Jernelöv, 1972; Jackson, 1998). Dimethylmercury is also very volatile, practically insoluble in water and with high Henry's law constant and therefore dimethylmercury, like Hg⁰, readily escapes into the atmosphere from water surfaces, whereas methylmercury, like HgCl₂, has a greater tendency to be retained in water (Jackson, 1998). The chemical affinities of methylmercury for ligands, including organic matter, is analogous to Hg⁺⁺ but the stability constants of methylmercury complexes with these ligands are consistently lower than for the corresponding Hg⁺⁺ complexes. Furthermore, unlike Hg⁺⁺, methylmercury easily and rapidly exchanges one thiol group for another, a property that has been suggested to explain why methylmercury spreads more easily through internal tissues of both plants and animals than inorganic Hg⁺⁺, which has a greater tendency to be retained at the point of entry (Jackson, 1998; Boudou *et al.*, 1991). Due to the complex speciation chemistry of mercury compounds in aquatic systems, apparent K_{ow}, water solubility, vapour pressure, and Henry's law constant are strongly affected by pH, salinity, concentration and nature of complexing ligands, temperature, ionic strength, and redox potential.

Table 1. Physical and chemical properties of major toxicologically relevant mercury compounds (adapted from ATSDR, 1999, except noted otherwise).

Chemical name	Elemental mercury ^a	Mercuric chloride	Mercurous chloride ^b	Methylmercury chloride ^c	Dimethyl mercury
Molecular formula	Hg ⁰	HgCl ₂	Hg ₂ Cl ₂	CH ₃ HgCl	CH ₃ HgCH ₃
CAS N°	7439-97-6	7487-94-7	10112-91-1	115-09-3	593-74-8
Oxidation state	0	+2	+1	+2	+2
Molecular weight	200.6	271.5	472.1	251.1	230.7
Water solubility, g/L	5.6×10 ⁻⁵ at 25°C	69 at 20°C	2.0×10 ⁻³ at 19°C	<0.1 at 21°C 5-6 at 25°C ^g	Practically insoluble, see text
Vapor pressure, Pa	0.27 at 25°C 0.18 at 20°C ^g	133 at 136.2°C 9×10 ⁻³ at 20°C ^g	≈10 ⁻⁵ at 25°C ^j	1.1 at 25°C 1.76 at 25°C ^g	7.8×10 ³ at 23.7°C ^d 8.3×10 ³ at 25°C ^g
Log K _{ow}	0.62 ^g	-0.215 ^e -0.30 ^g 0.52 ^h	No data	0.41 ^e 0.23 ^h	2.28
Henry's law constant, Pa m ³ /mol	729 at 20°C ^g	3.69×10 ⁻⁵ at 20°C ^g	No data	3.8×10 ⁻² at 15°C and pH 5.2 ^g	646 at 25°C ^g 340 at 0°C ⁱ

^aAlso known as metallic mercury

^bAlso known as calomel

^cMethylmercury chloride is used experimentally to investigate the effects of methylmercury

^dLong and Cattanchi, 1961; ^eHalbach, 1985; ^fGreenwood and Earnshaw, 1997; ^gSchroeder and Munthe 1998; ^hMason *et al.* 1995; ⁱSchlüter 2000; ^jLindqvist *et al.* 1991.

1.2. Production, uses, and environmental fate

1.2.1. Production

The terrestrial abundance of mercury is of the order of 50 µg/kg (range of 30-1000 µg/kg) (DeVito, 2005) and mainly found in the mercuriferous belt where most of principal mercury deposits are found (Schlüter, 2000).

The world production of mercury peaked in the early 1970s at about 10,000 tons annually. In 2000, the global primary production was about 2,000 tonnes/year with additional approximately 2,000 tonnes/year from secondary production (UNEP, 2002; RPA, 2002).

Mercury compounds used as pesticides are subject to the Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade, implemented in the Community by Regulation (EC) No. 304/2003¹⁰. This Regulation also bans the export of cosmetic soaps containing mercury, and requires notification of mercury compounds for all other uses. However, there are no Community or international restrictions on trading elemental mercury (EC, 2005).

1.2.2 Use

The current global mercury demand is around 3,600 tonnes per year. The main global uses are in batteries, gold mining, and the chlor-alkali industry, which together accounted for over 75% of the worldwide mercury consumption (EC, 2005). In 2003, the 15 EU Member States used around 300 tonnes annually (EC, 2005) as compared to estimated 448 tonnes per year in 1993 or 11.7% of the global usage (UNEP, 2002). Mercury has also been widely used in the production of dental amalgam.

Mercury compounds were widely used as pesticides and fungicides in agriculture since the beginning of the 20th century resulting in high concentrations of mercury in intensively cultivated soil. Various alkyl mercuric compounds were produced for use as disinfectants in agriculture but were banned or severely restricted in many countries around 1970. Mercury compounds are still in use for agricultural purposes in some countries, *e.g.* in Australia, Belarus, India, Benin, Burkina Faso, Ivory coast, Ghana, and Guinea (UNEP, 2002).

1.2.3 Environmental fate and levels

Atmosphere

Mercury exists in ambient air predominantly in gaseous form, *i.e.* 90-95% as monoatomic gas (Hg⁰) (Schroeder and Munthe, 1998). Small amounts of mercury are in the particulate phase (Lindqvist *et al.*, 1991) and minor quantities as methylmercury or up to 5% of total mercury in precipitation, usually around 1.5% (Downs *et al.*, 1998; Lindqvist *et al.*, 1991; Glass and Sorensen, 1999; Grigal, 2002). Dimethylmercury has also been found in air but it is expected to be very short-lived due to rapid oxidation with a half-life of only several hours (Niki *et al.*, 1983; Lin and Pehkonen, 1999).

The main natural sources of mercury to air are degassing of mercury from mineral deposits and aquatic and terrestrial systems, volcanic emissions, and forest fires. The total natural

¹⁰ Regulation (EC) No. 304/2003 of the European Parliament and of the Council of 28 January 2003 concerning the export and import of dangerous chemicals, OJ L 63, 6.3.2003.

emission was estimated to be about 2,500 tonnes annually in the late 20th century, where Europe accounts for 250-300 tonnes/year (Nriagu, 1989; Nriagu, 1990; Axenfeld *et al.*, 1991; Pacyna *et al.*, 2001).

The total global anthropogenic emission has been estimated to be about 2,000 tonnes in 1995-2000 where a decrease in emissions by about 60% in the last 20-30 years has been estimated (Pacyna *et al.*, 2006a; Pirrone *et al.* 1996; Lamborg *et al.* 2002; Nriagu, 1989; Nriagu and Pacyna, 1988). The main source is coal combustion accounting for two thirds of the global emission. Between 1990 and 2000, the emission rates have decreased most significantly in Europe and North America but an increase of more than 50% was observed in Asia of which half originated in China (Pacyna *et al.*, 2006a and 2006b). As regards Europe, countries in the central and eastern part generate the highest emissions (Pacyna *et al.*, 2006b).

Presently, the global average level of mercury in the atmosphere is 1.6 ng/m³ (Lamborg *et al.*, 2002). The total mercury levels in rain are usually in the range of 1-50 ng/L (Lindqvist *et al.*, 1991; Hall, 1995; Downs *et al.*, 1998), while results from unpolluted North Temperate areas indicate a volume weighted average of 5-15 ng/L (Grigal, 2002). The main form of mercury found in precipitation is Hg⁺⁺ following oxidation of elemental mercury by mainly ozone in the aqueous phase (Munthe *et al.*, 1991; Hall, 1995; Lin and Pehkonen, 1999). Several studies indicate a long-term decrease in levels of mercury in the atmosphere of Europe and North-America in the last 20-30 years (Iverfeldt *et al.*, 1995; Slemr and Schell, 1998; Kock *et al.*, 2005; Steffen *et al.*, 2005; Temme *et al.*, 2007; Wängberg *et al.* 2007).

Soil

Reflecting deposition from air, the dominant form of mercury in soil is Hg⁺⁺. Recent studies by Skylberg *et al.* (2006) show that inorganic mercury in soil is strongly complexed to organic matter. Methylmercury is typically present at 0.01-2% of the total mercury with most data <1% (Lindqvist *et al.*, 1991; Davis *et al.*, 1997; Grigal, 2003) with dimethylmercury levels at <1000 times the concentrations of methylmercury (Davis *et al.*, 1997). Hence, mercury has a long retention time in soils, and mercury accumulated may continue to be released to surface waters and other media for long periods of time, possibly hundreds or even thousands of years (UNEP, 2002; Hissler and Probst, 2006).

Volatilisation from soils is preceded by reduction of ionic mercury to elemental mercury (biotic and abiotic) (Zhang and Lindberg, 1999; Jackson, 1998) after which Hg⁰ is volatilised at rates dependent on temperature (Schlüter, 2000; Scholtz *et al.*, 2003), soil water content, pH, and clay and soil organic matter content (Ericksen *et al.*, 2006; Grigal, 2002; Zhang and Lindberg, 2002).

Agricultural soils, and the vegetation growing on them, usually contain very little mercury, although a considerable range of concentrations in soils has been reported. Archer and Hodgson (1987) suggested that a 'normal' range was 0.02 to 0.40 mg/kg; contents exceeding

these values should be considered contaminated from anthropogenic or other sources (Kabata-Pendias, 2001).

Urban soils contain higher and more variable levels of mercury than rural and agricultural soils, while soils close to natural or anthropogenic sources may contain very high levels (Schlüter, 2000; Tack *et al.*, 2005; Rodrigues *et al.*, 2006).

Vegetation

Uptake of mercury from soils by vascular plants is very limited with concentrations of mercury in plants being significantly lower than in soil where roots act as important adsorption sites and barriers for mercuric mercury transport (Grigal, 2002, 2003; Millhollen *et al.* 2006). In contrast, the atmosphere is almost the exclusive source of mercury in vegetation (Grigal, 2003; Ericksen *et al.*, 2003; Rea *et al.*, 2001; Millhollen *et al.*, 2006). Foliage not only receives mercury from air by dry deposition but also via uptake of gaseous Hg^0 (and gaseous Hg^{++} -compounds) (Grigal, 2002). The mercury accumulated in the leaves does not transport to other parts of trees or only to a very limited extent (Lindqvist *et al.*, 1991). The average ratio of methylmercury to total mercury in tree litterfall, predominantly foliage, is generally very similar to that in precipitation, indicating atmosphere as the main source (Grigal, 2002, 2003).

Total concentration of mercury in vegetation, excluding nonvascular plants, is generally less than 0.1 mg/kg dry weight in background areas (Lindqvist *et al.*, 1991). Reported foliar levels of trees differ widely depending on atmospheric concentrations and differences in uptake efficiencies.

Aquatic systems and sediments, methylation

Mercury is present in various physical and chemical forms in the natural aquatic environment. The main chemical species are complexes of the mercuric ion with various organic and inorganic ligands, elemental mercury, methylmercury and dimethylmercury.

Speciation of the Hg^{++} -ion in oxygenated water is largely dominated by organic complexes, and in freshwater, more than 90% of Hg^{++} is complexed by dissolved organic matter and most methylmercury as well (>70%) (Ullrich *et al.*, 2001). In anoxic waters, however, the speciation chemistry of Hg^{++} and methylmercury is governed by sulfide (Jackson, 1998).

Between 10 and 30% of dissolved mercury in oceans and lake water is elemental mercury (Ullrich *et al.*, 2001) and surface waters are usually supersaturated in Hg^0 with respect to the atmosphere, especially during summer (Gårdfeldt *et al.*, 2001; Anderson *et al.*, 2007). Hg^0 in aquatic systems derives from various biotic and abiotic reduction processes of Hg^{++} species.

Methylmercury concentrations of up to 10% of total mercury in lake water in Sweden have been reported (Lindqvist *et al.*, 1991), while dimethylmercury is normally not detected (Ullrich *et al.*, 2001). In ocean water, methylmercury usually accounts for between 10 and 40% of total mercury (Leermarkers *et al.*, 2001; Kotnik *et al.*, 2007; Horvat *et al.*, 2003;

Mason and Sullivan, 1999; Mason *et al.*, 1998). Methylmercury is formed by methylation of Hg^{++} -compounds by abiotic but mostly biotic processes, both in the water column and, most actively, in the sediments. The methylation process is not fully understood and a wide variety of factors may affect the rate of methylation and demethylation (Ullrich *et al.*, 2001).

Dimethylmercury is usually only found in deep ocean waters at very low levels, *e.g.* at <0.5% of total mercury in the Mediterranean Sea and only at depths below 20 to 40 m (Kotnik *et al.*, 2007; Horvat *et al.*, 2003). Dimethylmercury is predominantly found in some sediments, believed to be formed from methylmercury in the presence of sulfide (Quevauviller *et al.*, 1992; Baldi *et al.*, 1995; Weber *et al.*, 1998; Stein *et al.*, 1996).

Uncontaminated freshwaters generally contain <5 ng/L total mercury median values of 3.1 to 6.2 ng/L in 25 Swedish lakes were reported (Lindqvist *et al.*, 1991), although up to 10 or 20 ng/L can be found in humic lakes or rivers rich in particulate mercury (Ullrich *et al.*, 2001). Contaminated waters may, however, be in the $\mu\text{g/L}$ range (Ullrich *et al.*, 2001). Total mercury concentrations in the marine environment are much lower and range between 0.1 to 1 ng/L (Leermarkers *et al.*, 2001; Kotnik *et al.*, 2007; Horvat *et al.*, 2003; Mason and Sullivan, 1999; Mason *et al.*, 1998).

Since methylation of mercury occurs almost solely in aquatic systems, aquatic biota and fish eating birds and animals usually contain much higher levels of mercury than terrestrial animals. Additionally, the concentrations usually increase with trophic level and age. For example, Arctic zooplankton contains between 1 to 10 $\mu\text{g/kg}$ wet weight while top predators like beluga whale (toothed whale, *Delphinapterus leucas*), polar bears (*Ursus maritimus*) and ringed seals (*Phoca hispida*) may contain >10,000 $\mu\text{g/kg}$ in their livers (Dehn *et al.*, 2006). However, trophic status or age is not the only factors governing the mercury level. The highest levels of mercury in marine mammals are usually found in kidneys and livers. In muscle tissue, the main form of mercury is methylmercury, while the proportion of methylmercury - particularly in livers of many marine mammals and seabirds - decreases with increased total concentration of mercury indicating demethylation in these animals (Gaskin *et al.*, 1979; Falconer *et al.*, 1983; Chen *et al.*, 2002; Endo *et al.*, 2004; Thompson and Furness, 1989; Wagemann *et al.*, 1998, 2000).

