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OPINION OF THE SCIENTIFIC COMMITTEE ON ANIMAL NUTRITION ON UNDESIRABLE SUBSTANCES IN FEED

(Adopted on 20 February 2003, updated on 28 March 2003)

1. BACKGROUND

Council Directive 1999/29/EC of 22 April 1999 on the undesirable substances and products in animal nutrition is currently under revision.

The annex I of Directive 1999/29/EC lists the undesirable substances and fixes their maximum permissible levels in feed materials, premixtures, complete and complementary feedingstuffs. These substances are summarised hereafter.

Table 1. Substances listed in annex to Council Directive 1999/29/EC

A	IONS or ELEMENTS	B	PRODUCTS	C	BOTANICAL IMPURITIES
1	Arsenic	**1**	Aflatoxin B1	**1**	Apricots (Prunus armaniaca);
2	Lead	**2**	Hydrocyanic acid	**2**	Bitter almond (*Prunus dulcis var amara = Prunus amygdalus var. amara*),
3	Fluoride	**3**	Free gossypol	**3**	Unhusked beech mast (*Fagus silvatica),*
4	Mercury	**4**	Theobromine	**4**	Camelina (*Camelina sativa*)
5	Nitrites	**5**	Volatile mustard oil	**5**	Mourah, Bassia, Madhuca
6	Cadmium	**6**	Vinyl thiooxazolidone	**6**	Purghera (*Jatropha curcas*)
		7	Rye ergot (*Claviceps purpurea*)	**7**	Croton (*Croton tiglium*)
		8	Weed seeds and unground and uncrushed fruits containing alkaloids, glucosides or other toxic substances separately or in combination including *Lolium temulentum, Lolium remotum, Datura stramonium;*	**8**	Indian mustard (Brassica juncea ssp. Integrifolia)
		9	Castor oil plant (*Ricinus communis*)	**9**	Sareptian mustard (*Brassica juncea ssp juncea*)
		10	Crotalaria	**10**	Chinese mustard (*Brassica juncea ssp juncea var. lutea*)
		11	Aldrin	**11**	Black mustard (*Brassica nigra)*
		12	Dieldren	**12**	Ethiopian mustard (*Brassica carinata*)
		13	Camphechlor (Toxaphene)		
		14	Chlordane		
		15	DDT		
		16	Endosulfan		
		17	Endrin		
		18	Heptachlor		
		19	Hexachlorobenzene (HCB)		
		20	Hexachlorocyclo-hexane (HCH) (alpha, beta, gamma isomers)		
		21	Dioxins		

Some Member States as well as the European Parliament expressed the wish that the requirements for certain substances listed above be reviewed, in particular mercury, cadmium, lead and aflatoxins or drew the attention of the Commission on the need to assess new substances such as ochratoxin A, deoxynivalenol, fumonisins,

zearalenone or polycyclic aromatic hydrocarbons (PAH), for their possible inclusion as undesirable substances.

As a consequence, the Commission intends to review the provisions laid down in Annex I of the Directive. This exercise should be based on updated scientific risk assessments and should take into account the prohibition of any dilution of contaminated non-complying material intended for animal nutrition.

2. TERMS OF REFERENCE

As a consequence, the Commission requests the Scientific Committee on Animal Nutrition

2.1. to identify among the undesirable substances currently in annex I of Directive 1999/29/EC

- those substances, products or botanical impurities of which the listing as undesirable substance has become completely obsolete
- those substances, products or botanical impurities which can be on the basis of their toxicological profile considered as priority for evaluation

2.2. to evaluate all the undesirable substances and products identified under 2.1. starting with those identified as priority, and in any case, mercury, cadmium, lead and aflatoxin.

The evaluation should comprise for each undesirable substance the

(a) identification of feed materials which could be considered as sources of contamination for that contaminant and the characterisation, as far as possible, of the distribution of levels of contamination

(b) assessment of the contribution of the different identified feed materials as sources of contamination to the contamination of food of animal origin (taking into account dietary variations and carry over rates from feed to food)

(c) impact on animal health

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

2.3. to identify and evaluate possible new undesirable substances. This evaluation should consider the aspects (a) to (d) listed under 2.2.

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ACKNOWLEDGEMENT

SCAN adopted this opinion on the basis of the preparatory work done by the SCAN ad'hoc Working Groups, which included members of the Committee and the following external experts:

Ions and elements

- Prof.Dr. H. Schenkel

Mycotoxins

- Prof.Dr. J. Bauer
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- Dr S. Dänicke
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GENERAL INTRODUCTION

The mandate given to the Scientific Committee requests and requires:

- a review of the the items (substances/organisms) already classified as undesirable with the intention of establishing whether there is a need for their continued listing (paragraph 2.1 of the terms of reference)
- the identification on the basis of available knowledge of items that should be considered for addition to the existing list (paragraph 2.3 of the terms of reference), and
- a detailed risk assessment of all the items retained under 2.1 or newly identified under 2.3 (paragraph 2.2 of the terms of reference).

