Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to Fluorine as undesirable substance in animal feed

(Request N° EFSA-Q-2003-034)

Adopted on 22 September 2004

SUMMARY
Fluorine is one of the most abundant elements in the environment. Animals are exposed to the ionic form of the element (fluoride) which may be present in feed materials and drinking water. Moreover, ingestions of feed materials contaminated with soil for examples by ruminants and horses contribute to exposure in geographic areas with high natural fluorine concentrations, as the average concentration in soils is higher than the average concentration in plants. Fluorine is considered as an essential element in various animal species as experimental diets, low in fluorine resulted in growth retardation, impaired fertility and reduced dental enamel strength in various animal species, and also in humans. Excessive exposure, which is observed regularly in distinct geographic areas and incidentally in the proximity of industrial sites with high fluoride emission, is associated with dental and skeleton abnormalities. Fluoride accumulates in calcifying tissues (including egg shells) and stimulates calcium deposition in connective (peri-articular) tissues. The limited data available provided no evidence for toxic fluoride concentrations in natural pastures and mixed feeds in Europe. Incidental high concentrations in herbage grown in the proximity of industrial areas can, however, not be excluded. Transmission into edible tissues including milk and eggs is limited. Hence, the fluoride concentrations in foods from animal origin contribute only marginally to human exposure.

KEY WORDS
Fluorine, fluoride, animal feed, fluorosis
# TABLE OF CONTENTS

SUMMARY ............................................................................................................................... 1

BACKGROUND ......................................................................................................................... 3
  1. General background ............................................................................................................. 3
  2. Specific background ............................................................................................................. 4

TERMS OF REFERENCE ........................................................................................................ 5

ASSESSMENT .......................................................................................................................... 5
  1. Introduction ....................................................................................................................... 5
  2. Methods of analysis and current legislation for feed materials ...................................... 7
  3. Occurrence of fluorine in feed materials and animal exposure ..................................... 9
  4. Adverse effect on livestock ............................................................................................. 11
    4.1. Ruminants ............................................................................................................... 12
    4.2. Pigs ......................................................................................................................... 12
    4.3. Horses ..................................................................................................................... 13
    4.4. Rabbits .................................................................................................................... 13
    4.5. Poultry ..................................................................................................................... 13
    4.6. Fish ......................................................................................................................... 13
  5. Toxicokinetics .................................................................................................................. 14
    5.1. Absorption ............................................................................................................... 14
    5.2. Tissue Distribution ................................................................................................. 14
    5.3. Excretion ................................................................................................................. 15
  6. Carry over and tissue concentrations ............................................................................... 15
  7. Human dietary exposure ............................................................................................... 16

CONCLUSIONS ...................................................................................................................... 17

REFERENCES ......................................................................................................................... 17

SCIENTIFIC PANEL MEMBERS ......................................................................................... 22

ACKNOWLEDGEMENT ......................................................................................................... 22
BACKGROUND

1. General background


- extension of the scope of the Directive to include the possibility of establishing maximum limits for undesirable substances in feed additives.

- deletion of the existing possibility to dilute contaminated feed materials instead of decontamination or destruction (introduction of the principle of non-dilution).

- deletion of the possibility for derogation of the maximum limits for particular local reasons.

- introduction the possibility of the establishment of an action threshold triggering an investigation to identify the source of contamination (“early warning system”) and to take measures to reduce or eliminate the contamination (“pro-active approach”).

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

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

---

\(^1\) OJ L140, 30.5.2002, p. 10

\(^2\) OJ L 115, 4.5.1999, p. 32

\(^3\) Summary record of the 135\(^{th}\) SCAN Plenary meeting, Brussels, 21-22 March 2001, point 8 – New questions (http://europa.eu.int/comm/food/fs/sc/scan/out61_en.pdf)

http://www.efsa.eu.int

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

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

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

2. Specific background

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

A detailed risk assessment of the presence of fluorine in animal feed and the possible effects for animal health and public health is necessary and urgent as it appears that fluorine present at the maximum levels established in legislation for fluorine may affect the health of poultry, horse and rabbit as they are higher than their tolerance levels. Consequently a complete review of the maximum levels for fluorine on the basis of a detailed risk assessment is urgently necessary.

\(^4\) OJ L 38, 11.2.1974, p. 31
\(^6\) Opinion of the Scientific Committee on Animal Nutrition on Undesirable Substances in Feed, point 6.11. Conclusions and recommendations.

http://www.efsa.eu.int
TERMS OF REFERENCE

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

This detailed scientific opinion should comprise the

- determination of the toxic exposure levels (daily exposure) of fluorine for the different animal species of relevance (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 fluorine from the feed to the products of animal origin results in unacceptable levels of fluorine in the products of animal origin in view of providing a high level of public health protection.