1.3. Hazard assessment for humans

This chapter is not intended to be an exhaustive review of the voluminous literature published on health effects of mercury. Rather, the purpose is to present a brief survey of the available data regarding the three forms of mercury. Because organic mercury is the predominant form to which humans are exposed via food, the sections related to elemental and inorganic mercury only focus on major issues.

Mercury is highly toxic to most forms of life but its toxicity depends on its chemical form, and thus symptoms and signs are rather different after exposure to elemental mercury,

inorganic mercury compounds, or organic mercury compounds. Elemental mercury is relatively inert and not readily taken up by the gastrointestinal tract in vertebrates, but it is volatile and its vapour is toxic. Mercuric salts are also highly toxic, but of even greater concern is the ability of micro-organisms to methylate mercury and its salts to produce species, such as methylmercury (CH_3Hg^+) and dimethylmercury ($((\text{CH}_3)_2\text{Hg})$) (Rowland *et al.* 1980).

1.3.1. Elemental mercury

In animals, as in humans, effects on the nervous system appear to be the most sensitive toxicological endpoint observed following exposure to elemental mercury. Symptoms associated with elemental mercury-induced neurotoxicity include tremors, irritability, nervousness, excessive shyness, insomnia, neuromuscular changes, polyneuropathy, memory loss and performance deficits in test of cognitive function (US-EPA, 1997). At higher concentrations, adverse renal effects and pulmonary dysfunction may also be observed. However, the toxicity of elemental mercury is essentially due to the vapour, and, therefore, of limited concern in this opinion.

1.3.2. Inorganic mercury

The kidney appears to be the critical target organ for the effects of acute ingestion of inorganic mercury compounds, although there are several animal studies in which inorganic mercury-induced neurotoxicity has been reported.

Acute oral exposures of rats and mice to inorganic mercury at 2-5 mg/kg b.w. per day resulted in an increased kidney weight. Higher doses induced tubular necrosis (US-EPA, 1997). Males showed increased sensitivity, resulting in more severe histological changes than females (Fowler, 1972; NTP, 1993).

Long-term studies have also demonstrated histopathological effects affecting the tubules and glomeruli, including thickening of basement membranes and degeneration of tubular cells (Carmignani *et al.*, 1989; Jonker *et al.*, 1993; NTP, 1993). A no observed adverse effect level (NOAEL) for rat of 0.23 mg/kg b.w. per day has been identified for renal effects in a 26 week study (ATSDR, 1999). Autoimmune glomerular nephritis has been induced in genetically susceptible strains of rats and mice. When rodents are treated with mercuric chloride, they produce antibodies which attack the kidneys causing an autoimmune glomerulonephritis (NRC, 2000). Evidence exists that human exposure to inorganic mercury can trigger an autoimmune response. Tubbs *et al.* (1982) reported deposits of IgG and complement C3 were found in the glomeruli of two workers exposed to inorganic mercury.

Other commonly reported effects in rodents include signs of cardiovascular toxicity (e.g. increased blood pressure and changes in the contractility of the heart), irritation of the gastrointestinal mucosa, reproductive toxicity (e.g. changes in the estrous cycle and ovulation), and developmental toxicity (e.g. increased number of abnormal foetuses) (US-

EPA, 1997). Such effects were seen at doses of 0.3 mg/kg b.w. per day (cardiovascular toxicity, only one dose tested) and ≥ 2 mg/kg b.w. per day (other effects).

In a recent study, male mice were repeatedly orally dosed with mercuric chloride during the pre-mating and mating periods, whereas females were similarly exposed during pre-mating, mating, gestation and lactation periods (Khan *et al.*, 2004). The results showed that oral exposure to between 0.25 and 1 mg/kg b.w. per day of mercuric chloride produced adverse effects on reproductive performance of mice but without overt mercury toxicity in dams.

Mercuric chloride has produced some positive results for clastogenicity in a variety of *in vitro* and *in vivo* genotoxicity assays. Conflicting results regarding its mutagenic activity have been reported (WHO-IPCS, 2003)

DNA damage (single strand breaks) has been reported in rat and mouse fibroblasts as well as CHO cells and human cells. There are positive results for induction of chromosomal aberrations in mice exposed by gavage (Ghosh *et al.*, 1991) but contrasting data for chromosome aberrations and SCE induction in rodent and human cells *in vitro*. Mercuric chloride was not mutagenic in *Salmonella typhimurium* but it was positive for the induction of gene mutations in mouse lymphoma cells (NTP, 1993; IARC, 1997; US-EPA, 1995).

Studies in rats administered with mercuric chloride orally gave weakly positive results for dominant lethal mutation (Zasukhina *et al.*, 1983) and a slight reduction of the numbers of implants and living embryos in female mice administered by intraperitoneal injection (Suter, 1975; WHO-IPCS, 2003).

There is equivocal evidence of carcinogenicity of mercuric chloride in animals. Focal papillary hyperplasia and squamous cell papillomas of the forestomach, together with thyroid follicular adenomas and carcinomas, were observed in male rats gavaged with 3.7 mg mercuric chloride/kg b.w. for 2 years (NTP, 1993). In the same study, evidence for increased incidence of squamous cell forestomach papillomas in female rats and renal adenomas and carcinomas in male mice were observed. However, the forestomach tumours did not progress to malignancy and were thought to arise from the hyperplastic response of the tissue (US-EPA, 1997). The kidney tumours observed in mice occurred at doses that were also nephrotoxic, and would be expected to arise by a non-genotoxic mechanism (ATSDR, 1999). There are no data available on the carcinogenic effects of inorganic mercury in humans.

1.3.3. Organic mercury

Nearly all of the available toxicity studies for organic mercury compounds are for methylmercury. Toxic effects have been demonstrated in animal studies and observed in humans. Mitochondrial changes, induction of lipid peroxidation, microtubule disruption, and disrupted protein synthesis have all been proposed as possible mechanisms of methylmercury neurotoxicity (ATSDR, 1999; NRC, 2000).

The severity of the symptoms may depend on the concomitant presence of other environmental contaminants able to enhance the oxidative damage induced by organic mercury (Yoneda and Suzuki, 1997). It has been observed in experimental animals that the presence of dietary antioxidants (i.e. Vitamin E and selenium) could mitigate the toxic effects. The significance for humans is uncertain (Stohs and Bagchi, 1995).

1.3.3.1 *In vitro* and animal data

Oral exposure of laboratory animals to methylmercury levels >0.5 mg/kg b.w. resulted in damage to the kidneys, stomach and large intestine, changes in blood pressure and heart rate, as well as adverse effects on sperm, and male reproductive organs. In addition, several studies have reported an increase in embryonic lethality, decrease in foetus body weight and teratogenicity in rats (cleft palates, vertebral defects, histological abnormalities in the cerebellum, effects on lachrymal glands and ribs) (ATSDR, 1999).

Although there is emerging evidence that the cardiovascular and immune systems might also be sites of its toxicity, the critical organ for methylmercury adverse effects is the brain. Both the adult and foetal brains are susceptible to methylmercury toxicity. In adult rodents, the major clinical effects include motor disturbances, such as ataxia, tremors and paralysis, as well as signs of sensory dysfunction, such as impaired vision. The predominant neuropathological feature is degenerative changes in the cerebellum, which is likely to be the mechanism involved in many of the motor dysfunctions (US-EPA, 1997). The developing nervous system appears to be more sensitive. Animal studies provide evidence of damage to the nervous system from exposure to methylmercury during development, and these effects remain/continue to develop during aging, even after the exposure stops. Developmental neurotoxicity has been observed in offsprings of rats, mice and guinea pigs treated orally with levels of methylmercury <1 mg/kg b.w. per day during gestation, lactation and/or post weaning. Some studies suggest that cats and monkeys are more susceptible to the neurotoxic effects of organic mercury than rodents. Visual defects have been reported in monkeys (NRC, 2000).

Mutagenicity and genotoxicity of methylmercury have been investigated *in vitro* and *in vivo*. In reviews of WHO-IPCS (1990), NTP (1993), IARC (1997), US-EPA (1995), and NTP (2000), methylmercury was not found to be a weak mutagen, but appears to be capable of causing chromosomal damage and DNA strand breaks in a variety of systems including yeast, bacteria, fish cells, mammalian cells, human lymphocytes and brain cell lines. Tests for unscheduled DNA synthesis, sister chromatid exchange, chromosomal aberrations and dominant lethal mutations in mammals *in vivo* have given conflicting results. Tests for clastogenicity in fish and amphibians have provided more convincingly positive results. Strain-specific differences exist with respect to the ability of methylmercury to produce dominant lethal effects in mice (Suter *et al.*, 1975). Nondisjunction and sex-linked recessive lethal mutations were reported in *Drosophila melanogaster* treated with methylmercury in the diet (Ramel, 1972). There are data showing induction of changes in chromosome number in

oocytes of Syrian hamsters treated by i.p. with methylmercuric chloride (Mailhes, 1983). The doses of methylmercury chloride that induced sister chromatid exchange in cultured human lymphocytes were 5-25 times lower than those needed of mercuric chloride, whereas 5-10 times lower doses of methylmercury chloride than mercuric chloride were required to induce polyploidy (NTP, 1993; IARC, 1997; US-EPA, 1995). In summary, these data indicate that methylmercury is clastogenic but not a potent mutagen.

Data from animal studies show some evidence of carcinogenicity in two strains of mice but studies in rats are negative. In ICR and B6C3F1 mice exposed orally to methylmercuric chloride, only males were observed to have an increased incidence of renal adenomas, adenocarcinomas, and carcinomas. Renal epithelial cell hyperplasia and tumours, however, were observed only in the presence of profound nephrotoxicity suggesting that the tumours may be a consequence of reparative changes to the damaged kidneys. No increase in tumor incidence was observed in studies conducted in rat and cat. Tumours were observed at a single site, in a single species and sex. Therefore they are considered to provide limited evidence of carcinogenicity (US-EPA, 1997; NRC, 2000).

1.3.3.2 Human data

Accidental methylmercury poisoning in humans has been reported on a number of occasions. From the methylmercury poisoning episodes in Japan (Minamata Bay and Niigata, 1956-1965) and Iraq (1956 and 1959-1960) it appeared that the most severe effects take place in the development of the brain and nervous system of the foetus. The reports on the Minamata outbreak described only slight symptoms in the mothers whose children had been exposed *in utero*. These children had cerebral palsy and/or microcephaly, and it was concluded that the foetus was more sensitive to the effects of methylmercury than adults (WHO-IPCS, 1976). Further analysis of the Japanese and Iraqi data revealed additional information on the effects of prenatal methylmercury exposure, such as the limitation of the growth of the foetal brain and the inhibition of the migration of neurones from the embryological generation layer to the final destination in the cortex. Clinical examination revealed behavioural changes and reduced cognitive and motor ability in children exposed *in utero*.

The primary human exposure to methylmercury is from fish consumption. Research efforts have therefore focused on individuals consuming large amounts of seafood with the aim to determine if chronic exposure from this source could present a health risk. A series of large epidemiological studies have provided evidence that methylmercury present in pregnant women's diets appears to have subtle, persistent effects on the children's mental development as observed at about the start of the school age (NRC, 2000).

In 1989-1990, a cohort of 779 children in a fish-eating population of the Seychelles Islands was enrolled to study the developmental effects of prenatal methylmercury exposure (Davidson *et al.*, 1998). The cohort was prenatally exposed to methylmercury from maternal fish consumption, and the children started consuming fish products at about 1 year of age.

Prenatal exposure was measured in maternal hair and recent postnatal exposure in the child's hair. The cohort was examined six times over 11 years using an extensive battery of age-related developmental tests. Mean maternal hair mercury concentration was 6.8 µg/g hair (range 3-26.7 µg/g hair)¹¹. Analyses of a large number of developmental outcomes showed no convincing evidence for an association between prenatal exposure and child development in this fish-eating population (Myers *et al.*, 2003). More recent analyses however have suggested that latent or delayed adverse effects might be emerging at maternal exposure above 10-12 µg/g (measured in maternal hair) as the children mature. This suggests that the association between prenatal exposure and child development may be more complex than originally believed (Davidson *et al.*, 2006). A subsequent study of another Seychelles cohort showed that a negative mercury effect was present when the neurodevelopmental outcomes were adjusted for the positive effects of n-3 fatty acids (Strain *et al.*, 2007).

In 1986-1987, a cohort of 1,022 births was studied in the Faroe Islands, where increased methylmercury exposure occurs from traditional seafood diets that include pilot whale meat. Cohort members underwent detailed neurobehavioral examination, and blood and hair samples obtained from the participants were analysed for mercury. The neuropsychological test battery was designed for assessing motor speed, visuospatial function, attention, language, and verbal memory. Median maternal hair mercury concentration was 4.5 µg/g hair (range 0.17-39.1 µg/g hair). At seven years of age, clear dose-response relationships were observed for deficits in attention, language, and memory. An increase in blood pressure was also associated with the prenatal exposure level (Sørensen *et al.*, 1999). At the age of 14 years, methylmercury exposure was significantly associated with deficits in tests of motor, attention, and verbal ability. Postnatal methylmercury exposure had no discernible effects (Debes *et al.*, 2006), but the current exposure at age 14 years was associated with an increased latency for peak V on the brainstem auditory evoked potentials (Murata *et al.*, 2004). These findings are similar to those obtained for the age of 7 years and an analysis of the test score difference between results at 7 and 14 years suggested that mercury-associated deficits had not changed between the two examinations. The most recent report from this cohort showed that, when adjusting for the beneficial effects of maternal fish intake during pregnancy, the mercury effects tended to increase, with the greatest impact on mercury-associated deficits on motor function (Budtz-Jørgensen *et al.*, 2007).

A smaller prospective study in Boston showed that visual recognition memory in children aged 6 months decreased at increasing maternal hair-mercury concentrations, but this association was only statistically significant after adjustment for maternal fish consumption during pregnancy (Oken *et al.*, 2005). All these observational studies confirmed that the

¹¹ A daily average methylmercury intake of 0.1 µg/kg b.w. per day by an adult woman is estimated to result in hair mercury concentrations of about 1 µg/g (NRC, 2000). According to research at the Center for Air Toxic Metals (CATM) there is linear relationship between intake and concentrations of methylmercury in hair <http://www.undeerc.org/catm/pdf/area4/MercuryMetabolism2004.pdf>

developing foetus is the most sensitive sub-population and that nervous system domains involving motor function, attention, verbal learning and memory can be affected by methylmercury exposure. Overall, the published evidence suggests that mercury toxicity may in some cases be hidden by the beneficial effects of nutrients from fish.

1.4. Evaluations and classifications

JECFA re-evaluated the PTWI for methylmercury and lowered it from 3.2 to 1.6 µg/kg b.w. per week, based on two epidemiology studies (see above, chap. 1.3.3.2.) that investigated the relationship between maternal exposure to mercury and impaired neurodevelopment in their children (FAO/WHO, 2003).