The detailed risk assessment of the substances already listed, and any new additions considered undesirable, requires the collection and analysis of a substantial amount of data. To facilitate this process the Scientific Committee on Animal Nutrition has elected to approach this exercise in two consecutive stages:

– the first stage is limited to addressing the requirements of paragraphs 2.1 and 2.3

– the second and subsequent stage will concentrate on the detailed evaluation on the basis of priorities established in agreement with the risk manager.

The present opinion represents the outcome of the first stage of this exercise. It is therefore not a detailed risk assessment but a general review of the substances already listed and of those that could also be considered for listing, on the basis of the current scientific knowledge.

Although not a detailed risk assessment, this document is intended to highlight issues that SCAN considers important and to provide sufficient information to enable the risk managers to establish priorities for the further evaluation of the items considered undesirable. Four categories of substances were distinguished among those currently listed and addressed independently:

- ions and elements,
- mycotoxins,
- organic chemical contaminants, and
- botanical impurities.

These categories differ from the three identified in the annex to the present Directive. Consequently some items are to be found under a different heading in this Opinion than in their original listing.

IONS AND ELEMENTS

6.1. Introduction

Probably all elements mentioned below are essential, but the requirements are very low (ultra-trace elements) and deficiences have never been observed (unless provoked under experimental conditions) because these elements are ubiquitous and present to a sufficient extent in feeding materials. Like all trace elements, these elements are also tolerated only up to a certain limit by animals and humans. Above that limit, their intrinsic toxic potential (for animals and/or humans) leads to detrimental effects. In most cases the toxicity depends to a great extent upon the chemical form (for instance organic or inorganic). For the evaluation of the toxicity this aspect needs particular considerations.

Being either from geologic origin or anthropogenic (air, soil contamination), elements have an uneven distribution. As a consequence, their occurence in feed materials and, therefore, in feedingstuffs is variable and may exceed tolerable levels. For instance, concomitant presence of elements in phosphates obtained from mining is well-known. For that reason the toxic aspect of these elements is more important than meeting the requirements. Therefore, these elements naturally present or brought by anthropogenic contamination are considered as undesirable not only because of toxic effects in animals fed contaminated diets, but also because of a possible increase of human exposure due to residues in food of animal origin.

6.2. Lead

Lead is found in nature mainly as sulfide, but also as carbonate, sulfate and chromate. Lead is widely used for technical purposes in both organic and inorganic forms. This has led to its widespread distribution in the biosphere (air dust, vapour). Consecutive to the ban of the use of organic lead in petrol (tetraethyl lead)[1], the contamination of the environment by this chemical form is decreasing.

In uncontaminated rural areas lead concentration in soils is lower than 50 mg/kg. In soil, anthropogenic pollution can lead to 1000 mg lead/kg soil. Further sources of lead contamination are use of higher contaminated sludges and wastes as fertilizers and industrial emissions. Soil/plant transfer is relatively low and translocation from the roots to the other plant organs is very limited. Higher concentration in plant material is in most cases a result of soil or dust contamination. Some aerial deposits of lead are absorbed through the cuticle (Höll and Hamp, 1975). Higher body burden of lead in domestic

[1] Directive 98/70/EC of the European Parliament and of the Council of 13 october 1998 relating to the quality of petrol and diesel fuels and amending Council Directive 93/12/EEC (E.C.O.J. n° L 350 of 28/12/1998, p. 58. (Article 3.3: Pb < 0,15 g/l)

animals is mostly caused by airborne deposition of lead on the surface of plants and on soil.

Lead in drinking- and groundwater in most European countries is lower than 20 µg/l. Higher concentration could be observed in areas with soft water and the usage of leaded pipes. The concentration of lead in seawater is lower than in freshwater, and is in the range of 0.03-0.4 µg/l (WHO, 1977). According to Evers and Schlipköter (1991) the average lead concentration in surface ocean water is about 0.001 – 0.03 µg/l. Newer values from the east Adriatic cost near Croatia were at 0.29 µg/l for total lead and 0.0031 µg/l for organic lead (Mikac *et al*., 2001). Although lead exists in many different forms in marine and fresh waters, most of the lead found in fish is bound to proteins.

Lead impurities are often present in mineral feed material, like phosphates, and can contribute significantly to the diet contamination, even within the fixed limits. Recent analytical data from Germany indicate that feedinstuffs contain on an average the following lead contents (mg/kg dry matter): grass: 1.2; grass silage: 3.9; corn silage: 2.0; cereals: 0.2; soybean meal: 0.3; rapeseed meal: 0.2; milk replacer: 0.2; complete feed for pigs and poultry: 0.2-0.7; concentrates for dairy cows: 0.9; mineral mixture for cattle: 5.2 (KTBL, 2003). In Germany 0.7 % of feedingstuffs and 0.4 % of compound feed exceeded the legal limits in 2000 (Bundesministerium für Verbraucherschutz, Ernährung und Landwirtschaft, 2000). The lead content reported in fish feed is between 0.04 and 0.6 mg/kg.

Lead is a chronic and cumulative poison. Its effects have been extensively studied in humans. It affects enzymes, provokes anemia, renal toxicity, carcinogenicity, has cardiovascular and neurological/behavioural impact or negative consequences on the reproductive system.