- identification of feed materials which could be considered as sources of contamination by fluorine and the characterisation, insofar as possible, of the distribution of levels of contamination.

- assessment of the contribution of the different identified feed materials as sources of contamination by fluorine to the overall exposure of the different relevant animal species to fluorine.

- to the impact on animal health.

- to the contamination of food of animal origin (the impact on public health), taking into account dietary variations and variable carry over rates (bio-availability) depending on the nature of the different feed materials.

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

ASSESSMENT

1. Introduction

Fluorine belongs to the most abundant elements occurring in different chemical forms in the environment and in living organisms. Under normal conditions, fluorine (F; atomic weight 18.9984) is a gaseous element, with a strong, typical odour. The major source of fluorine is volcanic activity, resulting in an atmospheric emission of 1 - 9 million tons per year. Volcanic emissions also release acid smokes that contain hydrofluoric acid. Another natural source is

http://www.efsa.eu.int
deep well water. Fluorine almost exclusively occurs in the environment, food, water and plants in its ionic form (fluoride). However, the amount of fluoride present naturally in water is highly variable, depending upon the individual geological environment from which the water is obtained. In geographic areas of for example central Asia and India, in which endemic fluorosis is well documented, water may contain up to 20 mg fluoride/L (for review see Merian et al., 2004).

Due to its high electron affinity, fluorine is easily forming salts, and subsequently more than 100 fluoride minerals have been described. The most abundant inorganic fluorides in the earth’s crust are fluoroapatite (Ca$_5$(PO$_4$)$_3$F), fluorite (CaF$_2$) and cryolite (Na$_3$AlF$_6$)(CEPA, 1993). These inorganic fluorides are much more abundant than organic fluorides.

Fluorine in the form hydrofluoric acid (HF), silicon tetrafluoride (SiF$_4$), and fluoride derivatives can also be released from industrial sites associated with aluminium or phosphate processing, as well as steel and glass production (Cronin et al., 2000). These emissions can contaminate surface water, soil, and plants in the proximity of industrial sites and have resulted in Europe to unexpected high exposure rates in livestock in certain distinct areas (Bunce, 1985). Moreover, fluorides are natural components in phosphate and super-phosphate fertilizers used in agricultural practice. Fluoroapatite (rock phosphate) sources vary widely in their fluorine content, depending on their geographic origin. Fluoride-bearing rock phosphate is used in mineral supplements for livestock. High fluoride rock phosphates can be injurious to livestock when used over long periods, even in the amounts commonly applied to meet the calcium and phosphorus requirements of the animals. For this reason, rock phosphates should be (and are now routinely) de-fluorinated.

Uptake of fluorides by plants from the soil occurs via the roots and uptake from air through the stomata of the leaves. The availability of soil fluoride for plants depends on soil characteristics (pH, water content, organic matter content, cation and anion exchange capacities), the chemical form of the fluoride, and the individual plant species (Longanathan et al., 2001).

The average concentration of fluoride in soils is approximately 300 mg/kg, but may exceed 1000 mg/kg in soils on basic rocks. Fluorides are retained in roots and only poorly translocated to other parts of the plant. The relative accumulation index in herbage varies from 0.0001 to 0.1, according to the concentration of fluorides in soil (Geeson et al., 1998). Soluble fluorides taken up from the soil are converted into carbon-fluorine compounds, including monofluoroacetic acid, monofluorooleic acid, monofluoropalmitinic acid and monofluoromyristic acid, respectively. The significance of this conversion remains unknown. Since soil usually contains far higher fluoride concentrations than the plant, soil intake may be a major source of fluorine exposure to animals under certain feeding regimes.
Soluble fluorides are bio-accumulated by some aquatic and terrestrial biota, however, the significance of this biomagnification of fluorine in the aquatic or terrestrial food chains remains to be elucidated.

In mammals, fluorine is considered to be an essential element, as a low fluoride diet causes growth retardation and impairment of fertility in various animal species (Anke 1998, 2001). Fluorine deficiency in humans results in early tooth decay and probably in osteoporosis (Gabovich and Ovrutskyim, 1969, Anke et al., 1990, Avtsyn et al., 1991). Of medical importance is also the positive effect of fluoride in the prevention of dental caries, as it strengthens the enamel of teeth. Hence, in geographic regions with low natural fluorine levels, the drinking water is often supplemented with fluoride, and it is added routinely to health products for dental care. Moreover, fluoride is added to calcium products, used in the prevention and treatment of age-related fluorosis.