In a previous evaluation, the NRC (2000) used benchmark dose level from the Faroes study (12 µg mercury/g maternal hair) and used a composite uncertainty factor of 10, to take into account interindividual variability and incompleteness of the data base, to derive an exposure limit of 0.1 µg/kg b.w. per day or 0.7 µg/kg b.w. per week.

An International Programme on Chemical Safety (IPCS) Working Group (WHO-IPCS, 2003) recommended a TDI of 2 µg/kg b.w. for inorganic mercury, based on the NOAEL of 0.23 mg/kg b.w. per day for kidney effects in a 26-week study in rats (NTP, 1993) and applying an uncertainty factor of 100 (for inter- and intra-species variation) after adjusting for 5 days per week dosing. A similar TDI was obtained by applying an uncertainty factor of 1000 (an additional uncertainty factor of 10 for adjustment from a lowest observed adverse effect level (LOAEL) to a NOAEL) to the LOAEL for renal effects of 1.9 mg/kg b.w. per day in a 2-year study in rats (NTP, 1993).

Mercuric chloride was classified by IARC in group 3 (not classifiable as carcinogenic to humans), based on limited evidence in experimental animals, and by US-EPA in group C (possible human carcinogen), based on the absence of data in humans and limited evidence of carcinogenicity in rats and mice. Methylmercury was classified by US-EPA in group C and by IARC in group 2B (possibly carcinogenic to humans) (IARC, 1993; US-EPA, 1995).

The available human data are inconclusive regarding the carcinogenicity of methylmercury in humans exposed by the oral route (US-EPA, 1997).

2. Methods of analyses

No analytical methods are prescribed by the European Commission for the determination of mercury in animal feed.

2.1. Determination of total mercury

Most data regarding mercury in feed relate to total mercury. Total mercury is most frequently analysed by cold vapour atomic absorption spectrometry (CV-AAS) after acidic digestion of the biological samples as described by Hatch and Ott (1968). The sensitivity is about 1 ng mercury (corresponding to a limit of quantification (LOQ) of less than about 0.030 mg/kg dry weight in compound feedingstuffs and biological samples) where further sensitivity enhancement may be obtained by amalgamation. However, sensitivity enhancement is usually not necessary for feeds. A further enhancement of sensitivity by two orders of magnitude and better selectivity may be obtained by cold vapour atomic fluorescence (CV-AFS) instead of atomic absorption (Sánchez Uria and Sanz-Medel, 1998).

The main advantage of the cold vapour technique is the separation of the analyte from the potentially interfering sample matrix. The most frequently occurring interference in CV-AAS is that of nitrites and nitric oxides reducing the signal of mercury (Jones, 1997; Nunes *et al.*, 2005) requiring either stripping the sample digest with inert gas or treating it with reducing agents. Samples rich in iodine, like kelp, may require removal or sequestering of iodine to prevent it from interfering with the analysis.

Another technique, offering somewhat better sensitivity than CV-AAS (by a factor of about 3) and greater selectivity, is direct analysis of the sample digest by inductively coupled plasma mass spectrometry (ICP-MS) (Krata and Bulska, 2005; Palmer *et al.*, 2006), a technique that is increasingly being used. Recently, an interlaboratory study was reported by the Nordic Committee on Food Analysis (NMKL) where ICP-MS was used for total mercury in foodstuffs after pressure digestion of the samples in nitric acid (Julshamn *et al.*, 2007). However, it has been shown that nitric acid may suppress the signal of mercury during analysis by ICP-MS (Quevauviller *et al.*, 1993; Jian *et al.*, 2000; Krata and Bulska, 2005). The method gave very satisfactory results for total mercury down to 40 µg/kg dry weight, while the LOQ was at 10 µg/kg dry weight.

CV-AAS and CV-AFS and increasingly ICP-MS have been used for a wide variety of organic and inorganic samples with good results although some modifications or care may be required for certain types of samples. Since maximum levels of the current EU-legislation (see Chapter 3) are well above the limits of detection (LODs) and LOQs of these techniques, the data obtained must be considered as satisfactory. However, participation in proficiency testing programmes and intercomparison exercises of appropriate sample matrices is highly recommended for laboratories producing results for mercury in feed materials as an integral part of their quality control schemes.

2.2. Determination of organic mercury compounds

Gas chromatography (GC) with both packed and capillary columns has been the most widely used technique for the separation of mercury species while high performance liquid

chromatography (HPLC) is increasingly being applied (Sánchez Uria and Sanz-Medel, 1998; Carro and Mejuto, 2000; Harrington, 2000). The detection of mercury species by GC has mainly been carried out by electron capture detector (ECD) which is, however, not specific to mercury. Cold vapour atomic absorption spectrometry (CV-AAS) and cold vapour atomic fluorescence spectrometry (CV-AFS) are therefore more appropriate for detection, together with microwave induced plasma atomic emission spectrometry (MIP-AES), inductively coupled plasma atomic emission spectrometry (ICP-AES), mass spectrometry, and increasingly ICP-MS (Sánchez Uria and Sanz-Medel, 1998; Carro and Mejuto, 2000; Willoud *et al.*, 2004).

Extraction procedures vary but most are based on the initial work of Westöö (1966, 1967, 1968) where the sample is treated with hydrochloric acid to release methylmercury from sulfhydryl groups and sodium chloride to enable its recovery into the organic phase (benzene or toluene). Inorganic mercury remains in the aqueous phase. The organic phase is further back extracted to aqueous cysteine solutions to purify the extract. Modifications have included other organic phases, thiosulfate instead of cysteine, application of copper(II) to release methylmercury from proteins, use of bromide or chelating agents to improve extraction, further purification by back-extraction into organic phase, and defatting the samples prior to digestion to prevent emulsifications (Carro and Mejuto, 2000; Sánchez Uria and Sanz-Medel, 1998).

Some workers have analysed the extracted mercury species as for total mercury, denoting it as organic mercury, and the aqueous phase of the sample for Hg^{++} . Other workers differentiate between inorganic and organic mercury compounds by selective reduction where the samples are treated with stannous chloride, reducing Hg^{++} to Hg^0 and leaving Hg-C bonds intact. After complete purging of Hg^0 it is analysed by CV-AAS or CV-AFS, while the remaining sample, assumed to contain only organic mercury, is analysed as for total mercury.

Instead of extraction, biological samples treated with sulfuric acid and iodoacetic acid have been subjected to steam distillation where volatile methylmercuryiodide is distilled off. The distillate is usually derivatised with sodium tetraethylborate (forming methylethylmercury) to improve sensitivity and performance of the GC-analysis. However, the steam distillation may produce methylmercury from Hg^{++} as an artefact (Bloom *et al.*, 1997).

Alkaline digestions, usually in the presence of cysteine to avoid losses of methylmercury hydroxides and to stabilise the Hg-C bond, with subsequent acidification and extraction of methylmercury as above, have also been used. The hydroxide releases methylmercury quantitatively from proteins. This procedure is often followed by derivatisation with sodium tetraethylborate prior to GC-analysis. However, in the presence of high levels of inorganic mercury, Hg^{++} may be converted to methylmercury during derivatisation (Delgado *et al.* 2007).

By using HPLC instead of GC for separation, the derivatisation procedure may be omitted and the cleanup becomes less critical. Digestion may be carried out in aqueous cysteine hydrochloride directly at 60°C and the solution analysed for methylmercury and Hg⁺⁺ with reversed-phase HPLC after simple filtration (Hight and Cheng, 2006; Chiou *et al.*, 2001; Percy *et al.*, 2007). Precision and accuracy in single-laboratory validations have been shown to be satisfactory, but validation by way of intercomparison and/or interlaboratory studies is required. Although these methods appear promising, they have only recently been introduced and are therefore currently not in widespread use. Detection by CV-AAS, CV-AFS, MS or ICP-MS methods are all suitable as regards sensitivity for samples of feeds. The advantage of MS and ICP-MS are their multi-element and multi-isotope capabilities, whereas CV-AAS and CV-AFS have the advantage of being comparatively low cost and simple operations (Armstrong *et al.*, 1999; Cai *et al.*, 2000; Krata and Bulska, 2005).

Once in solution, methylmercury may decompose when exposed to light. pH, ionic strength, acidity, temperature, type of containers etc. may also affect the stability (Yu and Yan, 2003; Hight and Cheng, 2006; Delgado *et al.*, 2007; Devai *et al.*, 2001).

Dimethylmercury is, for several reasons, not reliably determined by most of the methods above (Puk and Weber, 1994; Leermakers *et al.*, 2005).

3. Statutory limits

Mercury is listed in the Annex to Directive No (EC) 2002/32 on undesirable substances in animal feed¹². The maximum levels (MLs) are shown in Table 2 below.

Table 2. EU legislation on total mercury in feed materials.

Product intended for animal feed	Maximum content in mg/kg relative to a feedingstuff with a moisture content of 12%
Feed materials with the exception of: - feedingstuffs produced by the processing of fish or other marine animals - calcium carbonate	0.1 mg/kg 0.5 mg/kg 0.3 mg/kg
Complete feedingstuffs with the exception of: - complete feedingstuffs for dogs and cats	0.1 mg/kg 0.4 mg/kg
Complementary feedingstuffs except - complementary feedingstuffs for dogs and cats (Article 6 of 2002/32)	0.2 mg/kg

¹² OJ L140, 30.5.2002, p. 10

No information on national or international standards for mercury in feed outside the EU has been identified.

4. Occurrence in feed and animal dietary exposure

As described above, mercury exists in elemental, organic and inorganic forms. The determination of mercury concentrations in feed materials are undertaken by Member States as part of routine surveillance programmes. Because legislation specifies MLs of total mercury, differentiation into the different forms of mercury are not normally undertaken. Therefore, data provided by Member States and presented in this section refer to total mercury.

4.1. Occurrence in feeding materials

SCAN (EC, 2003) concluded that mercury uptake by plants from soil is low, and that levels of mercury in plant material is independent of the soil mercury concentration. Studies by Ericksen *et al.* (2003) confirmed that nearly all of the mercury found in the foliage originated from the atmosphere. In general, therefore, it appears that mercury levels in plants are more likely to be related to atmospheric levels than soil concentrations. For non-plant feed materials, SCAN identified fishmeal to be the most common source of mercury for farmed animals under normal farming conditions .

In order to estimate levels of exposure to mercury by farmed livestock and fish within Europe, European countries were invited to provide information on levels of mercury in feedingstuffs acquired as part of routine surveillance programmes. Data on levels of mercury in 3,253 samples of feed were received from 13 European countries (Table 3) for the period 2002-2006.

Table 3. Number of samples of animal feedingstuffs analysed for mercury in the period 2002-2005 as reported by Member States, Iceland and Norway.

Country	Number of samples analysed in year						% of samples received
	2002	2003	2004	2005	2006	Total	
Belgium			25	183	75	283	8.7
Cyprus			3		2	5	<1
Czech Republic				69	184	253	7.8
Denmark	21	10	51	52	55	189	5.8
Finland				18	75	93	2.9
France		82	84	139		305	9.4
Hungary			265	270	257	792	24.3
Iceland	21	36	8			65	2.0
Ireland	101	99	67	88		355	10.9
Norway	33	58	68	60	82	301	9.3
Slovak Republic					451	451	13.9
Slovenia		4	13	28	8	53	1.6
Spain					108	108	3.3
Total	176	289	584	907	1,297	3,253	

Qualitative information (compliant/non-compliant) was provided by the UK, but the data could not be included in the above analysis. FEDIAF, The European Pet Food Industry Federation, also provided data on concentrations of mercury in samples of pet food; these data are not included in the table above but are discussed in the section on pet food (below).

There was a significant increase in the number of samples analysed for mercury over the period 2002-2006. However, the data are not evenly distributed across the EU; almost 25% originated from Hungary, while a further 20% came from the Nordic countries. In contrast, relatively few data originated from southern European/Mediterranean region.

Data were provided for a wide range of feed materials. Table 4 provides a summary of the total levels of mercury (average, median and MLs) reported in each of the main commodity groups. For many of the samples analysed, levels of mercury were reported as being less than the LOD or of LOQ for the particular method of analysis employed. In addition to the absolute values reported, the European countries were requested to provide information on the LOD or LOQ; where concentrations were reported as <LOD or <LOQ, these were considered equal to LOD/2 or LOQ/2 respectively.

Table 4 also provides information on the number of samples (total and as a percentage) that exceeded the ML for each particular commodity group.

Table 4. Levels of mercury reported in feedingstuffs (moisture content of 12%), categorised by feed commodity, and the number and percentage of samples analysed in each category in the period 2002-2006 that exceeded the maximum levels (MLs).

Food commodity	No. of samples	Mercury concentration (mg/kg)			ML (mg/kg)	Samples exceeding ML	
		Average	Median	Max		n	%
Additives and premixtures	290	0.03	0.01	1.3	0.1	5	1.7
Complete feed	539	0.03	0.04	0.12	0.1	11	2.0
Forage crops	368	0.02	0.002	0.19	0.1	2	0.5
Minerals and mineral feedingstuff	530	0.02	0.005	0.59	0.1	7	1.3
Other feedingstuffs	319	0.01	0.005	0.13	0.1	2	0.6
Unspecified feeds and raw materials	238	0.03	0.01	0.22	0.1	26	10.9
Complementary feed	228	0.02	0.01	0.34	0.2	1	0.4
Calcium carbonate	42	0.01	0.01	0.03	0.3	0	0
Complete feed for dogs and cats	126	0.02	0.01	0.18	0.4	0	0
Fish meal	193	0.10	0.10	0.26	0.5	0	0
Fish and bone meal	13	0.15	0.15	0.22	0.5	0	0
Fish oil	63	0.03	0.03	0.21	0.5	0	0
Fish silage	23	0.06	0.05	0.17	0.5	0	0
Complementary feed for fish	1	0.01	0.01	0.01	0.2	0	0
Complete feedingstuff for fish	280	0.06	0.05	0.4	0.1	23	8.2
Total	3253	0.03	0.01	1.3		86	2.6

Although the total number of samples that exceeded the ML tended to increase over time (detailed data not reported here), this was a reflection of the greater number of samples

analysed. Over the period 2002-2006 there was no apparent trend, with the percentage of samples that exceeded the ML ranging from 1.3 (2006) to 4.7 (2005). For the period as a whole, 2.6% of all samples exceeded the maximum level.

Average values for complete feedingstuffs for fish in each of the years 2002-2006 were 0.044, 0.061, 0.065, 0.062 and 0.051 mg/kg, respectively, suggesting that there was no trend in this particular category.

Additives and premixtures

Almost half (42%) of all samples in this category were described as premixtures. Although some authorities specified the livestock category for which the premixture was intended, the majority did not, and therefore it has not been possible to identify livestock species that have been exposed to the highest concentrations.