Limit in complete feedingstuffs fixed by Council Directive 99/29/EC is 5 mg/kg for all animals. Most farm animals tolerate *ca.* 30 mg lead /kg feed (NRC, 1980), with the exception of sheep which seems to tolerate only 10 mg lead / kg feed (Puls, 1994). Fish (rainbow trout (*Oncorhynchus mykiss*) appears to be more tolerant (up to 210 mg lead/kg feed for two months) (Mount *et al*., 1994). Clinical symptoms in animals fed higher levels are mainly inappetence, anorexia, growth depression, anaemia, constipation or diarrhea, nephropathy, blindness, muscle tremor, difficulties in suckling, reduced immune response, soft eggshell.

Lead is absorbed to a different extent depending on various factors (intake, interaction with other elements, age, species…). Lead is primarily deposited in less metabolically active cortical bones where it may persist without substantially influencing the concentrations of lead in blood and other tissues, but also in liver and kidneys. At the occasion of mineral mobilisation from the bone, lead may be released. Therefore carry-over in edible tissues, egg and milk is low and only significant at higher intake. Human exposure from products of animal origin is expected to be limited.

Recent estimates of lead intake based on data from different European countries (Finland, France, Sweden and the UK) indicate values ranging from

0.001 to 0.008 mg/kg body weight/week for the adults and up to 0.019 mg/kg for children (WHO, 2000). Based on a worst case scenario considering the maximum limits of lead established by the Codex Alimentarius (1999) for different food commodities, and the *per capita* consomption in Europe of raw and semi-processed agricultural commodities defined within the WHO food contamination and monitoring assessment program (GEMS Food), a 0.020 mg/kg body weight/week exposure has been calculated. Theses values are lower than the PTWI of 0.025 mg/kg body mass proposed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (WHO, 2000). The respective contribution of the different food commodities to the lead load of the human diet has been calculated by the Australian authorities. Although this may not correspond to the European diet, the calculation shows that meat contributes to about 5% of the whole contamination of the adult diet only, while milk contributes to 16% of the children diet (Australia-New Zealand Food Authority, 1998). In a UK study, food from animal origin including fish contributed about 7 % to total lead intake in the general UK population (Ysart *et al*., 2000). Therefore, the implementation of the actual legislation fixing maximum lead contents in feeds ensures a lead load in food of animal origin that contributes to a limited extent to the whole exposure of the human consumer.

6.3. Mercury

Mercury in the natural environment is found in both inorganic (metallic, monovalent and divalent) and organic (aryl and short chain alkyl) forms. The inorganic forms are less toxic. Inorganic mercury can be converted into organic form by the micro-flora and micro-fauna in the environment (Jonnalagada and Prasada-Rao, 1993). 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 (cold vapor technique). The limit of detection is 0.01µg/kg.

Mercury is widely used for industrial purposes and is released under both chemical forms from industrial sites into the environment, especially into rivers. Concentrations of total mercury measured in the environment are <0.5 µg/l in surface water, 0.0005-0.003 µg/l in the open ocean, 0.002-0.015 µg/l in coastal seawater, <0.01-0.07 µg/l in ground water, <0.2 mg/kg in soil and <0.001 µg/m^3 in atmosphere (WHO, 1989; Müller, 2002).

Mercury uptake by plants from soil is low and therefore concentration of mercury in plant feedstuffs is limited. Most pastures and crops contain less than 0.1 mg mercury /kg dry matter. Typically, animal feed derived from plants contain mercury levels between 0.001 and 0.03 mg/kg dry matter (Dudka and Miller, 1999).Limits in complete feedingstuffs fixed by Council Directive 99/29/EC are 0.1 mg/kg for all animals, except pets where 0.4 mg/kg is tolerated.

In former times mercury compounds were used as fungicides in seeds. Cases of subacute and chronic intoxications were reported after intake of mercury

treated seeds by farm animals. Since the use of these compounds is forbidden intoxication of animals by feedingstuffs does not occur. Because of the low concentration of mercury in pastures and crops the mercury content of edible tissues and products of farm animals is rather low (e.g mg/kg fresh tissue or product: meat: 0.001, liver: 0.006-0.010, kidney: 0.023-0.045, chicken: 0.010, milk: 0.010, eggs: 0.005; Weigert, 1988).

Fish eating plankton such as herring and sardines have total mercury concentrations less than 0.1 mg/kg wet weight where approximately 80 % of the total mercury is in the form of methyl mercury (Bloom, 1992). Mercury is concentrated up the food chain, and subsequently high concentrations (typically 0.5-1 mg/kg muscle wet weight) can be found in long-living marine predators, such as tuna, dogfish, halibut and shark (Clarkson, 1993). Farmed fish (rainbow trout, *Oncorhynchus mykiss*, and Atlantic salmon, *Salmo salar*) have shown mercury levels of 0.008 to 0.052 mg/kg fillet (Santerre *et al.*, 2001; Julshamn *et al.*, 2002).

Mercury is known to be teratogenic and carcinogenic in mammals.