In contrast, in various regions of central Asia and the Indian subcontinent, fluorosis, mainly induced by too high concentration of fluoride in well water, is endemic (for review see WHO, 2002). Clinical signs of excessive fluoride intake comprise skeletal fluorosis (chronic joint pain, osteosclerosis and calcification of ligaments, progressing into skeletal deformities, intense calcification and muscle wasting), as well as and neurological deficits, reproductive effects (spontaneous abortion, no apparent malformations, but congenital cardiac diseases in children) and adverse respiratory effects (for details see WHO, 2002).

Symptoms of acute toxicity of fluorides in laboratory animals are generally non-specific. Experimentally induced chronic toxicity resulted in skeletal and dental fluorosis, associated with nephrotoxicity in rodents. Fluoride is genotoxic (clastogenic but not mutagenic) in human and animal cells in vitro, but do not appear to induce direct mutagenicity in vivo in laboratory animals. However, high concentrations may alter the response to mutagens. The evidence regarding the carcinogenicity of fluoride in laboratory animals is inconclusive (WHO, 2002; EC, 1996). IARC (1987) stated that the limited animal data available are inadequate for a reliable risk assessment. More recent NIH studies performed in rats and mice have only shown an increased incidence of osteosarcomas in male rats, and this effect was evaluated by NIH as equivocal evidence (NIH, 1990, US-NRC, 1993, EC, 1996).

2. Methods of analysis and current legislation for feed materials

The determination of fluoride in biological materials is carried out by alkali fusion and fluoride ion-selective electrodes (Malde et al., 2001). Sodium hydroxide is used as an ashing aid.

Trace levels of fluoride in biological media are determined primarily by potentiometric (ion selective electrode [ISE]) and gas chromatographic (GC) methods. Colorimetric methods are available, but are more time consuming and lack the sensitivity of the other methods.
(Kakabadse et al., 1971; Venkateswarlu et al., 1971). Alternative methods that have been used include fluorometric, enzymatic, and proton activation analysis (Rudolph et al., 1973). The latter technique is sensitive to trace amounts of sample, and requires minimal sample preparation. The most accurate method of sample preparation is microdiffusion techniques, such as the acid-hexamethyldisiloxane (HMDS). These methods allow the liberation of fluoride from organic or inorganic matrices (WHO 2002). Bone fluoride levels can be measured using the ISE technique after ashing of the sample (Boivin et al., 1990).

Numerous national regulations for fluorine exist with respect to drinking water, and the concentrations permitted in health products. As fluoride is also present in virtually all animal feed materials, currently the following maximum levels have been set in the European Union.

Table 1. Prescribed limits for total fluorine in feedingstuffs, mg/kg, at a moisture content of 12%7

<table>
<thead>
<tr>
<th>Feed materials with the exception of:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>- feedingstuffs of animal origin with the exception of marine crustaceans such as marine krill</td>
<td>500</td>
</tr>
<tr>
<td>- phosphates and marine crustaceans such as marine krill</td>
<td>2000</td>
</tr>
<tr>
<td>- calcium carbonate</td>
<td>350</td>
</tr>
<tr>
<td>- magnesium oxide</td>
<td>600</td>
</tr>
<tr>
<td>- calcareous marine algae</td>
<td>1000</td>
</tr>
<tr>
<td>Complete feedingstuffs with the exception of:</td>
<td></td>
</tr>
<tr>
<td>- complete feedingstuffs for cattle, sheep and goats in lactation</td>
<td>30</td>
</tr>
<tr>
<td>other</td>
<td>50</td>
</tr>
<tr>
<td>- complete feedingstuffs for pigs</td>
<td>100</td>
</tr>
<tr>
<td>- complete feedingstuffs for poultry</td>
<td>350</td>
</tr>
<tr>
<td>- complete feedingstuffs for chicks</td>
<td>250</td>
</tr>
<tr>
<td>Mineral mixtures for cattle, sheep and goats</td>
<td>2000</td>
</tr>
<tr>
<td>Other complementary feedingstuffs</td>
<td>125</td>
</tr>
</tbody>
</table>

3. Occurrence of fluoride in feed materials and animal exposure

Concentrations of fluoride in soils vary considerably depending on geographic conditions as well as industrial pollution. In contrast to many heavy metals, mobility of fluorine in soil is very limited. Furthermore, most plant species have a limited capacity to absorb fluoride from the soil, even when fluoride-containing fertilisers are applied.