Complete feedingstuffs other than for pets and fish

Of the 11 complete feeds that exceeded the ML (0.1 mg/kg) for this category, two were for mink while the target species for others were unspecified. For 366 of the feeds in this category (68%), it was possible to identify the target species, and the data for the main species are summarised in Table 5.

Table 5. Average and maximum concentrations of total mercury in complete feedingstuffs for terrestrial animal categories.

Target species	Number of samples	Average (mg/kg)	Median (mg/kg)	Maximum (mg/kg)
Pigs	123	0.032	0.050	0.050
Poultry	96	0.039	0.050	0.10
Ruminants ¹³	56	0.012	0.004	0.10
Horses	9	0.022	0.010	0.10
Mink	39	0.053	0.054	0.12
Rabbits	18	0.031	0.050	0.10
Rodents	25	0.050	0.050	0.10

Forage crops

Although data on 368 samples of forage crops were provided, they generally lacked information with which to further classify them, with the majority being variously described as 'green feed', 'pasture crops', 'plant raw material' etc. Only two samples exceeded the ML (0.1 mg/kg), with the highest concentration (0.19 mg/kg) in a sample described as 'various

¹³ Includes both complete and complementary feedingstuffs

fodder'. For 28 samples of alfalfa (lucerne), the average and maximum mercury concentrations were 0.005 and 0.02 mg/kg, respectively, while for forage maize (n=42) they were 0.007 and 0.05 mg/kg, respectively. In general the concentrations of mercury in forages appear to be low, and similar to the values reported by SCAN (EC, 2003). Since the diet of many ruminants consists almost entirely of forages, it seems reasonable to assume that their exposure to mercury is low.

Minerals and mineral feedingstuff

Data on 530 samples of minerals were provided. In only seven samples did mercury concentrations exceed the ML (0.1 mg/kg), with the highest concentration of 0.59 mg mercury/kg in a sample of manganese oxide. Very few authorities provided information on the type of mineral, feedingstuff or target livestock species, and so it has not been possible to establish risks for particular livestock types resulting from the consumption of these feedingstuffs.

Other feedingstuffs

This category consisted of named feed materials, e.g. wheat, rapeseed meal, vegetable oil etc. For the major feed materials, the average, median, and maximum concentrations are given in Table 6. Overall, mercury concentrations were low, with only two samples in this category exceeding the ML (0.1 mg/kg). The highest concentrations of mercury were reported in a sample of distillers dried grain (0.13 mg/kg) and a sample of shrimp meal (0.12 mg/kg).

Table 6. The average, median and maximum total mercury concentrations (mg/kg) in a number of common feed materials.

Feedingstuffs	Number of samples	Average mercury concentration (mg/kg)	Median mercury concentration (mg/kg)	Maximum mg/kg
Barley	29	0.006	0.001	0.078
Wheat	48	0.003	0.001	0.030
Oil seed rape	42	0.007	0.002	0.100
Sunflower meal	13	0.003	0.001	0.010
Soya bean meal	13	0.022	0.011	0.050
Distillers dried grains	8	0.047	0.020	0.130
Maize gluten feed	15	0.026	0.015	0.100
Vegetable oils	16	0.021	0.020	0.050

Unspecified feeds and raw materials

The greatest number of data (238) and highest proportion of non-compliant samples (10.9%) was in this category. The returns from the European countries described these as ‘raw materials’, ‘animal feed’ etc., and therefore it is not possible to further characterise the specific feeds or livestock that might be at risk from consuming them.

Complementary feed

A complementary feed will not, on its own, provide all the nutrients required on a daily basis but is intended to be fed with other feed materials. For this reason, the ML can be higher than for complete feedingstuffs. The ML for mercury in complementary feed is 0.2 mg/kg. In one sample (target species unspecified) analysed in 2005, the concentration of mercury was 0.34 mg/kg; in the remaining 227 samples levels were all <0.2 mg/kg. For 160 samples, the target species was indicated. The average concentrations of mercury in complementary feeds for pigs (n=44), poultry (n=23) and cattle (n=80) were 0.006, 0.007 and 0.011 mg/kg, respectively.

Calcium carbonate

The average and median mercury concentrations of the 42 samples of calcium carbonate analysed in 2002-2006 were 0.01 and 0.005 mg/kg, respectively, and in none of the samples did mercury concentrations exceed the ML of 0.3 mg/kg

Complete feed for dogs and cats

Mercury concentrations in 126 samples of pet food were provided. The highest reported concentration in this category was 0.18 mg/kg in a sample of compound feedingstuff for cats. This was well below the maximum permitted level of 0.4 mg/kg in this category. The majority of samples were simply designated “Complete feed – dogs and cats” and so it was not possible to calculate average and median values for each species. Furthermore, since 80% of the samples analysed originated from one country (Hungary), it is not clear to what extent these results are representative of the EU as a whole. In addition to the information obtained from the European feed authorities, FEDIAF also provided data on 78 samples of canned pet food and 119 of dried pet food analysed in the period 2003-2006. The average (and maximum) concentrations were 0.021 (0.026) and 0.033 (0.110) mg/kg for the canned and dry pet foods, respectively (12% moisture basis).

Feed for fur-producing animals

Denmark provided data on 25 samples of complete feedingstuffs for mink. The average, median and maximum concentration of these samples was 0.053, 0.054 and 0.12 mg/kg, respectively.

Fishmeal

As discussed elsewhere in this report, mercury accumulates in the food chain, particularly in fish, and in recognition of this the ML for fishmeal is higher (0.5 mg/kg) than in other feed materials. The average and median concentrations in the samples analysed between 2002 and

2006 were 0.10 mg/kg, with the highest concentration reported of 0.26 mg/kg. Information on samples in this category were provided, predominantly (but not exclusively) by the Nordic countries (Denmark, Iceland and Norway).

Fish and fish bone meal

Information on mercury concentrations in 13 samples of fish and bone meal were provided by Iceland. The average and highest concentrations were 0.15 and 0.22 mg/kg, respectively, which were well below the maximum permitted concentration (0.5 mg/kg).

Fish oil

The highest reported concentration of mercury in fish oil was 0.21 mg/kg, although the average for the 63 samples was 0.03 mg/kg,

Fish silage

Fish residues and unwanted fish may be ensiled – rather than dried – for storage before being used as livestock feed. Data for fish silage analysed in this period were provided by the Danish (n=7) and Norwegian (n=16) Food Authorities. The highest concentration reported was 0.17 mg/kg, with an average concentration for all samples of 0.06.

Complete feedingstuff for fish

Fishmeal constitutes the major ingredient in most complete feeds for fish, and therefore concentrations of mercury in this category tend to be higher than in complete feeds for land animals and birds. Information on 280 samples of complete feedingstuffs for fish was provided. The average and median concentrations were 0.06 and 0.05 mg/kg, respectively, which compares with the ML of 0.1 mg/kg approximately 8% of fish feeds exceeded the ML. Twenty three samples exceeded the ML, with the highest concentration being 0.4 mg/kg. The highest concentration of mercury found in fish feed for marine larvae which typically contain high inclusion levels of fishmeal.

Few data were provided on the proportion of methylmercury in fish feed. In Norway, the average concentration of methylmercury in fish feed analysed in 2004 was 0.044 mg/kg (and ranged between 0.03-0.06 mg/kg, n=49) representing approximately 81% of the total mercury. In 2005 methylmercury represented approximately 86% of the total mercury in fish feed (n=19). The average concentrations of total mercury and methylmercury in fish feed in 2006 were 0.06 mg/kg and 0.05 mg/kg respectively (concentrations of total mercury and methylmercury ranged between 0.02-0.18 mg/kg (n=49) and 0.03-0.13 mg/kg (n=17) respectively), the proportion of methylmercury representing approximately 89% of total mercury (Måge *et al.*, 2005, 2006, 2007).

Protein hydrolysates from feathers

Hair and feathers can accumulate a large amount of methylmercury. The practice of recycling poultry (chicken, turkey) feathers as feather meal (as protein hydrolysate) to feed back to farmed animals could represent an additional source of methylmercury contamination (Plummer and Bartlett, 1975; Soares *et al.*, 1973). According to Regulation No (EU)

1774/2002 and Regulation No (EU) 1292/2005, only feathers originating from animals that are slaughtered in a slaughterhouse, after undergoing ante-mortem inspection, can be used to produce protein hydrolysate with a molecular weight of <10,000 daltons. Protein hydrolysate from feather meal can be used in animal feeding with the following indicative figures for maximum amounts used in complete feeds: pigs 3%, chickens for fattening 5%, ruminants 6%, fish 15% (Animal Feed Resources Information System¹⁴).

Water

In addition to mercury in feed materials, livestock and poultry may also be exposed to mercury in drinking water. Although no information was provided by Member States on mercury in drinking water for livestock, IPCS (WHO-IPCS, 1990) suggest that the concentration range for mercury in drinking water is the same as in rain, with an average of approximately 25 ng/L. Therefore, water does not make a significant contribution to the exposure of livestock except in highly polluted areas.

Summary

A substantial number of feed materials have been analysed for total mercury in recent years, and for the large majority the concentrations of mercury were below the MLs specified in feedingstuffs legislation. Less than 3% exceeded the MLs, including additives and premixtures. In the category of feedingstuffs produced by the processing of fish or other marine animals, which normally contain higher mercury concentrations, no sample exceeded the maximum level. However, approximately 8% of the complete feedingstuffs for fish exceeded the ML.

For a large proportion of all the samples for which data were provided by European countries, however, there was insufficient information to allow the data to be usefully used. For example, 10% of all samples were categorised as “Other feedingstuffs” without any meaningful description. Even in well defined categories, information was frequently lacking; for the 288 sample described as “Complementary feeds”, for example, only 158 included information on the target species. Given the considerable amount of effort associated with collecting and analysing the samples, it is unfortunate that a full description of the sample is not available.

4.2 Animal exposure

Land animals and poultry

The extent to which land animals and poultry are exposed to mercury is a function of the concentration in feed and the amount of feed consumed. In an attempt to estimate levels of exposure by different categories of livestock, a number of assumptions have had to be made

¹⁴ <http://www.fao.org/ag/AGA/AGAP/FRG/afris/>

regarding the level of feed intake and concentrations in different dietary ingredients. Even within livestock categories, the amount of feed consumed can vary considerably as a result of a wide range of animal, environmental and management factors. In the estimates of exposure, the assumptions made for each category have been given in Annex Tables 2 and 3. Similarly, concentrations of mercury in feedingstuffs vary widely; in the calculations that follow both the average and maximum concentrations in feedingstuffs described above have been used provide an indication of 'typical' and 'worst case' levels of exposure. This method of estimating exposure by land animals and poultry is similar to that used for other opinions of the CONTAM panel.

Ruminants

Ruminant rations consist predominantly of forages, supplemented where necessary by concentrate feeds e.g. cereals, oilseed meals and minerals, vitamins etc. Concentrations of mercury in forages vary considerably. Flachovsky (2006) reported values that range from 0.005-0.03, while data provided by Member States for this report had a range of 0.0002 to 0.19 mg/kg. In estimating likely exposure from forages (Table 7), the average and maximum levels from Table 4 have been used. The non-forage component of the diet consists of feedingstuffs within the categories other feedingstuffs, unspecified feeds and raw materials or complementary feeds, which may be fed as individual feeds separately, given as a loose mix of ingredients – either separately or mixed with the forage – or provided in a compound feed. Where concentrate feeds are fed separately or are mixed on-farm, the choice of feed, and the proportions used varies considerably throughout the EU, making it difficult to describe a 'typical' ration. On many farms, however, forages are supplemented with complete feedingstuffs – usually as compound feeds – and therefore the data presented in Table 5 have been used to estimate the exposure to mercury from the concentrate component of the ration. The animal and feed intake data used to calculate these exposures are given in Annex Table A2.

For comparison, levels of exposure by livestock consuming feeds with the maximum permitted concentrations (see Table 2) are also given. Since the highest mercury concentration in forages (0.19 mg/kg) exceeded the ML (0.1 mg/kg), estimates of mercury intake by ruminants consuming forages with this concentration exceed the regulatory maximum. However, the likelihood of this occurring in practice is extremely small; only two (of 368) samples exceed the ML, while the average for all samples was 0.02 mg/kg.

Table 7. Likely intake of mercury, as mg/day or mg/kg body weight, by different classes of ruminant livestock, when consuming forages and concentrates containing the average or maximum concentrations of mercury calculated from data provided by European countries (see Tables 5 and 6) or the maximum levels (MLs).

Livestock type	Mercury intake average ^a		Mercury intake max ^b		Mercury intake ML ^c	
	mg/d	mg/kg LW ^d	mg/d	mg/kg LW ^d	mg/d	mg/kg LW ^d
Growing cattle	0.051	0.0006	0.391	0.0043	0.240	0.0027
Growing cattle	0.103	0.0005	0.883	0.0044	0.500	0.0025
Growing cattle	0.178	0.0005	1.632	0.0047	0.880	0.0025
Dairy cow-dry	0.280	0.0004	2.660	0.0043	1.400	0.0022
Dairy cow-lactating (20 kg/d)	0.378	0.0006	3.015	0.0048	1.800	0.0029
Dairy cow-lactating (40 kg/d)	0.497	0.0008	3.542	0.0057	2.300	0.0037
Sheep-growing lamb	0.016	0.0005	0.152	0.0051	0.080	0.0027
Sheep-lactating ewe	0.049	0.0007	0.299	0.0043	0.220	0.0031
Goats-lactating	0.060	0.0008	0.307	0.0038	0.260	0.0033

^a forage = 0.02 mg Hg/kg, concentrate = 0.024 mg Hg/kg

^b forage = 0.19 mg Hg/kg, concentrate = 0.10 mg Hg/kg

^c forage = 0.10 mg Hg/kg, concentrates = 0.10 mg Hg/kg

^d life weight

As discussed above, concentrations of mercury in fishmeal and other fish products are often higher than in feeds derived from vegetable material. The period for which data for this report have been provided (2002-2006) cover the period during which it has been illegal to feed fishmeal to ruminants¹⁵. The lifting of the ban, were it to occur, might result in higher concentrations of mercury in the diets of ruminant livestock, but in practice the extent to which this is likely to occur would be determined by the cost of fishmeal relative to other feed ingredients and the demands of consumers.

Non-ruminants and fish

In contrast to ruminants, rations for pigs and poultry consist almost entirely of concentrate feeds. These are normally fed in the form of compound feeds, but individual feed materials may be fed, separately or in a loose mix. For all poultry and most pigs the concentrate is fed in a dry form, either as meal or in pellets. In some areas pigs are given feed in liquid form, but since no data were provided on concentrations of mercury in liquid feeds, no attempt has been made to estimate exposure of pigs given these feed in this way.