Organic mercury is readily absorbed in the gastrointestinal tract (more than 80% in monogastric animals/ 31 to 71% in trout (Lock, 1975)) and passes other barriers such as the blood/brain and the placental and oviduct barriers. Therefore, organic mercury, in particular methyl mercury, accumulates in the brain and is known to cause damage to the central nervous system. Inorganic mercury is poorly absorbed (less than 10% (Neathery and Miller, 1975) also in fish) and stored predominantly in the kidney

Most farm animals tolerate between less than 0.5 (pigs), 2 (chicken & laying hens), 5 (calves) (NRC 1980) and less than 5 (Atlantic salmon, Berntssen *et al*., 2003) mg mercury from organic mercuric compounds /kg feed. Tolerance levels for inorganic mercury are expected to be correspondingly higher. Clinical symptoms in animals fed higher levels are inappetence, anorexia, ataxia, abnormal behaviour, fatty liver, enlargement of lymph nodes, necrosis of gastro-intestinal tract and nephrosis, reduced fertility, reduced egg shell stability, and in Atlantic salmon, elevated metallothionein, brain pathology and altered blood parameters.

In the absence of occupational exposure, human intake of mercury is dominated by the diet and amalgam dental fillings (Horvat, 2001). Methyl mercury in fish and fish products represents up to 85% of the mercury in total intake from the diet. To protect consumers, maximum levels have been set for mercury in fish, these are based on the position of fish in the food chain. The maximum limit of mercury in non-predatory fish is 0.5 mg/kg wet weight whereas certain predatory fish (including tuna, halibut, shark) can contain up to 1 mg/kg wet weight (WHO, 1990, Directive 2001/466/EC).

The provisional tolerable weekly intake (PTWI) for mercury, set by the JECFA, is 0.005 mg/kg body weight (JECFA, 1978) of which no more than 0.0033 mg/kg body weight should be in the form of methyl mercury (JECFA, 1987; WHO, 1972, re-evaluated and confirmed in 1978). Estimates of typical total mercury intakes for some European countries indicate values ranging

from 0.0007 to 0.0135 mg/day/person which represent 1.6 to 32% of the PTWI for total mercury (Nasreddine and Parent-Massin, 2002). An estimate of the methylmercury human intake has been calculated based on typical concentrations measured in fish and GEMS Food consumption figures for Europe. A value of 0.0011 mg/kg body mass/week was found that represents 33 % of the specific PTWI for methylmercury. The mercury intake of consumers with a high intake of fish (95^{th} percentile) still remains below the PTWI as long as fish contain "typical" below regulatory limits of methylmercury (JECFA, 2000). Therefore, the implementation of the actual legislation fixing maximum mercury contents in feed ensures a limited mercury load (estimated as total mercury) in animal products.

6.4. Cadmium

Cadmium occurs naturally in the environment as a result of volcanic emissions. Background soil cadmium concentration is relatively low (about 0.1 mg/kg) and depends on the type of soil and on the parent rock for the soil.

Industrially produced contamination (deposition from the air) and fertiliser use (phosphates, sewage sludges etc.) has increased the background levels of cadmium in soil, water, and organisms (WHO, 1993a). High concentrations of cadmium (up to 10 mg/kg) have been found in forages grown in fields near industrial zinc-plating sites, where urban sludge has been used as a fertiliser, and where silt from industrial areas were deposited (Smith, 1986).

In contrast to other elements, cadmium is rather mobile and can be absorbed by plants *via* roots and its concentration decreases in the following order: root>leaves>stem>subterranean storage organs>fruits/grain. Crössmann (1986) mentions a decreasing order of cadmium concentrations between species in grains: oat>wheat>barley>rye>maize. Whithin the grain, most of the cadmium is bound in the epidermis.

Cadmium impurities are often present in mineral feed material, like phosphates, and can contribute significantly to the diet contamination, even within the fixed limits.

The current limits for cadmium are shown by animal category in table 2.

Table 2: Limits of cadmium in complete feedingstuffs fixed by Council Directive 99/29/EC

Adult ruminants	1 mg/kg
Calves, lambs and kids	0.5 mg/kg
Other animals except pets	0.5 mg/kg

The toxic effects of cadmium in food are largely related to long-term exposure to low doses. Pathologic changes of morphologic and functional nature have been observed in the kidney. Excess of cadmium in food, namely in rice, has been associated with a severe bone disease (itai-itai disease). Cadmium may interfere with calcification, decalcification and bone remodelling due either to a direct action in bone, or to the inhibition of the activation of vitamin D

metabolite in the renal cortex. The available experimental data indicate that these effects are observed with doses above 1 mg/kg body weight per day. Cadmium is also a potential neurotoxin, although some level of protection is provided by metallothionein in the brain. Cadmium has been shown to be carcinogenic in the rat (prostate). Teratogenic effect was reported in ruminant.

Cadmium is absorbed at different extent depending on cadmium speciation, animal species, dose and frequency of administration, age or stage of development, nutritional status and interactions with various nutrients but especially minerals (iron, zinc, calcium). Studies on experimental animals have shown that 0.5 to 8% cadmium is absorbed. A similar value (5%) was observed in humans. Cadmium bound to metallothioneins in food was shown to be absorbed at a lesser extent than the ionic form in mice. Selective accumulation of cadmium occurs in the kidney (through re-absorption), liver and to a much lesser extent in the muscle, representing approximately 50, 15 and 20% of the body storage, respectively. In mammals, cadmium is virtually absent at birth. Transfer to the milk and egg is very limited.