Data of the occurrence of fluoride in feed materials have been made available by a number of individual EU member states or taken from reports published within the EU. Some of these data are difficult to evaluate because of limited information on the nature of the samples (e.g. compound feed without any detailed information on designated species) or inadequate sample description. The data for feed materials that can reliably be categorised are summarised in table 2, and for commercially manufactured compounds or complementary feeds in Table 3.

Table 2. Fluoride concentrations (mg/kg dry matter) in certain feed materials, expressed as fluorine.

<table>
<thead>
<tr>
<th>Feed Material</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>n =</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish meal</td>
<td>159</td>
<td>57.7</td>
<td>164</td>
<td>109</td>
<td>250</td>
<td>5</td>
</tr>
<tr>
<td>Meat and bone meal</td>
<td>180</td>
<td>23.5</td>
<td>178</td>
<td>132</td>
<td>201</td>
<td>7</td>
</tr>
<tr>
<td>Palm kernel expeller meal</td>
<td>46</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Rapeseed meal</td>
<td>10</td>
<td>0.8</td>
<td>10</td>
<td>8.6</td>
<td>10.3</td>
<td>5</td>
</tr>
<tr>
<td>Soya bean meal</td>
<td>11</td>
<td>2.4</td>
<td>12</td>
<td>5</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>Sugar beet pulp (fresh and dried)</td>
<td>244</td>
<td>145.3</td>
<td>261</td>
<td>6</td>
<td>588</td>
<td>192</td>
</tr>
</tbody>
</table>

With the exception of sugar beet pulp, none of the samples analysed contained fluorine levels in excess of the maximum permitted level for that category of feed material. Insufficient numbers of forage analyses were reported to allow any meaningful summary to be made. Forages from uncontaminated pastures usually contain between 5 and 16 mg fluorine/kg dry matter (Allcroft et al., 1965), while concentrations in cereals and cereal by-products are generally < 3 mg/kg dry matter (US-NRC, 1980). Exceptions to this are crops that have been contaminated by fumes or dust from industrial processes or by irrigation with fluoride-rich water. Therefore, with the exception of the latter, fluoride in feed materials are unlikely to exceed maximum permitted levels.

Since it is stored in the bones of animals, it is not surprising that elevated levels of fluoride have been reported in fishmeal and meat and bone meal.

---

8 Where data have been reported as being below the level of detection, e.g. < 0.1 mg/kg, a value of half of the level of detection (in this case (0.05 mg/kg) has been used in calculating the mean and standard deviation.
Exposure of animals to fluoride is a function of the concentration of fluorides in the feed, and the amount of feed consumed. In order to estimate the intake of, and level of exposure to fluoride, it is necessary to have estimates of both the likely intake (g or kg of dry matter per day) of feed material for each class of livestock, and typical concentrations.

In estimating dietary exposure to fluoride, two approaches were considered. The first was to describe typical inclusion rates for feed materials used in the manufacture of compound feeds, and to calculate the final fluoride concentrations. However, this approach had to be rejected, primarily because information on many individual raw materials was scarce or not available. The alternative approach was to use data for manufactured compound feeds. This is the approach that was used previously by SCAN in its reviews of zinc and copper, and it has been adopted in this report. Information on the fluoride concentrations in complete feedingstuffs and complementary feedingstuffs, obtained as part of routine surveillance in a number of Member States, are summarised in Table 3. The maximum permitted levels of total fluorine in these feeds are given in Table 1.

Table 3. Mean concentrations of fluorine (mg/kg dry matter) in commercial compound feeds for farm livestock and fish (data reported by EU member states)\(^9,10\)

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>n =</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poultry - Layers</td>
<td>24</td>
<td>10.7</td>
<td>22</td>
<td>11</td>
<td>42</td>
<td>9</td>
</tr>
<tr>
<td>Poultry - Broilers</td>
<td>24</td>
<td>8.9</td>
<td>25</td>
<td>15</td>
<td>39</td>
<td>7</td>
</tr>
<tr>
<td>Poultry - unspecified</td>
<td>32</td>
<td>14.1</td>
<td>31</td>
<td>22</td>
<td>42</td>
<td>2</td>
</tr>
<tr>
<td>Fish</td>
<td>30</td>
<td>10.8</td>
<td>29</td>
<td>8</td>
<td>88</td>
<td>354</td>
</tr>
<tr>
<td>Pigs &lt; 17 weeks</td>
<td>14</td>
<td>13.0</td>
<td>11</td>
<td>2</td>
<td>33</td>
<td>4</td>
</tr>
<tr>
<td>Pigs &gt; 16 weeks</td>
<td>23</td>
<td>6.9</td>
<td>24</td>
<td>12</td>
<td>31</td>
<td>7</td>
</tr>
<tr>
<td>Pigs - unspecified</td>
<td>16</td>
<td>7.9</td>
<td>11</td>
<td>3</td>
<td>33</td>
<td>37</td>
</tr>
<tr>
<td>Ruminants - unspecified</td>
<td>17</td>
<td>12.5</td>
<td>22</td>
<td>2</td>
<td>33</td>
<td>8</td>
</tr>
</tbody>
</table>