Complete feed consist of a range of feed materials, selected on the basis of price, availability, and the contribution that they may make to the supply the nutrients required by the target

¹⁵ The prohibition on feeding fishmeal to ruminants was introduced in December 2001 as part of the European Commission's programme to control BSE, as laid down in regulation 999/2001. OJ L 147 31.5.2001 p 1-40.

animals. Fishmeal may be included in rations for non-ruminants, largely because of its superior amino acid profile relative to other feed materials. Estimates of the average or maximum mercury concentrations (from Table 5) have been used to estimate the likely intakes of mercury (mg/day or mg/kg body weight) by pigs and poultry consuming complete feeds (Table 8). It is acknowledged that the composition of compound feeds differ for different types of livestock within the same category. For example, there will be differences in formulation and composition between feeds for broilers and layers or young and old pigs. In practice, however, the differences are generally relatively small, and variation in the use of raw materials is likely to be greater between manufacturer and between regions of the EU. In the information provided by Member States, the descriptions of the feeds were generally insufficient to permit further differentiation of the data.

The relative contribution of methyl mercury from food versus water to rainbow trout in controlled laboratory conditions was examined by Phillips and Buhler (1978). Nearly 70% of the methylmercury ingested was assimilated while approximately 10% of the methylmercury that passes over the gills was assimilated. The main source of mercury in fish is from the diet, waterborne exposure does not contribute significantly under normal farming conditions. Fishmeal is currently the main source of protein in fish feed, however the inclusion level depends on the species farmed and marine predatory fish species have a particularly high requirement for fishmeal for normal development. Considering that the protein content may be as high as 56% in salmon feed (Måge *et al.*, 2006) and assuming inclusion of fish meal with a maximum mercury concentration of 0.26 mg/kg (Table 4) the resulting feed would contain 0.12 mg mercury/kg. Consequently the ML in fish feed of 0.1 mg/kg (88% dry matter) and the ML of 0.5 mg/kg (88% dry matter) in feedingstuffs produced by the processing of fish or other marine animals are not harmonized. This is supported by the data submitted to EFSA, that the exceedence of the maximum level of 0.1 mg mercury/kg is most frequently reported for fish feed (see Table 4).

Table 8. The intake of mercury, as mg/day or mg/kg body weight (bw), of different classes of pigs and poultry when complete feedingstuffs containing the average or maximum concentrations of mercury calculated from data provided by Member States (see Table 5), or the maximum level (ML) (Table 2)*.

Livestock type	Hg concentrations in complete feedingstuffs average		Hg concentrations in complete feedingstuffs maximum		Hg concentrations in complete feedingstuffs ML	
	mg/day	mg/kg b.w.	mg/day	mg/kg b.w.	mg/day	mg/kg b.w.
Growing pigs	0.0480	0.0016	0.0750	0.0025	0.1500	0.0050
Growing pigs	0.0928	0.0015	0.1450	0.0024	0.2900	0.0048
Growing/ fattening pigs	0.1056	0.0012	0.1650	0.0018	0.3300	0.0037
Growing/ fattening pigs	0.1088	0.0009	0.1700	0.0014	0.3400	0.0028
Dry sow	0.0864	0.0004	0.1350	0.0007	0.2700	0.0014
Lactating sow	0.2080	0.0010	0.3250	0.0016	0.6500	0.0033
Broilers (finishing)	0.0059	0.0023	0.0150	0.0060	0.0150	0.0060
Laying hens	0.0041	0.0012	0.0115	0.0033	0.0115	0.0033
Turkeys	0.0234	0.0015	0.0650	0.0041	0.0650	0.0041

* The animal and feed intake data used to calculate these exposures are given in Annex Table A3.

Pets

The average, median and maximum concentrations of mercury in complete feeds for dogs and cats were 0.02, 0.01 and 0.18 mg/kg, respectively. Unfortunately, information on the target animal was provided for only 29 of the 126 samples. The average, median and maximum mercury concentrations in 13 samples of complete feed that were clearly identified as being for cats were 0.037, 0.010 and 0.18 mg/kg, respectively. For 16 samples of dog food, the average, median and maximum concentrations were 0.037, 0.010 and 0.02 mg/kg, respectively. The reasons for the higher concentrations in cat feed are not clear, but it may be unwise to draw any conclusions from this relatively small population of samples, the majority of which originated from one country.

Fur-producing animals

Based on the feed concentration data provided by Denmark, exposure of mink consuming feed containing either the average or maximum mercury concentrations are given in Table 9.

Table 9. The intake of mercury by mink consuming feed containing average and maximum concentrations of mercury observed in 25 samples of mink feed .

	Age (weeks)	Body weight (g)	Feed intake (g/day)	Hg exposure at average dietary concentrations (0.053 mg /kg)		Hg exposure at maximum dietary concentrations (0.12 mg/kg)	
				mg/day	mg/kg b.w.	mg/day	mg/kg b.w.
Male	7	630	40	0.002	0.003	0.005	0.008
	31	2400	130	0.007	0.003	0.016	0.007
Female	7	450	30	0.002	0.004	0.004	0.008
	31	1300	85	0.005	0.003	0.010	0.008
	Lactating	1300	200	0.011	0.008	0.024	0.018

5. Adverse effects on fish, livestock and pets, and exposure-response relationship

While there is a large amount of data on mercury dose-response effects in laboratory animals, few and rather old data are available for farmed animals, mostly focused on clinical signs of toxicity observed in acute situations.

Toxicological data for inorganic and organic mercury are summarised for the different species in Table A1 in the Annex.

5.1. Fish

A four month study was conducted with triplicate groups of Atlantic salmon (*Salmon salar*) exposed to methylmercury chloride at levels of 0.03, 0.12, 0.63, 4.4 and 8.5 mg mercury (expressed as total mercury)/kg feed (dry weight). Metallothionein levels were elevated and adverse effects in terms of monoamine oxidase activity, brain pathology and altered blood parameters were evident in fish exposed for four months to 4.4 mg methylmercury/kg feed, equivalent to 1.2 mg of methylmercury (as total mercury)/kg body weight. Growth appeared to be an insensitive parameter, and was not affected in Atlantic salmon parr exposed to a dietary concentration of 8.5 mg methylmercury/kg feed for four months (Berntssen *et al.*, 2004a). Elevated blood packed cell volume and hyperplasia of gill epithelium was seen in rainbow trout (*Oncorhynchus mykiss*) exposed to 16 mg/kg feed for 3.5 months (Wobeser, 1975). A NOAEL of 0.17 mg methylmercury (expressed as total mercury)/kg b.w. can be established for salmonids corresponding to 0.63 mg methylmercury (expressed as total mercury/kg feed (dry weight)).

5.2. Ruminants

Goats experimentally exposed to mercuric chloride added to drinking water (average 150 mg mercury/head per day (7 – 7.5 kg b.w.)) developed signs of toxicity after 43 days, such as gastrointestinal disturbances and renal dysfunction (Pathak and Bhowmik, 1998).

Palmer *et al.* (1973) produced acute mercury toxicosis in yearling cattle and sheep with an ethyl-mercury fungicide, administered in capsules at 0.48 mg/kg b.w. (equivalent to 0.15 mg/kg elemental mercury), with deaths recorded between 7 and 27 days.

Chronic methylmercury intoxications were experimentally achieved in 4 week old calves (Herigtad *et al.*, 1972), cattle and sheep (Wright *et al.*, 1973; D'Itri, 1971). Main manifestations were dysfunction of the central nervous system (CNS) (incoordination and unsteady gait) and of the digestive and genito-urinary systems, as well as skin and visual problems (Annex, Table A1). Young animals are more susceptible to methylmercury intoxication as compared to adults.

The NOEL values (Annex, Table A1), usually expressed as mg/kg feed were estimated on the basis of a dry matter intake corresponding to 2% of the body weight in non lactating ruminants. They range from 5 (calves) to 12 mg/kg feed (yearlings) for exposures that cover 10-30% of the expected economic life in meat producing animals.

5.3. Pigs

Weanling pigs exposed to methylmercury and ethylmercury salts via feed at doses of 0.19, 0.38 and 0.76 mg total mercury/kg b.w. (equivalent to 20 mg total mercury/kg feed) for 60-90 days showed anorexia, incoordination and liver degeneration (Tryphonas and Nielsen, 1970, 1973). The NOAEL based on liver failure, the most sensitive endpoint, was 0.19 mg/kg b.w. per day corresponding to 3.4 mg total mercury/kg feed.

5.4. Poultry

Fifty percent of one day old chicks exposed to methylmercury at 5.0 mg/kg feed died within 33 days, while 2.2 mg/kg feed resulted in no appreciable signs of intoxication (Soares, 1973).

Scott (1975) observed reduced weight gains, a drop in egg production and infertility in hens fed methylmercury at 10 mg/kg diet. More recently, Lundholm (1995) reported a significant drop in egg production of hens exposed for 50 days to methylmercury at 0.75 mg/kg b.w. (corresponding approximately to 10 mg/kg feed for a 3 kg hen, eating daily 200 g feed containing 12% moisture).

Gardiner (1972) reported that 5 day-old ducks fed on a diet containing 3.3 mg methylmercury/kg feed showed a reduced growth rate. At the same concentration level, Heinz

(1979) reported embryo toxicity associated to methylmercury exposure over two breeding seasons. More recently, in mallard duck, Heinz and Hoffmann (2003) derived a LOAEL of 5 mg total mercury/kg feed, based on embryo deformations resulting from the carry-over of methylmercury into eggs. Considerable differences in the sensitivity of mallard embryos, especially from different parents, were recorded.

Incoordination and weakness were provoked in 16 week-old turkeys, fed a feed containing a ethylmercury fungicide at a 5 mg/kg b.w. for 13-42 days (Palmer *et al.*, 1972) equivalent to a 0.16 mg/kg total mercury/kg b.w. and to 24 mg total mercury/kg feeds.

5.5. Cats

Over a period of two years, Charbonneau *et al.* (1976), exposed groups of adult cats (male/female ratio 1:1; control, n =10; exposed n=8 for each dose) to diets based on natural methylmercury in fish at doses of 0.05 (control), 0.14, 0.33, 0.76, 1.23 and 2.95 mg total mercury/kg (methylmercury was not measured), corresponding to 3.0 (control), 8.4, 20.0, 46.0, 74.0, and 176.0 µg total mercury/kg b.w. per day. During the same period other groups of cats were fed the control feed (containing 0.05 mg methylmercury/kg feed) contaminated with exogenous methylmercury chloride, at the same levels reported above. The feeding rate was 60 g feed/kg b.w. per day, with selenium present at 0.13 mg/kg in the diet. Haematological, and biochemical investigations, together with neurological and clinical examinations were performed at regular intervals. At 1.23 mg/kg feed marked signs of methylmercury neurotoxicity were recorded after 40 weeks of exposure in all animals (loss of balance, ataxia, impaired hopping, hypalgesia, motor incoordination, muscle weakness). At 0.76 mg/kg feed (46 µg methylmercury/kg b.w. per day) one animal out of eight developed neurological signs of toxicity and was sacrificed after 38 weeks of exposure. Another died due to acute renal failure after 68 weeks of treatment. The remaining animals all showed slight neurological damage (mild impairment of the hopping reactions and hypalgesia) after 60 weeks of treatment, and their condition did not deteriorate in the remaining period of the study. No treatment-related effects were noted in the groups exposed to 0.14 and 0.33 mg/kg feeds. No difference in toxicity was observed between methylmercury naturally present in fish and methylmercury added in pure form to the diet. Therefore the NOAEL was 0.33 mg methylmercury (expressed as total mercury)/kg feed.

Cats fed tuna fish showed a modified behaviour: they were less active, vocalized less, and spent more time on the floor and more time eating than cats fed commercial beef cat food (Haupt *et al.*, 1988). In this study, possible additive effects between mercury and thiaminase present in raw fish cannot be excluded. Several types of raw fish, including carp and herring, contain thiaminase that cause thiamine (vitamin B1) deficiency in cats. Clinical cases of thiamine deficiency (anorexia, ataxia, vomiting, dilation of the pupils, ventroflexion of the neck and convulsions have been reported in cats and mink fed raw fish (i.e. herring and carp) containing thiaminase (Davidson, 1992). The presence of a sulfur atom in the thiamine

structure determines interaction with divalent mercury and possibly methylmercury, thus causing the denaturation and the subsequent loss of vitamin B1 activity.

5.6. Dogs

Mongrel dogs (estimated body weight around 30 kg) were orally exposed to different doses of methylmercury (1.2 (n=1), 12 (n=1), 60 (n=1), 120 (n=1), 430 (n=4), 640 (n=4) µg/kg b.w. expressed as total mercury) for 385 days (Davies *et al.*, 1977). The dose of 430 µg/kg b.w. per day resulted in neurological signs of toxicity in all animals within 60 days of exposure, and disseminated cerebral lesions. No clinical signs were observed up to 120 µg/kg b.w. although histological examination revealed degeneration of brain tissues at 120 µg/kg b.w.. Due to the weakness of the toxicological database and only single animal experiments no NOAEL could be derived for dogs. The LOAEL was 0.12 mg methylmercury (expressed as total mercury)/kg b.w. corresponding to 8 mg/kg feed.

5.7. Horses

The acute toxic dose of inorganic mercury (calomel) in horses is 8-10 grams. Chronic toxicity was observed following ingestion of 0.4 mg inorganic mercury (calomel)/kg b.w. per day over a period of several weeks (Guglick *et al.*, 1995). The main clinical signs were renal failure and ulceration of the digestive apparatus.

No relevant information is available for methylmercury toxicity.

5.8. Fur animals

Woebeser *et al.* (1976) exposed four groups of adult mink (5 animals/group) to methylmercury chloride at levels of 0.1 (control), 1.1, 1.8, 4.8, 8.3 and 15 mg/kg feed (expressed as total mercury), for 93 days (corresponding to around 30% of the production cycle). Mink exposed to feed contaminated at 0.1 mg/kg did not show appreciable clinical symptoms, whereas in the 1.1 mg/kg group (equivalent to 0.18 mg/kg b.w. per day) a tendency to ataxia was noted in two animals on the last three days of the experiment. Small necrosis foci were noted during the histological investigation in brain. Since the nature of the mercury species in the control feed (0.1 mg/kg) is not known, the NOAEL for methylmercury cannot be derived from this experiment. The LOAEL was 1.1 mg methylmercury (expressed as total mercury)/kg feed.

As mentioned for cats, also for mink a possible additive effect of thiaminase and methylmercury present in raw fish offals cannot be excluded if the diet is based on fish.

It is worth noting that fur animals are excluded from the restrictions in the use of processed animal protein in feedingstuffs, including fishmeal, to prevent the spread of Transmissible Spongiform Encephalopathies (TSE) (Regulation No (EC) 1774/2002)¹⁶.