Experiments were conducted with cadmium chloride, sulphate, acetate and succinate. Impaired growth, anemia, hypertension, impaired renal, reproductive and hematopoietic functions, depressed immune response, were reported with cadmium (Puls, 1994). Additionnally congenital defects and abortion were observed in cattle and sheep exposed to cadmium succinate fed for 49 and 41 weeks, respectively (Wright *et al.*, 1977). In pigs, changes in haematology and in kidney and liver biochemistry were shown with 0.47 mg cadmium/kg feed for 8 weeks (Hansen and Hinesly, 1979). In laying hens, reduced egg production was seen with 3 mg cadmium / kg feed for 2 months (Leach *et al.*, 1979; Prinbilincova and Marettova, 1996). In fish (Atlantic salmon, *Salmo salar*) increased cell proliferation, apoptosis and metallothionein and decreased nutrient digestibility were observed when exposed to 6.7 mg cadmium/kg feed for four months (Berntssen and Lundebye, 2001; Berntssen *et al.*, 2001;).

Data from Member States (EU report, 1996) indicate that meat products, fish, milk and eggs contain low amounts of cadmium (0.001 to 0.01 mg/kg), with the exception of horse meat (up to 0.27 mg/kg). Edible offals of cattle, sheep and pig contain 0.04 to 0.07 mg/kg, with the exception of cattle kidney (0.15 mg/kg). The highest concentration of cadmium was found in molluscs (up to 1.4 mg/kg). Cereals, leafy vegetables, roots and tubers contents range from 0.01 to 0.03 mg/kg. Oilseeds may contain higher amounts of cadmium (0.05 to 0.22 mg/kg) while vegetable oils and fats contain very low amounts (0.002 to 0.003 mg/kg). The provisional tolerable weekly intake (PTWI) for cadmium, set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), is 0.42 mg/person/week (JECFA, 2001). An estimate of cadmium intake in the EU Member States indicates a range of 0.054 and 0.34 mg/person/week, the highest value representing 80% of the PTWI.

The contribution of the different food commodities to the cadmium load of the human diet has been calculated based on the cadmium contents mentioned above and the GEMS Food *per capita* consumption in Europe. It appears that for a total intake of 0.253 mg/person/week, the animal products (including

fish but not molluscs) contribute to about 5% of the PTWI only. Therefore, the implementation of the actual legislation fixing maximum cadmium contents in feed ensures a cadmium load in food of animal origin that contributes to a limited extent to the whole exposure of the human consumer.

6.5. Arsenic

Arsenic can exist in different oxidative states and chemical forms, although in nature, it is mainly bound to metals or as As_2O_3. It occurs in tri- and pentavalent states, with arsenic trioxide being the most common compound. Arsenic is a major constituent of many minerals of the earth crust. Clays, phosphate rocks, sedimentary iron ores and coal are notably rich in arsenic.

Arsenic can be determined in biological materials, including feedingstuffs, by different methods. Routine analysis involves the determination of total arsenic concentrations although, due to the varying state of valency and the quantity of different organic arsenic compounds, different analytical methods for speciation exist (McSheehy *et al.*, 2002).

Arsenic is present in all types of soils. Apart from the geological origin, arsenic in soil also comes from emissions from coal fired power plants, smelters, use in wood preservation and the now discontinued use of arsenical pesticides. Average concentrations are about 5 to 6 mg/kg, but they vary considerably. For example, mean arsenic concentration in soils in Germany ranges from 1 to 12 mg/kg.

Wide ranges of arsenic concentrations have been found in rivers and lakes and drinking water. Extremely high values have been found in groundwaters from territories with thermal activities and with arsenic rich rocks. Common arsenic concentrations in surface- drinking- and groundwater in Germany were mostly lower than 10 µg/l. Arsenic concentrations in seawater are generally in the range of 1-8 µg/l (WHO, 1981).

The arsenic content of plants is determined by arsenic exposure *via* soil, water, air, fertilizers and other chemicals, the geological origin of the soil, and the species (Meharg and Hartley-Whitaker, 2002), part and age of plants. Concentrations vary and plants growing on arsenic rich soils can accumulate much higher levels. In practice, most feed of terrestrial origin contain less than 0.3 mg/kg and rarely exceed 1 mg/kg on dry matter basis whereas marine algae may have extremely high organic arsenic contents (40 to 50 mg/kg dry matter).

On the basis of limited data it has been estimated that the percentage of inorganic arsenic is about 75% in meats and in dairy products, and 65% in cereals (Yost *et al*., 1998). Most of the arsenic present in seafood is in organic forms, primarily as arsenobetaine (approximately 95% of total arsenic) in fish (Ballin *et al*., 1994).