On the basis of the data available, it would appear that compound feeds for farm livestock do not exceed maximum permitted concentrations. However, it should be noted that with the exception of fish feeds this conclusion is based on very few samples.

For the majority of non-ruminant livestock (pigs and poultry as well as farmed fish) in the EU, feed is provided as compounded feed, consisting of a mixture of individual feed components, to which additives and/or mineral supplements are added. Intake of fluorine may therefore be estimated by multiplying the concentrations given in Table 3 by the estimated intake of the compound for the particular class of livestock. Estimating fluoride intake by ruminants is less straightforward. For these animals, the daily ration usually consists of forage

---

\(^9\) Data obtained as part of routine surveillance of feed materials, and provided by Member States.

\(^{10}\) No data on horse or rabbit feeds have been provided.

[http://www.efsa.eu.int](http://www.efsa.eu.int)
(or mixture of forages), either fresh or conserved, to which may be added complementary feeds or individual feed materials as necessary to achieve the required level of production (growth rate, milk yield). The proportions of different feed materials in the diet are influenced by many factors, including their nutritional value and the required level of production. However, combining data for feed materials (Table 2), compound feeds for ruminants (Table 3), and assuming normal levels of fluoride in forages, suggests that typical dietary concentrations are well below the maximum permitted level for complete feedingstuffs as specified in Directive 2003/100/EC.

However, reference has been made to the fact that soils may contain significantly higher fluoride concentrations than plants growing on them. As a result, consumption of soil during grazing (or consumption of soil-contaminated feeds) is an additional factor contributing to total exposure of livestock (Loganathan et al., 2001 and references therein).

4. **Adverse effect on livestock**

The pathological results of skeletal fluorosis include dissociation of the normal sequences in osteogenesis, acceleration of bone remodelling, production of abnormal bone (exostosis, sclerosis) and in some cases accelerated bone resorption (osteoporosis). Clinical signs comprise stiffness and lameness and in severe cases animals refuse to stand, moving instead on their knees. The stiffness and lameness are primarily associated with osteofluorotic lesions and calcification of peri-articular structures and tendons preceding skeletal deformation.

Excess fluoride intake produces dental fluorosis in animals (like in humans) affecting the teeth during development. Specific ameloblastic and odontoblastic damage may be caused by high fluoride intake and varies directly with the levels consumed. Faulty materialisation results when the matrix laid down by damaged ameloblasts and odontoblasts fails to accept minerals normally. Once a tooth is fully formed, the amenoblasts have lost their constructive ability and the enamel lesions cannot be repaired. Oxidation of organic material in the teeth results in brown or black discoloration, which is a prominent sign of in dental fluorosis. This discoloration may serve as diagnostic parameter in veterinary medicine (Shupe et al., 1992).

Reduced uptake and loss of condition are most likely the consequences of these dental abnormalities. The tolerance of livestock towards fluoride in feeds varies depending on the form in which they are present, and the presence of calcium and phosphates, but also aluminium, in the diet, and the general nutritional status of the animal. Moreover, other sources than feed, including drinking and the intake of soils, have to be considered.
4.1. Ruminants

Cattle is generally described as the most frequently affected animal species, but this might reflect the relatively high exposure due to the typical feeding pattern of these animals (high plant, soil water and mineral intake) rather than a specific sensitivity.

In dairy cattle, rations containing more than 150 mg of fluorine/kg of diet for a period of 1 month were associated with reduced feed intake and slightly reduced milk production (Suttie and Kolstad, 1977). Minor morphological lesions can occur in young cattle receiving as little as 20 mg of fluorine /kg of diet when teeth are developing rapidly, but the relationship between these minor lesions and animal performance is unknown.

Fluoride crosses the placental barrier of cows, and fluoride levels in the bones of the offspring are correlated with the fluoride concentrations of maternal blood (US-NRC, 1980). However, bone fluoride concentrations of calves delivered from cows consuming as much as 108 mg/kg dietary fluoride were low (Hobbs and Merriman, 1962), and it appeared that neither placental fluoride transfer nor milk fluoride concentrations were sufficient to adversely affect the health of these calves.