5.9. Rabbits

Limited data are reported concerning mercury exposure via feedingstuffs in rabbits under farming practices. Most of the experiments deal with the rabbit as a laboratory animal model to study inorganic and organic mercury toxicity on target organs such as kidney, brain and the immune system following non-oral routes of exposure (Petersson, 1991; Dock, 1994; Moszczynski, 1997).

Abdelhamid (1988) studied the effects of HgSO₄ administered via feed to rabbits for 7 weeks at concentrations of 0, 150, and 300 mg total mercury/ kg feed (6 animals/group). Diarrhoea, haemorrhage, oedema, liver and stomach necrosis and mortality were observed in the treated groups. The contaminated diets significantly increased feed intake, drinking water consumption and body weight gain. The most affected organ was the liver, which showed a slight dry weight increase, as well a severe reduction in vitamin A and iron content for the animals fed the 300 mg total mercury/kg diet. The highest level of mercury also caused a significant rise in glycemia and an increase in bone magnesium.

Ultrastructural changes were described by Jacobs *et al.* (1977) in different districts of the nervous system of rabbits administered methylmercury at an oral daily dose of 7.5 mg total mercury/kg b.w. within 1-4 days.

No NOAEL or LOAEL for mercury after oral exposure could be established for rabbits.

Conclusions

The toxicological database for farmed animals is limited in terms of proper dose-response experiments, toxicological endpoints (reproductive toxicity studies (except for poultry), immunotoxicity, length of study, type of mercury species, etc.). Some observational studies may have been affected by the presence of confounding factors (i.e. the simultaneous exposure to metals other than mercury, and/or to persistent organic pollutants), and the exposure time has not always encompassed the full production cycle of the animals.

The most sensitive domestic animal species to methylmercury toxicity are cats and mink.

New-born animals (calves, chickens) are more susceptible to methylmercury intoxication as compared to adults (Annex, Table A1).

¹⁶ OJ L 273, 10.10.2002, p. 1–95.

6. Toxicokinetics and tissue disposition

The knowledge of the toxicokinetics of mercury is mainly based on experimental studies carried out in humans and laboratory animals 20 to 30 years ago. These data have been assessed by a number of national (US-EPA, 1997; ATSDR, 1999; NRC 2000) and international (WHO-IPCS, 1990, 1991, 2000) bodies. A summary of these data is given below, completed with either more recent studies (e.g. carried out on marine mammals or seabirds) or data obtained on farmed animals, including fish, that were not taken into consideration in these assessments.

The absorption, distribution, metabolism and excretion of mercury are largely dependent on its chemical form, i.e. elemental mercury, inorganic mercury and organic mercury.

6.1. Absorption

Elemental mercury (Hg^0) in vapour phase is absorbed to a large extent (80%) through inhalation. Hg^0 and mercurous salts (Hg_2^{++} , e.g. Hg_2Cl_2) are poorly absorbed (<0.10%) following oral exposure or contact with the gills.

Mercuric salts (Hg^{++} , e.g. HgCl_2) are absorbed to a limited extent in the gastrointestinal tract. The extent to which inorganic mercury is transported across the intestinal tract is largely dependent on its solubility and its dissociation in the lumen. Mercuric compounds are more readily absorbed than mercurous forms because of their solubility. Their absorption varies according to the species (e.g. 20% for the adult mice, 30% for the goat, 7% for humans), age (38% in the 1 week-old mice), individuals, nutritional factors (organic ligands such as phytate, proteins/aminoacids, micronutrients like selenium) and physiological factors (feed intake, gut passage time and physiology).

Organic mercurials are absorbed much more extensively and rapidly after oral intake than are inorganic forms. More than 80% methylmercury and phenylmercury have been shown to be absorbed by humans, laboratory animals and farmed animals (poultry, ruminants and fish) following oral exposure. Feed composition has a major influence on the digestion and release of mercury from feed components in the intestinal lumen and subsequently bioavailability. Association with organic ligands such as phytates or proteins and/or amino acids can affect the absorption of mercury over the intestinal tract. Other factors such as feed intake, gut passage time and gut physiology also contribute to the large inter- and intraspecies differences in bioavailability of mercury (Schlekat *et al.*, 2005). In fish (trout) absorption of methylmercury dissolved in water through the gills occurs at a limited extent as compared with digestive absorption following ingestion of contaminated feed and was shown to be dependent on the metabolic rate (e.g. related to water temperature) (de Freitas and Hart, 1975). Evidence of reduced bioavailability of inorganic mercury for fish with increasing salinity has been given, which cannot be attributed unequivocally to either the decrease of the

bioproduction of methylmercury from inorganic mercury and/or the intrinsic decrease of fish absorption.

6.2. Distribution

Absorbed elemental mercury vapor readily distributes through the body and crosses the blood-brain and placental barriers. However, the distribution of the very small amounts absorbed through the intestine is limited primarily by the oxidation of Hg^0 to Hg^{++} that occurs in tissues.

Inorganic mercury does not easily cross the blood-brain or placenta membranes. Kidneys exhibit the greatest concentration of mercury (bound to metallothioneins) following exposure to inorganic mercury salts (50-90% of the body burden in the rat). Liver and carcass, in decreasing order, contain lower amounts whereas brain harbours very limited quantities (about 1%). It has been shown in mice, goats and humans that Hg^{++} crosses the mammary barrier.

Organic mercury absorbed through the intestine or the gills is distributed throughout the animal body. In blood, most of methylmercury is found within the red blood cells, bound to hemoglobin, whereas a minor part is largely bound to plasma proteins and thiol compounds, L-cysteine and reduced glutathione (GSH). Mercury in blood only reflects recent exposure to methylmercury and inorganic mercury.

In mammals, methylmercury has been shown to cross the blood-brain and placental barriers, the mammary gland and the pilous follicle (hair, feathers). For example, the whole body retention in mice, 14 days after methylmercury oral administration, is inversely proportional to the dose applied and the mercury is distributed as follows: carcass 65-75% (including the hair which represents the major deposit), liver 8-10%, kidneys 5-20% and brain 10%. In similar conditions in the rat, it has been established that methylmercury represented 97% and 92% of the whole mercury in brain and liver respectively, whereas inorganic mercury amounted for 65 to 80% in the kidney. In human milk, 16% of total mercury was found to be methylmercury. Mercury in hair is approximately 90% methylmercury. Hair measurements provide a record of methylmercury exposure but do not accurately reflect exposure to inorganic mercury (ATSDR, 1999).

In fish, a link exists between mercury distribution in tissues and water/food regimes and contamination, with comparatively high Hg concentration ratios between gills and muscle for the periphytophagous and benthivorous species and, in contrast, ratios less than 1 for the piscivorous and omnivorous species. Methylmercury is mainly deposited (99%) in muscle of piscivorous/carnivorous species that ingest fish. In benthivorous species that ingest biofilms and small benthic vertebrates with quite low methylmercury burden (18-52% of total mercury), the highest mercury levels are observed in the liver and kidneys, the two principal

organs for the deposition of inorganic mercury in fish (Régine *et al.*, 2006). Another study on inorganic mercury accumulation in salmon showed concentrations in intestine, kidney, liver, gill, and brain in decreasing order (Berntssen *et al.*, 2004a).

In chickens, organic mercury is distributed in tissues, crosses the oviduct of the laying hen to the egg and is deposited in the feathers (March *et al.*, 1974 and 1983). Female mallards (*Anas platyrhynchos*) fed diets containing high levels of methylmercury (5 to 20 mg/kg) laid eggs containing 7 to 55 mg total mercury/kg of which 95 to 100% was methylmercury, which is preferentially deposited in the egg albumen rather than the yolk (Heinz and Hoffman, 2004). In laying hens fed diets contaminated with high levels of phenylmercury, methylmercury represented about 95% of the residues found in the egg white and 15% of those found in the yolk (Cappon and Smith, 1981).

6.3. Metabolism

The metabolism of mercury and mercury compounds appears to be similar for animals and humans and involves an oxidation-reduction cycle. Moreover, bacteria (rumen and gut flora) harbour an organomercurial resistance system based on an organomercurial lyase which catalyses the demethylation of methylmercury to Hg^{++} . Some seabirds may be capable of demethylating organic mercury in a species dependent way (Thompson and Furness, 1989), while animal and human studies have provided data suggesting that Hg^{++} may be further reduced to elemental mercury by a mercuric catalase. There is no evidence in the literature for the synthesis of organomercury compounds in human and mammalian tissues. It appears that methylation of inorganic mercury does not occur in fish (trout) (Huckabee *et al.*, 1979), but may do so to a very limited extent (0.17% the administered dose) in the rumen of the cow (Neathery *et al.*, 1974).

Organic mercury contaminants entering the animal body are converted to Hg^{++} by cleavage of the carbon-mercury bond, with subsequent metabolism occurring via the oxidation/reduction cycle. This occurs in the rumen and the intestine, where it involves the bacterial flora, but also in red blood cells and tissues. The rate of demethylation is generally very slow. Aryl mercury compounds (e.g. phenylmercury) undergo this conversion more readily than do the short-chain (methyl) mercury compounds. For example, the rat rapidly converts phenylmercury to phenol and Hg^{++} , a reaction involving *p*- or *o*-hydroxyphenylmercury as an intermediary compound (Daniel *et al.*, 1972). The conversion of phenylmercury to methylmercury has been observed in the laying hen, where the latter represents the main metabolite excreted in the egg (Cappon and Smith, 1981). Once absorbed, methylmercury undergoes a first pass metabolism in the liver and is excreted into the bile as a methylmercury-glutathione complex (CH_3Hg-SG). It has been shown that GSH is involved in the disposition and excretion of methylmercury (Strange *et al.*, 2001). Higher levels of mercury contamination in the hair have been found in human populations harbouring a null glutathione S-transferase (GST) genotype (GSTM1 0/0) (26% frequency) when compared with the counterpart population for which the

null genotype frequency was 0%. This study and others (Klautau-Guimarães *et al.*, 2005; Gundacker *et al.*, 2007) suggests that GSTs polymorphism plays an important role in the disposition of mercury in humans.

The chemical identity of mercury species in skeletal muscle of wild fish has been partly established (Harris *et al.*, 2003). Linear bonds between mercury, methyl groups and sulfur donors have been identified. Among sulfur donors cysteine is the most likely candidate as the predominant biological thiol, either in the free form or as a constituent of glutathione or proteins. More than 99% of methylmercury in both salmon and cod muscle was found in the protein fraction (Amlund *et al.*, 2007). The most commonly used “model” of methylmercury species in fish experiments is aqueous methylmercury chloride, where the Hg-Cl bond is highly covalent (see chapter 1.1). Moreover, methylmercury chloride is relatively hydrophobic and therefore expected to exhibit membrane crossing properties superior to many other methylmercury species. However, the affinity of methylmercury for sulfhydryl groups is much stronger than for the chloride (see chapter 1.1), and is therefore more likely to survive in this form in, for example, the intestinal tract, or is less effectively absorbed. The higher toxicity of methylmercury chloride compared with thiol bonded species is consistent with the physicochemical differences between these methylmercury species, and could partly explain the toxicological differences observed (Harris *et al.*, 2003; Oyama *et al.*, 2000; Berntssen *et al.*, 2004b).

The selenium dose, form (oxidation state, organic or inorganic) and exposure route may affect tissue deposition of methylmercury in the body and consequently modulate mercury toxicity in animals. The mechanism by which selenium influences the deposition of mercury has not been established. Proposed mechanisms include the formation of seleno-methylmercury complexes, a selenium-induced release of methylmercury from sulfhydryl bonds in the blood, and tissue-specific mechanisms that influence intracellular intake (Glynn and Lind, 1995). It has been shown that in marine mammals (i.e. ringed seal) about 50% of the mercury deposited in the liver is in the form of insoluble mercury selenide (HgSe), with inorganic mercury and methylmercury representing about 40% and only 2%, respectively (Wagemann *et al.*, 2000).

6.4 Excretion

The main pathway of excretion of inorganic mercury is via the urine and faeces. Due to the poor absorption of orally administered inorganic mercury, the majority (in the order of 80%) of the ingested dose in humans is excreted in the feces. The half-life of the absorbed Hg⁺⁺ is approximately 40 days (humans) (Clarkson *et al.*, 1988). Elimination of inorganic mercury from the blood and brain is a biphasic process encompassing an initial rapid elimination phase followed by a slower phase. Inorganic mercury may also be reduced to form elemental mercury which is exhaled as elemental mercury vapour or excreted in the breast milk. Inorganic mercury is also excreted in milk during lactation, as shown in mice, guinea-pigs and

humans. In ruminants (goat), following intraruminal administration of $^{203}\text{HgCl}_2$ for 9 days, the half-time retention (carcass measurement) was 78 days (Sell and Davison, 1975).

Berlin *et al.* (2007) recently reviewed the fate of organic mercury compounds in mammals. The major part of the excretion is by the fecal route (about 90%). Much of the methylmercury excreted in the bile is absorbed in the gut, producing an enterohepatic circulation of methylmercury. In the rat, methylmercury in the bile is bound to glutathione and cysteine. A part of the mercury in the bile (approximately 30-80%) of the monkey is inorganic mercury derived from the demethylation of methylmercury in the body. This part, less effectively absorbed in the gut, is excreted. In the gut, methylmercury can be decomposed by the microflora to inorganic mercury. As inorganic mercury is absorbed to approximately 5-10%, this factor contributes to an increased excretion.

The elimination of organic mercury compounds generally follows first-order kinetics, with whole body clearance times and blood clearance times being longer than for inorganic mercury. The biological half-life of methylmercury in the human is about 1.5 – 2 months (EFSA, 2004). Milk, egg, saliva, sweat, hair and feathers have been identified as other elimination routes of mercury compounds. It has been shown that after injection of equivalent doses of inorganic and methylmercury, the concentration of total mercury in milk was 5 times higher when in the inorganic form in lactating mice and 2.5 times higher in guinea-pigs (Sundberg *et al.*, 1998). In ruminants, following a single intraruminal administration of $\text{CH}_3^{203}\text{HgCl}_2$ to a milking cow and a milking goat, the cumulative secretion of ^{203}Hg over a 13-day period was negligible in the cow and amounted 0.28% in the goat. The half-time retention (carcass measurement) was 22 days in the goat (Sell and Davison, 1975). Another study performed on milking cows which received a single dose of ^{203}Hg -methylmercury (Neathery *et al.*, 1974), confirmed that the excretion of radioactivity in milk was very limited (0.17% of the administered dose over the 15-day milk collection period).