Some organic arsenic compounds (*e.g.* arsanilic acid, 4-nitrophenylarsonic acid and 3-nitro-4-hydroxyphenylarsonic acid and their salts) have been used as feed additives for disease control and improvement of weight gain in swine and poultry in concentrations of 100 mg/kg feed since the mid 1940s (Frost,

1967). Their use has been abandoned in Europe but they are still in use in third countries such as USA. Inorganic arsenic as well as its organic metabolites are extensively absorbed and excreted in the urine (Underwood and Suttle, 1999). In mammals, inorganic arsenic undergoes reduction and oxidation reactions which interconvert arsenate and arsenite, but also methylation reactions which convert arsenite to methylarsonic acid and dimethylarsinic acid. Accumulation of arsenic in tissue is slow and occurs mainly in liver, kidney and skin. Withdrawal of exposure led to a decrease of tissue contamination (Underwood and Suttle, 1999). In fish, low retention was found for arsenate (approximately 1% (Cockell and Hilton, 1988), whereas no retention was observed for dimethylated compounds. In contrast, almost 40% of the arsenic administered as arsenobetaine and arsenocholine was accumulated in fish (Francesconi and Edmonds, 1989).

Experimental evidence shows that all animal species tolerate much higher levels of arsenic than the limit fixed for feedingstuffs (Table 3).

Table 3 Limits of arsenic in complete feedingstuffs fixed by Council Directive 99/29/EC

For all animals except fish	2 mg/kg
For fish	4 mg/kg

Ruminants do not show any sign of toxicity unless exposed to more than 200 to 300 mg inorganic arsenic /kg feed. Pigs fed 100 mg arsenic from arsanilic acid/kg diet for 6 weeks lowered their feed intake (Morrison and Chavez, 1983). Laying hens decreased their feed intake (-24%) and egg production (-20%) when fed a diet containing 44 mg arsenic from 3-nitro-4-hydroxyphenylarsonic acid/kg feed (Chiou *et al*., 1997). Egg mass was reduced with 15 mg arsenic from arsenic oxide/kg diet (Holcman *et al.*, 2001). Quails fed up to 30 mg arsenic from arsenite/kg diet expressed no effect (El Begearmi *et al.*, 1982). Tolerated single oral doses of arsanilic acid, 3-nitro-4-hydroxyphenylarsonic acid and phenylarsonic acid in chicken relate approximately as 1:0.25:0.1. Toxic effects in fish following dietary exposure to arsenate concentrations between 32 and 160 mg/kg diet included elevated hepatic metallothionein levels, histopathological alterations in liver and gall bladder, and decreased growth rate (Cockell *et al*., 1992; Pedlar *et al*., 2002a; Pedlar *et al*., 2002b). Rainbow trout (*Oncorhynchis mykiss*) exposed to inorganic arsenic (180 mg arsenic trioxide/kg diet and 137 mg disodium arsenate heptahydrate/kg diet) for 8 weeks showed similar toxic responses, including altered feeding behaviour and reduced growth (Cockell and Hilton, 1988). In contrast, no toxic effects were observed in fish exposed to ten fold higher dietary concentrations (1 500 mg/kg diet) of the organic arsenic forms dimethylarsinic acid and arsanilic acid for 8 weeks (Cockell and Hilton, 1988).

The toxicity of arsenic is dependent on chemical form and valency. Trivalent arsenic is much more toxic than pentavalent arsenic compounds. Sodium arsenite, which is more soluble than arsenic(III)oxide, has been shown to be ten times more toxic than arsenic(III)oxide. The toxicity of organic arsenic

compounds is inversely related to their degree of methylation. Inorganic forms are much more toxic than organic arsenic (OyaOhta *et al*., 1996). Taking into account the NOAEL established for different toxic end points (skin lesions, neurological effects) on human populations a reference dose considered without effect of 0.0003 mg/kg body weight/day has been retained by the U.S. EPA (2001). The EU Scientific Committee on Toxicology, Ecotoxicology and Environment considered that the available evidence on inorganic arsenic indicated that arsenic is genotoxic both in vitro and in vivo, and that there was also some information to suggest that it be genotoxic for humans (CSTEE, 2001). A sub-chronic toxicity study of organic arsenic in the rat has shown that "fish arsenic" (mainly arsenobetaine) up to 3 mg/kg body weight/day did not produce toxic effects (Siewicki, 1981). Arsenobetaine was shown to be neither toxic nor carcinogenic to mammals (Neff, 1997).

The PTWI for inorganic arsenic set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is currently 0.015 mg/kg body mass/week (WHO, 1989). The reported survey of arsenic in food in the United Kingdom carried out as part of the 1997 total diet study (MAAF, 1999; Ysart, 2000) indicates that foodstuffs of animal origin, with the exception of sea food, contribute only 0.6 % of the daily intake. Arsenic concentrations are greatest in seafood that made also the greatest contribution to the total arsenic intake (94%) of which only 1 to 3% is in the inorganic form. This study indicated that the mean daily intake from food for the total population was estimated to be 0.065 mg total arsenic/day/person. Considering the figure above (3% inorganic arsenic) it can be calculated that the mean daily intake is about 0.006 mg inorganic arsenic /day/person. This intake represents 10% of the PTWI. A total diet study conducted by the US FDA has confirmed seafood accounts for approximately 90% of the arsenic dietary exposure of the adults and children but only 42% of that of the infants (Tao and Bolger, 1999). Other data from different European countries indicate exposure values ranging from <0.030 to 0.286 mg total arsenic/day/person (Nasreddine and Parent-Massin, 2002 ; Robberecht *et al*., 2002 ; Noël *et al*., 2003). Therefore, the implementation of the actual legislation fixing maximum arsenic contents in feed ensures an inorganic arsenic load in food of animal origin that contributes to a limited extent to the whole exposure of the human consumer.