In 8 - 12 months old sheep receiving a concentrate mixture containing 25, 50, 75, 100 or 200 mg/kg fluorine, respectively, over a period of 140 days, the growth rate was significantly reduced only in the animals receiving the highest dose of 200 mg/kg. In animals of the same age receiving 100 mg/kg feed over a period of 3 years, the weights of ewes and their lambs remained normal (Hobbs et al., 1954). Experiments on 9 month-old animals given water containing 30 mg/L fluorine during 25 months resulted in adverse effects on growth after 32 weeks and signs of fluorosis after 72 weeks (Said et al., 1977).

On the basis of observations made on animals coming from polluted areas and showing fluorosis, Milhaud et al. (1983) claimed that the sensitivity of goats to fluorine is comparable to that of sheep, whereas lambs proved to be more resistant to fluorine as compared to goat kittens.

4.2. Pigs

Pigs seem to tolerate feed concentrations of more than 100 mg fluoride/kg dry matter (Gueguen and Pointillart, 1986). Previous studies of Spence et al. (1971) described already that a dose of 1 mg F/ kg bodyweight had no influence of weight gain, feed intake or reproduction. Typical signs of intoxication at very high doses include irregular calcification of teeth and jaw bones, constipation and loss of appetite.
4.3. Horses

No controlled studies have been conducted to determine the sensitivity of horses to graded amounts of dietary fluoride. Early reports from Shupe and Olson (1971) and Spencer et al. (1971) describe the typical dental lesions induced by fluorine in horses, but provide no data on the rate of exposure in these cases.

4.4. Rabbits

Data on the sensitivity of rabbits towards fluoride exposure are scarce. Experimental data indicated that a concentration of 150 mg F/L in drinking water given over a period of 6 months, induced no gross pathological symptoms, and failed to alter antioxidant enzyme activity (Reddy et al., 2003). In contrast, the application of 10 mg NaF/kg body weight daily for a period of 18 months provokes severe abnormalities of sperm cells, suggesting a role of fluoride in impaired fertility of the male rabbit (Kumar et al., 1994).

4.5. Poultry

Sodium fluoride (150, 300 or 600 mg/kg feed) was added to the basal ration of male and female chickens when they had reached the age of 98 days. No alterations in body weight gain, total feed consumption, feed conversion and mortality (as measured until the age of 158 days were observed (Mehdi et al., 1983). Subsequently, egg production started on day 157 - 158 in all groups. The egg production rate over 70 days showed a tendency to decrease as the level of added fluoride rose. When broilers and laying hens were fed sodium fluoride at a level of 1000 and 1500 mg/kg feed, respectively, for 3 months, mean egg weight, feed consumption and body weight gain decreased (Seddek et al., 1977). Feeding studies with turkeys indicated that levels up to 400 mg F/kg feed are tolerated over the entire lifespan, whereas feed consumption was decreased when fluorine was present in a concentration of 800 mg/kg feed (Anderson et al., 1955). In layers, fluoride accumulates not only in skeletal tissues, but also in the egg shell (Machalinski, 1996, Nogareda et al., 1990).

4.6. Fish

Although several papers deal with waterborne exposed fish, few studies have been conducted on the dietary toxicity of fluoride in fish. Rainbow trout tolerated high fluoride concentrations (more than 2500 mg/kg for 82 days) in their diet (Tiews et al., 1982).

Fluoride concentrations in fresh water are relatively low (about 0.2 mg/L), but are several fold higher in sea water (approximately 1.3 mg/L). Hence fluoride concentrations are high in certain marine organisms such as krill (Julshamn et al., 2003), which is a natural feed source for wild fish (Grønvik and Klemetsen, 1987). The fluoride content in krill meal ranges from

http://www.efsa.eu.int
1000 to 3000 mg/kg, most of the fluoride is associated with the exoskeleton (Julshamn et al., 2003).