Total mercury accumulates in bird tissue following methylmercury administration in feeds, and is excreted when the source is removed. In chickens for fattening receiving 0.05, 0.15, 0.45 and 1.35 mg methylmercury/kg feed for 8 weeks, the elimination half-times of total mercury in tissues after withdrawal increased in proportion to the amounts of mercury retained, i.e. of the dose applied in the diet. The values were similar for the liver and pectoral muscle (4 to 8 days) but higher for kidneys (7 to 23 days). In comparison to the chicken for fattening, the elimination half-time of total mercury in tissues of laying hens that received the same range of concentrations in feed was similar for the lowest dose and proportionally higher for increasing dosages. The elimination half-times were much higher (27, 14 and 49 days for the kidneys, liver and pectoral muscle, respectively) for the lowest dose, but proportionally lower for the increasing doses (March *et al.*, 1983).

In fish, the elimination half-life of methylmercury from muscle was found to be 377 days in the Atlantic cod (Amlund *et al.*, 2007) and between 202 and 516 days in the rainbow trout

(*Oncorhynchus mykiss*), depending on dose and water temperature (Rouhtula and Miettinen, 1975).

7. Carry-over and tissue/products concentration

The carry-over of an orally administered compound to animal tissues and products (milk, eggs) is dependent on the absorption, distribution, metabolism and excretion/deposition of the compound (and its eventual metabolites). These biological phenomena are dose and/or time-dependent, but are also influenced by other factors such as the interaction with other compounds (e.g. selenium contents in the case of mercury). No dose-response studies are available concerning the transfer of inorganic or methylmercury into target species. In general, very limited or only partial data are available.

7.1 Transfer into animal products

In laying hens fed diets containing 0.05, 0.15, 0.45 and 1.35 mg methylmercury/kg feed for 28 weeks, total mercury concentration in the eggs reflected dietary concentrations and reached a plateau after 4 weeks, with the exception of the highest dose for which the concentration in the eggs increased at a much slower rate until week 28. On the basis of approximate values taken from a graph, the following linear relationship for the carry-over of methylmercury to whole egg at plateau has been established: y (mg mercury/kg egg) = 0.133 x (mg mercury/kg feed) covering the range of doses 0.05 to 0.45 mg methylmercury/kg feed (March *et al.*, 1983).

In chickens for fattening given the same range of methylmercury concentrations in feeds (see above) for 8 weeks, total mercury retention in tissues reached a steady state after 1 week. The transfer ratio for the pectorial muscle (concentration in the tissue relative to the concentration in the diet) was between 4.1 (for 1.35 mg/kg feed) and 13.8 (0.05 mg/kg feed). Transfer ratios calculated for kidneys and liver were similar and varied from about 5 to 33 according to the mercury contents of feeds (March *et al.*, 1983).

The only data available for ruminants concerns the comparative carry-over of $[^{203}\text{Hg}]\text{Cl}_2$ and $\text{CH}_3[^{203}\text{Hg}]\text{Cl}$ in goat, following intraruminal administration of 0.5 mg mercury/kg b.w. equivalent for 9 days to a single animal. Cumulative excretion (36-day period) into milk represented 0.22% and 1.12% of the intake, respectively. However, as the dose applied represents 20 to 50 times the maximum level in complementary feed, no conclusion can be drawn concerning normal levels of exposure (Sell and Davison, 1975).

7.2 Tissue levels and bioaccumulation

Terrestrial domestic animals

Experimental data available in the literature indicate that the highest mercury levels are present in the skin, nails, hair and feathers. Among the internal organs, kidneys generally contain the highest mercury concentrations, usually at approximately 100-fold the levels found in other tissues including liver or muscle (Clarkson, 1992).

A number of biomonitoring studies have been carried out during the last decades in farm species from relatively unpolluted areas, mainly associated with cattle, pig and poultry production (Korsrud *et al.*, 1985; Vos *et al.*, 1986; Jorhem *et al.*, 1991; Niemi *et al.*, 1991; Salisbury *et al.*, 1991; Kluge-Berge *et al.*, 1992; Falandysz, 1993a,b; Raszyk *et al.*, 1996; Ulrich *et al.*, 2001; López-Alonso *et al.*, 2003, 2007). The results show that total mercury concentrations in meat and meat products are generally below 10-20 µg/kg wet weight, being below the LOQ (generally 1-5 µg/kg wet weight) in many liver and muscle samples. In addition, a tendency for declining total mercury content in meat products has been observed in recent decades, largely reflecting the decrease in environmental burden (Jorhem *et al.*, 1991; Falandysz, 1993a).

Data on mercury accumulation from experimental studies in domestic animals given diets with known mercury concentrations are sparse. A large number of studies have been published (e.g. Wright *et al.*, 1973; Kacmar *et al.*, 1992; Raszyk *et al.*, 1992; Krupicer *et al.*, 1996; Pathak and Bhowmik, 1998) but the information was inappropriate for inclusion in this opinion due to either the lack of information on mercury sources or because exposure doses were much too high.

Dórea (2006) has recently reviewed the transfer of methylmercury from fishmeal to animals. Depending on the concentration of methylmercury in fishmeal, feathers concentrate four to seven times more methylmercury than in breast muscle (Plummer and Barlett, 1975). In laying hens, the incorporation in complete feed of 5, 10 and 17% fish (herring) meal containing 0.17 or 0.22 mg mercury/kg resulted in total mercury concentrations in feathers that increased proportionally to the mercury content of the diet. The transfer ratio (concentration in feathers *vs* concentration in the diet) was 22. The maximum value measured (17% incorporation) was 0.85 mg mercury/kg feathers, compared to 0.09 mg for the control (soybean) diet (March *et al.*, 1974).

Fish

Estimates for whole body assimilation efficiency of dietary methylmercury in fish vary considerably among studies (from 10 – 95% of the fraction of methylmercury ingested absorbed) and depends on source (natural prey versus formulated feed), fish species, fish size (Phillips and Gregory, 1979; Leaner and Mason, 2002; Wang and Wong, 2003), dose and exposure duration (Lock, 1975; Houck and Cech, 2004).

In juvenile Atlantic salmon, whole body assimilation efficiencies for inorganic mercury chloride varied between 6 – 27% depending on whether the mercury was in live prey or formulated feed (Berntssen *et al.*, 2004a; Wang and Wong, 2003). The variability in assimilation efficiencies of mercury may possibly be due to increased bioavailability of inorganic mercury in prey species compared to inorganic mercury salts.

Transfer of methylmercury into flesh of Atlantic cod (*Gadus morhua L.*) administered a dose of 0.95 mg/kg feed (i.e. equivalent to about 10 times the maximum level in complete feed) showed a linear increase during the 3-month experiment at a rate of 0.005 mg/day; the fraction of methylmercury deposited in flesh to methylmercury ingested was approximately 38% (Amlund *et al.*, 2007). This supports earlier findings that methylmercury preferentially accumulates in fish muscle (Giblin and Massaro, 1973; Julshamn *et al.*, 1982; Boudou and Ribeyre, 1985; Berntssen *et al.*, 2004a; Houck and Cech, 2004; Leaner and Mason, 2004). Mean muscle mercury concentrations in Atlantic salmon fed methylmercury (0.1, 0.5, 5 or 10 mg methylmercury/kg feed) for four months were 0.05, 0.14, 1.1 and 3.1 mg total mercury/kg wet weight. In comparison, mean muscle mercury concentration in Atlantic salmon fed inorganic mercury chloride (0.1, 1, 10 or 100 mg inorganic mercury/kg feed) for four months were 0.04, 0.03, 0.06 and 0.31 mg total mercury/kg wet weight (Berntssen *et al.*, 2004a).

Since experimental feeding trials do not last for the duration of an entire production cycle, mercury concentration in fish fillets was modelled using one-compartment first-order rate kinetics (Sijm *et al.*, 1993; Berntssen *et al.*, 2007). Uptake (assimilation efficiency of $38 \pm 1\%$ and elimination rate constant ($0.18 \pm 0.08 \text{ } 10^{-2}/\text{d}$) described by Amlund *et al.* (2007) for mercury in Atlantic cod were used to predict the mercury concentration in farmed fish. Fish raised on feed containing 0.1 mg mercury/kg feed would contain approximately 0.05 mg mercury/kg cod fillet assuming a growth rate of 0.006 body weight/per day and a production cycle of 2.5 years. In comparison, the mercury concentration measured in farmed cod has been found to be in the range of 0.003-0.35 mg/kg wet weight (mean concentration 0.1 mg/kg wet weight, n=24¹⁷).

The calculation above indicates that the current maximum level of total mercury in fish feed would result in a mercury concentration in farmed cod approximately ten fold below the EU maximum level for mercury in fish (0.5 mg/kg in most species and 1 mg/kg in a limited list of fish species). The maximum mercury concentrations reported to date in farmed salmonids raised on commercial feed contain approximately 0.1 mg/kg, i.e. about 20% of the EU maximum level for mercury in fish for human consumption. The maximum mercury level measured in farmed cod represents approximately 70% of the maximum level; however limited data are available for cod and other farmed species since salmonids are currently the only major category of farmed fish in the EU.

¹⁷ <http://www.nifes.no>

8. Animal risk assessment

The present limit for total mercury in complete feedingstuffs is 0.1 mg/kg feed (containing 12% moisture) for all animal species, except cats and dogs (0.4 mg/kg feed). Among pets, cats and dogs have been identified as the most sensitive species, based on longterm studies (>1 year). For cat, a NOAEL of 0.33 mg/kg feed (corresponding to 20.0 µg total mercury/kg b.w. per day) based on neurobehavioral effects has been identified. Although not clearly indicated in the study from which the NOAEL was derived (Charbonneau *et al.*, 1976), the Panel considered that the water content of the diet was 41%. Accordingly, a NOAEL of 0.5 mg/kg feed (12% moisture), can be extrapolated. In dogs, no NOAEL was identified, and the LOAEL was 0.12 mg/kg b.w., which corresponds to about 8 mg/kg feed. Taking into account an uncertainty factor of 10 for extrapolation from LOAEL to NOAEL, a chronic oral maximum feed concentration of 0.8 mg/kg feed can be derived, which should not cause adverse effects in dogs. The current ML for pets seems to be protective for dogs, but for cats the margin between the NOAEL and the ML is very small. However, based on the available data on the occurrence of total mercury in complete feedingstuffs, it is unlikely that cats and dogs will be exposed to toxic levels from feed.

For pets, the consumption of raw fish and fish based home-made feeds may represent a relevant source of exposure when given over an extended period of time (i.e. more than just occasional meals).

Whilst mink will be able to tolerate the maximum level set for total mercury in complete animal feedingstuffs, it cannot be excluded that the extensive use of offal from fish or other marine animals could result in neurotoxic effects in this species. However, these effects are highly improbable in animals fed on commercial feedingstuffs owing to the relatively low average concentration of total mercury found in such commodities in Europe.

For other land animal species and poultry the maximum levels are well below the risk level for clinical toxicity.

For fish, only data regarding salmonids were identified. A NOAEL 0.17 mg methylmercury (expressed as total mercury)/kg b.w. corresponding to 0.63 mg methylmercury (expressed as total mercury/kg feed (dry weight)) was estimated. The current maximum level for complete feed for fish (0.1 mg/kg feed) is considered sufficiently protective.

In terrestrial livestock, the margin of safety for methylmercury, (as the ratio between the NOAELs and the maximum limits of contamination in feedingstuffs in place within the EU) is sufficient and may buffer possible changes in risk scenario, i.e. as a result of the withdrawal of the ban on the feeding of fishmeal to ruminants, and/or an increased use of hydrolysates from feather meals in feed formula.

9. Human dietary exposure

In the period 2004-2007, several opinions concerning human dietary exposure to mercury were issued (EFSA, 2004, 2005; UK-COT, 2004, 2007; Japan-FSC, 2005; Canada-BCS, 2007). All these documents indicate that fish (marine and freshwater) and seafood are the major source of mercury intake in humans. Depending on species, methylmercury accounts for 70-100% total mercury in fish (EFSA, 2005). However, for conservative assessment purposes, it is generally assumed that 100% of the mercury found in fish and shellfish is methylmercury.

Wild fish species that are low in the food-chain, such as herring and sardines (plankton eaters) typically have total mercury concentrations less than 100 µg/kg wet weight, whereas predatory fish such as tuna, dogfish, halibut and shark contain considerably more mercury (typically 500-1000 µg/kg wet weight). Mercury levels are also dependent on the size and age of the fish (e.g. Boudou and Ribeyre, 1985).

Farmed salmonids have been shown to contain total mercury levels of up to approximately 100 µg/kg (Knowles *et al.*, 2003). The mercury content of 274 farmed Atlantic salmon (*Salmo salar*) fillets has been shown to vary from between <4 and 52 µg/kg wet weight¹⁸ to up to 103 µg/kg (Knowles *et al.*, 2003). The average total mercury concentration found in farmed rainbow trout (*Oncorhynchus mykiss*) fillets was 44 µg/kg (range 10-80 µg/kg, n=21). The average mercury content in farmed cod (*Gadus morhua*) fillet and liver were 100 µg/kg (range 3-350 µg/kg, n=24) and 10 µg/kg (range 1-30 µg/kg, n=21), respectively. The mean total mercury level in farmed Atlantic halibut (*Hippoglossus hippoglossus*) fillets was 50 µg/kg (range 3-90 µg/kg, n=15)¹⁹. The very limited data on farmed tuna fed on defrosted herring and sardines indicate that contamination levels in fillets exceed those reported for other farmed fish (490-1809 µg/kg, n=29) (Srebocan *et al.*, 2007).

The average value reported for total mercury contamination of fish in Europe was 109 ±845 µg/kg (EFSA, 2004), the high standard deviation reflecting the wide variations in the analytical results. More recent data obtained in France and Catalonia (Leblanc *et al.*, 2005, Bocio *et al.* 2005) indicated that average concentration of total mercury in fish of 62 and 97 µg/kg, respectively, which confirms former data.

According to EFSA (2004), the range of average fish consumption is from 10 to 80 g per day for six European countries, corresponding to a mercury weekly intake from 1.3 to 92 µg, per person. This is markedly lower than the values reported for Faroe Islands (average 252 µg/week), while in the Seychelles the daily mercury intake was estimated to be 103 µg, assuming a per capita consumption of fish of 75 kg per year (205 g per day) (Robinson and Shroff, 2004).

¹⁸ Nifes, seafood data on undesirable substances: http://www.nifes.no/index.php?page_id=137&lang_id=2

¹⁹ Nifes, seafood data on undesirable substances: http://www.nifes.no/index.php?page_id=137&lang_id=2

Some additional data on intake of mercury have recently been published. The estimated average weekly intake of mercury by the French population is 68 µg for adults aged 15 years or more (corresponding to 1.1 µg/kg b.w. per week for a 60 kg person) and 55 µg for children aged 3-4 years (Leblanc *et al.*, 2005). Estimated weekly intake of total mercury in the population from Catalonia (Bocio *et al.*, 2005) is 148 µg, corresponding to 2.1 µg/kg b.w. per week, and is due principally to the high consumption of fish in this region.