6.6. Fluorine

Fluorine is ubiquitous in nature as calcium and sodium-aluminium fluorides. Other potential sources of fluorine include deep well water and volcanic soil. Fluorine in the form of hydrofluoric acid (HF), silicon tetrafluoride ($SiF_{4)}$ and fluorides can be released from industrial sites associated with aluminum or phosphate processing. These emissions can contaminate water, soil, and plants near these sites, resulting in fluorine intoxication in animals grazing in the areas (Bunce, 1985).

Most of the fluorine is present as calcium fluoride in the soil. Fluoride is absorbed to a limited extent, is mainly retained in roots and is not translocated at significant extent to other organs. The fluorine concentration of pastures and forages is therefore low, unless these have been contaminated by deposition of fumes and dusts of industrial origin or by irrigation with

fluoride-rich waters. Direct drying of feed materials can also represent a source of fluorine.

Since soil usually contains far higher fluoride concentrations than the plant, ingestion of fluoride-rich soil on overgrazed pastures can significantly contribute to fluorine intakes.

The principal source of fluorine for livestock is compound feedingstuffs containing fluoride-rich phosphate supplements. Fluoroapatite (rock phosphate) sources vary widely in their fluorine content, depending on their origin. The high fluoride rock phosphates can be injurious to livestock when used over long periods in the amounts ordinarily required as calcium and phosphorus sources. For this reason, these should be (and are now normally routineously) defluorinated.

Fluorine compounds with low solubility like calcium, magnesium or aluminium fluorides are poorly absorbed while fluoride ions released from readily soluble fluorine compounds such as sodium or hydrogen fluoride, fluorosilicic acid and monofluorophosphate are almost completely absorbed by the gastroinstestinal tract by passive diffusion. Fluoride in drinking-water is highly bioavailable. A limited number of studies indicate that the bioavailability of fluoride from fluoride-containing diets varies from 90% to 60% depending on the nature of the diet, due to the binding of fluoride with certain food constituents. Fluoride is rapidly distributed then excreted at a large extent in the urine. The major part (99% in the human and laboratory animal) of the total body burden of fluoride is retained in the bones and teeth. The concentration of fluoride in bone also varies with age, sex and the type and specific part of bone and is believe to reflect an individual's long-term exposure to fluoride. Fluoride is not irreversibly bound to bone and is mobilized continuously from the skeleton and subsequently excreted (IPCS, 2000).

In aquatic organisms (fish, invertebrates), fluorides are taken up directly from the water and *via* feed. However there appears to be species-specific differences in the ability to accumulate fluoride in bone tissue or exoskeleton. Fluoride does not appear to accumulate in fish muscle tissue (Grave, 1981; Tiews *et al*., 1982; Julshamn *et al*., 2003).

Fluorine is generally regarded as a toxic element with regards to domestic livestock because in large amounts fluorine will accumulate in bone to an extent that actually weakens bone, increasing lameness and increasing wear of teeth (Shupe, 1980; Crissman *et al.,* 1980). In case of chronic intoxication exostoses, osteoporosis and osteomalacia can be observed mainly in pelvic bones, ribs and vertebrae. In several parts of the world chronic fluorosis is enzootic as a consequence of the consumption of waters abnormally high in fluorine, usually from deep wells or bores. Intoxication occurs most frequently in cattle, rarely in horses and pigs. 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. It has been demonstrated that the pig species

is more resistant to fluoride than ruminant species, 100 mg fluoride/kg dry matter being well tolerated by the pig (Gueguen and Pointillart, 1986).

Table 4 Maximum recommended (Puls, 1994) or tolerated (Hapke, 1988) concentration of fluorine

Species	mg/kg feed	Species	mg/kg feed
Calf	30/40	Pig	70/150
Dairy cow	40/40	Horse	40/40
Fattening bull	100/140	Rabbit	-/40
Milking sheep	60/60	Turkey	150/150
Mutton	100/150	Chicken	200/200

Few studies have been conducted on the dietary toxicity of fluoride in fish. Rainbow trout (*Oncorhynchus mykiss*) have been shown to tolerate high fluoride concentrations (more than 2500 mg/kg for 82 days) in their diet (Tiews *et al.*, 1982).

Table 5: Limits of fluorine in complete feedingstuffs fixed by Council Directive 99/29/EC

Lactating ruminants	30 mg/kg
Non lactating ruminants	50 mg/kg
Pigs	100 mg/kg
Poultry	350 mg/kg
Chicks	250 mg/kg
Other animals	150 mg/kg

Fluorides are genotoxic (clastogenic but not mutagenic) in human and animal cells *in vitro,* but not *in vivo* in laboratory animals. The evidence regarding the carcinogenicity of fluoride in laboratory animals is inconclusive (IPCS, 2000). Effects on the skeleton (skeleton fluorosis and increased risk of fracture) and teeth (dental fluorosis and hypomineralization of the enamel) are the most consistent and best characterized toxic responses to fluoride that are observed at exposures below those associated with the development of other organ- or tissue-specific adverse effects. An increased risk of bone effects has been identified in the human for total intakes above 5 mg fluoride/day (WHO, 1996).