5. Toxicokinetics

5.1 Absorption

Fluoride compounds with low solubility like calcium, magnesium or aluminium fluorides are poorly absorbed while fluoride ions released from readily soluble fluoride compounds such as sodium or hydrogen fluoride, fluorosilicic acid and monofluorophosphate are almost completely absorbed from the gastrointestinal tract by passive diffusion in monogastric species. Conditions of high gastric acidity favour absorption, whereas alkalinity decreases fluoride absorption. Fluoride from various sources may be absorbed at different rates, as described by Clay and Suttie, 1985):

<table>
<thead>
<tr>
<th>Source</th>
<th>relative absorption rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaF</td>
<td>100 %</td>
</tr>
<tr>
<td>Raw rock phosphate</td>
<td>69 %</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>52 %</td>
</tr>
<tr>
<td>Defluorinated phosphate</td>
<td>20 %</td>
</tr>
</tbody>
</table>

As far as sodium fluoride or fluoride derivatives present in plants are concerned, the fraction actually absorbed by ruminants is approximately 75 % (Shupe et al., 1962). However, in assessing the bioavailability of fluoride contained in soil in sheep, Milhaud et al. (1990) found that digestive absorption ranged between 4.5 and 23 % of ingested dose. This percentage was between 30 and 41 % for bovine species (Wöhlbier et al., 1968). In humans, soluble fluorides are rapidly and extensively absorbed (virtually 100 % of the ingested dose). In the presence of other ions (calcium, magnesium, phosphorus, aluminium), the rate of absorption may be altered significantly (ATSDR, 1993).

5.2 Tissue Distribution

Absorbed fluoride is sequestered in bones and teeth were it interacts with the hydroxyapatite of calcified tissues (Kaminsky et al., 1992, Hamilton, 1992). The most efficient uptake of fluoride into bones and teeth occurs in juvenile animals during periods of rapid development. Thus mature bone takes up fluoride considerably slower than newly forming bone. Higher concentrations of fluoride are also found in surface layers of mineral structures than in deep layers, and fluoride released during bone remodelling is largely re-deposited (Guo et al., 1988). In goats receiving during 3 years 2.5 mg fluorine/kg/day as sodium fluoride orally in 10 mL water, fluorine residues in bone varied between 4400 – 6600 mg/kg. Results of long-term experiments with beef and dairy cattle indicate that after 7.5 years of exposure to 10, 30, 50 and 100 mg/kg dietary fluoride, fluorine concentrations of 1000, 2000, 5000 and 8000 mg/kg were found in rib and mandibule (Puls, 1994). In cattle grazing fluoride-contaminated pastures, bone ash concentrations may reach 10,000 mg fluorine per kg bone (about 5.5 % of

http://www.efsa.eu.int
the total concentration of phosphorus) (US-NRC, 1980). The concentration of fluoride in bones varies, however, with age, sex and the type and specific part of the skeleton and is believed to reflect an individual’s long-term exposure to fluoride. In contrast to chelating agents, fluoride is not irreversibly bound to bone tissues, and is mobilized continuously from the skeleton and subsequently excreted (Baars et al., 1987; WHO, 2002).

Only minor concentrations of fluoride are measurable in body fluid and soft tissues/Concentrations found in brain and kidneys were 7.9 and 2.6 mg/kg respectively, whereas in muscle, liver and milk the levels remained below 0.5 mg/kg (Milhaud et al., 1983). Placental transfer of fluoride to the developing foetus has been demonstrated in rats (Theuer et al., 1971) and humans (Gedalia et al., 1961). Nevertheless, a partial placental barrier may exist at high maternal fluoride levels (Gedalia et al., 1970).

Pharmacokinetic models for exposure to fluoride have been developed for the growing pig (Richards et al., 1985) and the ewe (Joseph-Enriquez et al., 1990). A three compartment open model was selected to describe fluoride disposition. The mean half-life was 0.7 hours in the pig and 2.6 hours in sheep.

5.3. Excretion

Ingested fluoride is rapidly eliminated with urine, but only about half of the ingested dose will be actually excreted, as the remainder accumulates in calcifying tissues, as mentioned above. Only a minor percentage of the given dose is excreted with the faeces, as demonstrated in sheep, in which renal excretion accounted for 2.1 – 9.6 % of fluoride dose (Milhaud et al., 1990). Experimental data indicated that the degree of saturation of skeletal tissue of animals affect the relative amount of fluoride retention. It has also been found that farm animals that shifted form high level of fluoride intake to low levels, reduced the urinary excretion of fluoride and at the same time start to mobilize some fluorine form skeletal tissues, maintaining a constant blood level (Mitchell et al., 1952).

6. Carry over and tissue concentrations

Results of experimental studies designed to assess residue formation, confirmed that fluoride mainly accumulates in bone and teeth (Patra et al., 2000), and skeletal retention of fluoride was approximately proportional to the concentration of fluoride in the diet. In soft tissues fluoride levels are very low (generally < 2.5 mg/kg wet weight,) even following high levels of dietary exposure (Puls, 1994). Only tendon (Armstrong and Singer, 1970), aorta (Ericsson and Ullberg, 1958) and placenta have higher fluoride concentrations than other soft tissue, possibly associated with their relatively high levels of calcium and magnesium.