The JECFA (2003) established a Provisional Tolerable Weekly Intake (PTWI) of 1.6 µg methylmercury/kg b.w. based on epidemiological studies that investigated the relationship between maternal exposure to mercury and impaired neurodevelopment in their children. This PTWI was used along with the Reference Dose by EFSA (2004).

Several recent European risk assessments (UK-COT, 2004; EFSA, 2005; Leblanc *et al.*, 2005) concluded that for the general adult population the calculated intake of methylmercury does not exceed the PTWI. Regular consumption of top predatory fish such as tuna could result in the methylmercury PTWI being exceeded. The data examined in this opinion indicate that the maximum concentration reported to date in farmed salmonids is approximately five times lower than the EU maximum level for mercury in fish for human consumption (0.5 mg/kg in most species including salmonids and 1 mg/kg in a limited list of fish species). However, this mercury concentration in salmonids would allow weekly consumption of two fish meals, as recommended by nutritionists, without appreciable health risk. Therefore, the current level of total mercury in fish feed does not pose a threat to consumer's health, confirming that fish farming offers the possibility of managing the contaminant levels in fish in order to minimize the risks while maintaining the benefits (EFSA, 2005).

CONCLUSIONS

Chemistry and environmental fate

- Mercury is a naturally occurring element in the environment and may occur as elemental, inorganic and organic mercury. In the majority of cases, analyses of feed or animal tissues involve the measurement of the sum of all mercury (or “total mercury”) in the sample, regardless of the chemical form in which it is present.
- Human activities have contributed significantly to the contamination of the environment. Currently, coal combustion is the main source. Anthropogenic emissions to air have decreased globally over the last decades and are lower in the atmosphere of Europe and North-America.
- Mercury compounds are still in use for agricultural purposes in some non-European countries.
- Methylmercury is the prevalent form in aquatic organisms and bioaccumulates in the food chain, particularly in aquatic animals.
- Analytical methods for total mercury are satisfactory and routine methods for methylmercury in feed are emerging.

Occurrence in feed

- The most common source of mercury in feed materials for farmed animals is fishmeal. Relatively few data are available on the speciation of mercury in fish feed, nevertheless the available data showed that it is mainly present as methylmercury.
- In feed materials derived from plants, average mercury concentrations are generally low (between 0.03 and 0.047 mg/kg dry matter). For all complete feedingstuffs, except those for fish and pets, the average value is 0.03 mg mercury/kg feed.
- For pets, the average concentration in complete feedingstuffs is 0.02 mg mercury/kg feed.
- Less than 3% of all feedingstuffs analysed exceeded total mercury MLs.
- Complete feedingstuffs for fish generally have the highest mercury content compared with feeds for other food producing animals. The average value was 0.06 mg mercury/kg feed, with approximately 8 % exceeding the ML. In the category of feedingstuffs produced by the processing of fish or other marine animals, no samples exceeded the ML. This indicates that the current MLs for complete feedingstuffs for fish

and feedingstuffs produced by the processing of fish or other marine animals are not harmonized.

General toxicological effects

- The three forms of mercury, namely elemental, inorganic and organic mercury, have different toxicological properties.
- Effects on the nervous system appear to be the most sensitive endpoints following inhalation, not oral (negligible absorption), exposure to elemental mercury.
- Nephrotoxicity is the most sensitive endpoint following chronic ingestion of inorganic mercury.
- Methylmercury is the form of greatest toxicological concern. Development of the central nervous system is affected by the chronic oral exposure to methylmercury. The cardiovascular, immune and reproductive systems are also affected at higher doses.

Adverse effects in target animals

- Following chronic oral exposure, the most sensitive species are cats (NOAEL for methylmercury: 0.5 mg/kg feed expressed as total mercury) and mink (LOAEL for methylmercury: 1.1 mg/kg feed expressed as total mercury).
- Due to the weakness of the toxicological database and only single animal experiments, no NOAEL could be derived for dogs. Only a LOAEL of 8 mg/kg feed expressed as total mercury) could be derived.
- For young chickens, young pigs and young calves, the NOAELs were 2.2, 3.4 and 5.0 mg/kg feed, respectively. For sheep, turkeys and ducks LOAELs of 7.7, 1.7 and 5 mg/kg feed, respectively, were established. For rabbit and horses no NOAEL or LOAEL could be derived.
- For cats on the basis on the available data on the occurrence of total mercury in complete feedingstuffs, no effects are expected. However, when cats are fed continuously with feedingstuffs containing a high proportion of top predatory fish, the current ML for complete feed for cats and dogs (0.4 mg/kg feed) appear as not sufficiently protective.
- For salmonids, the NOAEL for methylmercury is 170 µg (expressed as total mercury)/kg b.w. corresponding to 630 µg/kg feed (dry weight). The current ML for complete feed for fish (0.1 mg/kg feed) is considered sufficiently protective.

Fate in animals and carry-over to animal products

- The absorption, distribution, metabolism and excretion of mercury are largely dependent on its chemical form. Inorganic mercury is absorbed to a limited extent (10-30%) while methylmercury is absorbed extensively (typically 80%) following oral exposure.
- Inorganic mercury does not easily cross membranes, but concentrates in the kidney. Methylmercury distributes in all tissues (preferentially muscle in carnivorous fish), crosses blood-brain and placental barriers, and concentrates in hair and feathers.
- The metabolic fate of inorganic and organic mercury, which is similar for animals and humans, involves the bacterial (rumen, gut flora) demethylation of methylmercury and the oxidation-reduction cycle of Hg^{++} and Hg^0 . Inorganic and methylmercury are mainly excreted in the faeces as Hg^{++} which is less effectively absorbed in the gut than organic mercury.
- Transfer of of organic and inorganic mercury to milk is about 1.2 and 0.2% of the dose respectively. It is limited to eggs (below 1%).
- Due to the lack of appropriate experimental data on mercury accumulation in domestic animals, it is not possible to calculate a transfer ratio of mercury into animal tissues, except for chicken meat.

Human exposure

- Fish and seafood are the main sources of human dietary exposure to mercury, and this is predominantly as methylmercury.
- Wild fish species that are low in the food chain have usually total mercury concentrations of less than 100 $\mu\text{g}/\text{kg}$ wet weight, whereas predatory fish may contain more than 1000 $\mu\text{g}/\text{kg}$ wet weight. Farmed fish fed pellets typically contain total mercury levels in the range of 8-100 $\mu\text{g}/\text{kg}$ flesh. Higher levels have been found in farmed tuna.
- The maximum concentration reported in farmed salmonids is approximately five times lower than the EU maximum level for mercury in fish (500 $\mu\text{g}/\text{kg}$ for salmonids). This mercury concentration in salmonids would allow weekly consumption of two fish meals, as recommended by nutritionists, without appreciable health risk. The ML for fish feed is sufficient to ensure that contamination levels in farmed salmonids pose no appreciable risk to consumers, but the validity of the ML need to be ascertained for other farmed fish.

RECOMMENDATIONS AND DATA NEEDS

- Although appropriate analytical methods are available for total mercury in feeds, definition of their quality performance criteria are needed.
- The analysis of methylmercury in feeds should be encouraged. Furthermore, intercomparison exercises on the analysis of methylmercury are required as well as the quality performance criteria for their use.
- Monitoring programmes should be more informative with respect to feed composition, and more systematic monitoring in terms of feed categories in the EU is needed. More data on occurrence of mercury in feed materials originating from Mediterranean countries should be made available.
- The Member States should be encouraged to report mercury levels as methylmercury and total mercury along with their respective concentrations rather than report the results as compliant or non-compliant for total mercury.
- There is a lack of data on contamination of farmed fish, except salmonids. Additional data on farmed carnivorous species, as compared with equivalent wild animals could help in estimating the capability of fish farming to reduce contamination of fish for consumption.

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- Iceland. The Icelandic Food and Veterinary Authority
- Ireland. Department of Agriculture,
- Norway. Norwegian Food Safety Authority.
- Slovak Republic. Central Control and Testing Institute of Agriculture.
- Slovenia. Veterinary Administration of Republic of Slovenia and University of Ljubljana.
- Spain. Catalan Agency of Food Safety.
- UK. Animal Feed Unit, Food Standards Agency.

EMFEMA. International Association of the European Manufacturers of Major, Trace and Specific Feed Mineral Materials. Belgium.

FEDIAF. The European Pet Food Industry Federation. Belgium.

FEFAC. The European Feed Manufacturers' Federation. Belgium.

Vereinigte Kreidewerke Dammann KG. Germany.

Ecosyl Products Ltd. UK.

Magnesitas de Rubián, S.A. Spain.

LIST OF ABBREVIATIONS

AAS	Atomic absorption spectrometry
AES	Atomic emission spectrometry
AFS	Atomic fluorescence spectrometry
ATSDR	Agency for Toxic Substances and Disease Registry
CNS	central nervous system
CV	Cold vapour
EC	Electron capture
EC	European Commission
EPA	Environmental Protection Agency
FEDIAF	European Pet Food Industry Federation
GC	Gas chromatography
GST	Glutathione S-transferase
HPLC	High performance liquid chromatography
IARC	International Agency for Research on Cancer
ICP	Inductively coupled plasma
IPCS	International Programme on Chemical Safety
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOAEL	Lowest observed adverse effect level
LOD	Level of detection
LOQ	Level of quantitation
MIP	Microwave induced plasma
ML	Maximum level
MS	Mass spectrometry
NMKL	Nordic Committee on Food Analysis
NMR	Nuclear magnetic resonance
NOAEL	No observed adverse effect level
PTWI	Provisional tolerable weekly intake
RPA	Risk and Policy Analysts Limited
SCAN	Scientific Committee on Animal Nutrition
SCOOP	Scientific co-operation on questions relating to food
UNEP	United Nations Environment Programme
US	United States (of America)
WHO	World Health Organization

ANNEX

Table A1. Estimates of mercury NOAELs and LOAELs in farm animals, pets and fish

Species	Age/weight	Mercury species	NOAEL mg/kg b.w. (mg/kg feed)*	LOAEL mg/kg b.w. (mg/kg feed)*	Exposure in days	Clinical symptoms. Biochemical and histological findings	Reference
Calves	4 week old 45-57 kg	MeHg	0.1 (5)	0.2 (10)	90	Ataxia, prostration Nephrosis, cerebellar cells atrophy	Herigstad, 1972
Cattle	Yearlings	MeHg	0.225 (11)		56-65	Incoordination, stiffness, insteady gait	Wright <i>et al.</i> , 1973
Cattle	Yearlings 172-254 kg	EtHg		0.48 (24)	27	Weakness, incoordination Enlarged kidneys, congestion of cerebral vessels	Palmer <i>et al.</i> , 1973
Sheep	Yearling	MeHg		0.225 (7.7)	42-59	Incoordination, stiffness, insteady gait	Wright <i>et al.</i> , 1973
	Yearling 30-37 kg	EtHg		0.48 (17)	12	Anorexia, diarrhea Liver, kidney, cranial vessels and intestine mucosa congested	Palmer <i>et al.</i> , 1973
Pigs	5 weeks old	MeHg	0.19 (3.4)	0.38(6.8)	60	Liver degeneration	Tryphonas <i>et al.</i> , 1973
		MeHg		0.78 (8)	41-46	Anorexia, incoordination Liver degeneration	Tryphonas <i>et al.</i> , 1973
		MeHg		0.5	27	Liver degeneration	Chang <i>et al.</i> , 1977
		HgCl ₂	(5)	(50)	27	Liver degeneration	Chang <i>et al.</i> , 1977
Chickens	Day old	MeHg	(2.2)	(5)	33-49	50% death	Soares, 1973
	Adult	MeHg		(10)		Decreased weight gain, drop in eggs production and fertility	Scott, 1975
	Hens	MeHg		3.3 (44)	50	Drop in eggs production.	Lundholm, 1995

Species	Age/weight	Mercury species	NOAEL mg/kg b.w. (mg/kg feed)*	LOAEL mg/kg b.w. (mg/kg feed)*	Exposure in days	Clinical symptoms. Biochemical and histological findings	Reference
	1.4 -1.6 kg					Alteration in egg shell	
Turkeys	16 week 6-9 kg	EtHg		0.16 (1.7-2.4)	13-42	Incoordination, weakness	Palmer <i>et al.</i> , 1973
Duck	Adult	MeHg		0.8 (11.2)		Reproductive impairment	Heinz, 1979
	Adult	MeHg		(5)		Deformities in ducklings	Heinz and Hoffman, 2003
Mink	Adult	MeHg		(1.1)	59-93	Anorexia, ataxia	Wobeser, 1976
Dogs	Adult	MeHg		0.12 (8)	385	No clinical signs; neuronal damage at histological examination	Davies <i>et al.</i> , 1977
				0.43 (28)	41-46	Anorexia, gait unsteady Neuronal, kidney and intestinal damages	Davies <i>et al.</i> , 1977
Cats		MeHg	0.020 (0.33)	0.046 (0.76)	420	Impaired hopping, ataxia, renal failure	Charbonneau <i>et al.</i> , 1976
Fish Atlantic Salmon (<i>S. salar</i>)	Parr	MeHgCl	0.17 (0.63)	1.2 (4.4)	112	Increased cell proliferation and elevated metallothionein, altered haematology	Berntssen <i>et al.</i> , 2004a
Rainbow trout (<i>O. mykiss</i>)			1.04 (21.6)		84	No effects on growth	Lock, 1975
Rainbow trout (<i>O. mykiss</i>)			(8)	(16)	105	Elevated blood packed cell volume and hyperplasia of gill epithelium	Wobeser, 1975

* expressed as mg/kg b.w. if figure is given without brackets and mg/kg feed if the figure is in brackets.

Table A2. Animal, intake and diet values used to calculate ruminant exposure levels in Table 9.

Livestock type	Live weight (kg)	Dry matter intake (kg/day)	% forage	% concentrates
Growing cattle	90	2.4	70	30
Growing cattle	200	5	85	15
Growing cattle	350	8.8	95	5
Dairy cow-dry	625	14	100	0
Dairy cow-lactating (20 kg milk/day)	625	18	75	25
Dairy cow-lactating (40 kg milk/day)	625	23	60	40
Sheep-growing lamb	30	0.8	100	0
Sheep-lactating ewe	70	2.2	40	60
Goats-lactating	80	2.6	20	80

Table A3. Animal and intake values used to calculate pig and poultry exposure levels in Table 10.

Livestock type	Body weight (kg)	Feed intake (fresh weight)(kg/day)
Growing pigs	30	1.5
Growing pigs	60	2.9
Growing/fattening pigs	90	3.3
Growing/fattening pigs	120	3.4
Dry sow	200	2.7
Lactating sow	200	6.5
Broilers (finishing stage)	2.5	0.15
Laying hens	3.5	0.115
Turkeys	16	0.65