In fresh water fish (rainbow trout, given feed containing high amounts of fluorine (2538 mg/kg feed) for a period of 82 days, residues in muscle tissue were approximately 4.5 mg/kg
wet weight, whereas in the skeleton up to 2450 mg/kg tissue were found (Tiews et al., 1982). In Atlantic salmon tissues contained fluorine concentrations up to 1.4 mg/kg wet weight in muscle tissue, and 5.8 – 7.2 mg/kg in the skeleton, respectively, after being fed with a diet containing graded amount of fluorine varying from 18 – 358 mg/kg feed (Julshamm et al., 2003).

Milk fluoride concentrations are affected only to a minor extend by dietary fluoride. Greenwood et al. (1964) found that when Holstein cows were fed 10, 29, 55 and 109 mg/kg dietary fluoride over the entire life time (3 months to 7.5 years of age), milk fluoride concentrations were 0.06, 0.10, 0.14 and 0.20 mg/L respectively.

7. Human dietary exposure

In continental Europe, drinking water concentrations are generally below 3 mg F/L (only in some Scandinavian countries fluoride levels up to 9 mg/L have been found. In public water works in most cases the fluoride concentration is even below 1 mg/L. Hence fluoride had been added to drinking water in certain countries (EC, 1996). In contrast, in many other parts of the world, fluorosis occurs as an endemic disease condition, due to as high amounts of fluorine (20 – 45 mg/L) in well-water supplies (WHO, 1984, Kaminsky et al., 1990, US-DHHS, 1991, WHO, 2002). Reviewing common foodstuffs, it becomes evident that high fluorine concentrations may be found in fish (0.06 – 4.57 mg F/kg (Dubeka and McKenzie, 1995), vegetables form distinct regions (0.01 – 1.34 mg F/mg) (Chen et al., 1996), fruits and fruit juices (0.1 - 2.8 mg F/kg) (Kiritsy et al., 1996), beverages (0.02 - 1.28 mg F/kg) (Heilman et al., 1999), and tea (Wei et al., 1989, Bergmann, 1995), whereas edible tissues, as well as milk and eggs from farm animals contain only low amounts of fluoride.

In conclusion, whereas endemic areas of fluorosis have been identified, where an excessive exposure is related to local drinking water resources, in many industrialized countries with a complete water cleaning system dietary intake of fluoride might be rather low, implying that drinking water is sometimes even fortified with fluoride to achieve protection against dental caries.
CONCLUSIONS

- Fluorine (F) is an abundant element in the environment. It may affect human and animal health following exposure to high levels. Endemic outbreaks of fluorosis have been observed in certain geographic areas where water from natural wells contains high levels of fluorides.

- Fluoride contributes to teeth and dental enamel strength. In some animals, fluorine is considered to be an essential element, as diets low in fluoride impaired fertility and development.

- Adverse effects of fluoride are frequently reported resulting from locally high fluoride concentration in the soil, pastures and local drinking water supplies. In these cases, dental abnormalities can be observed, followed by reduced feed intake and subsequent production losses and ultimately skeletal fluorosis.

- In Europe, fluoride levels in the environment are generally low, and fluoride uptake by herbage is limited. Subsequently, exposure of animals to fluoride is generally below the limit causing detrimental effects. However, in certain industrial areas, fluoride emission can be high, contaminating surface water and plants, and should be monitored on a regular base.

- A detailed assessment of the likely exposure of livestock to fluoride is not possible due to the very limited amount of data on the fluoride concentrations in feed materials and compound feeds. Moreover, exposure of animals will also result from fluoride containing drinking water, and the ingestion of contaminated soils.

- Fluoride accumulates particularly in calcifying tissues, which are normally not consumed. In contrast, fluoride levels in edible tissues, including milk and eggs, are low, and do not contribute significantly to human exposure.

REFERENCES


Machalinski, B. 1996. Concentration and distribution of fluorine in eggs as an aspect of selected parameters. Ann Acad Med Stetin, 42, 25028,


http://www.efsa.eu.int


SCIENTIFIC PANEL MEMBERS


ACKNOWLEDGEMENT

The Scientific Panel on Contaminants in the Food Chain wishes to thank George Bories, Bruce Cottrill, Wolfgang Dekant, Johanna Fink-Gremmels, Jürgen Gropp, Karl Honikel, Gerard Keck, Martha Lopez Alonso and Anne-Kathrine Lundebye Haldorsen for the contributions to the draft opinion.