The consumption of foodstuffs and drinking-water is the principal route of intake of human consumer. Fluoride levels in drinking-water may reach approximately 2.0 mg/l. However in areas of the world with endemic fluorosis of the skeleton and/or teeth has been documented, these levels ranged from 3 to 20 mg/l. Virtually all foodstuffs contain at least trace amounts of fluoride. Results from a survey gathering data from Canada, China, Hungary, Germany and the USA indicate a range of concentrations of 0.01 - 1.34 mg/kg wet weight for vegetables, 0.01- 2.8 for fruit and fruit juice, 0.04 – 1.9 for cereals and baked goods and 0 .05-0.13 for fats and oils, 0.01 – 0.8 mg/kg for milk and milk products, 0.01 – 1.7 for meat and poultry, and 0.06 to 4.6 for fish (IPCS, 2000). Published estimates for intake of fluoride indicate that children and adolescents exposure does not exceed 2 mg/day, including the ingestion from water and fluorine-enriched toothpaste. The total consumption by adults of fluorine in western countries is in the range 0.6 – 4.1 mg/day (IPCS, 2000).

The human exposure to dietary fluorine covers the physiological needs and, with the exception of the contribution of the high fluoride contents of drinking-water or food commodities in specific geographical regions, is below the lowest dose above which toxicity signs are observed at the bone and teeth levels.

6.7. Chromium

Chromium is ubiquitous in nature. It occurs in air, water, soil and biological material over a great range of concentrations. Almost all forms of chromium in the earth's crust are in the trivalent state, hexavalent chromium compounds being man-made products (IPCS, 1988). Even if Cr(VI) is more absorbable than Cr(III), the transfer of both ion species to plants is quantitatively very limited. The chromium content of minerals used as feed ingredient are highly variable ; in phosphates, for example, this element can range from 60 to 500 mg/kg with average values around 200 mg/kg (Sullivan *et al.*, 1994). According to Mordenti and Piva (1997) it is likely that chromium levels are lower than 1 mg/kg in the case of feedingstuffs which do not contain phosphates, protein hydrolysates or hay, 5 mg/kg or 10 mg/kg in the case of feedingstuffs containing phosphates and/or chromium-enriched yeasts and/or hay, but less than 10% or 20% ash, respectively, 25 mg/kg in the case of low-dose premixes where the individual raw materials would be available even at very high concentrations. In addition to natural environmental sources of chromium, the technological processing of feed materials may contribute to feedingstuffs contamination.

Following oral ingestion by animals Cr(VI) is converted to Cr(III) in the digestive tract. The absorption of Cr(III) is poor, *e.g.* 2% for a 10 μg/day ingestion but only 0.5% for >40 μg/day. Furthermore, chromium kinetics indicates a small long-lasting compartment, but no cumulative process. Consequently tissue residues are very low. As far as humans are concerned the same considerations apply, *e.g.* chromium bioavailability is very limited which reduces systemic exposure.

Only in accidental cases chromium caused intoxication in animals. Chromium compounds showed toxic effects in different species (Hapke, 1988):

- lethal oral doses: horse: 15-30 g chromate, dog: 3 g chromate, cattle: 700 mg chromate/kg body weight
- toxic effects: calves: 30-40 mg chromate/kg bw/day; no toxic effects: broiler: 100 mg Na-chromate/kg feed.

Clinical signs of intoxication were gastroenteritis, nephritis, central nervous symptoms and dermatitis. Chronic intoxication caused pathological changes especially in the gastrointestinal tract (ulceration and erosion) and parenchymatous degenerations, especially in the kidneys.

The trivalent anionic Cr(III) is harmless.
Epidemiological and experimental evidence has clearly demonstrated the carcinogenicity of Cr(VI) through air borne exposure. The hexavalent oxidation state, which at physiological pH exists as an oxyanion (chromate), is actively transported into cells where it readily reacts with a number of

endogeneous reducing compounds of the cell and generates the stable Cr(III) ion plus intermediate oxidation states of chromium such as Cr(V) which are believed to be important in chromium genotoxicity (Canter, 1995). Therefore, any source of Cr(VI) may represent a risk for the humans.

A recent evaluation of foodstuffs chromium contents carried out in Italy in principal FAO food groups indicates that the most significant contribution to the average total amount of 198 μg/day/individual comes from wheat, maize, rye and oat category (52%), while animal products contribute to 27%, of which mostly from meat and some organs (liver, kidney) 16%, fish and sea food, eggs and milk representing 4%, 3.5% and 3.5% respectively (Santoprete, 1997). However, a great part of the total chromium in foods derives from food processing in stainless steel containers and processors which typically contain 18% chromium.

Total chromium intakes reported from different worldwide countries indicate values from 50 to 200 μg/day (IPCS, 1988). Similar figures have been found for chromium intake by the populations of different European countries, i.e. 132 to 206 μg/day/individual, while their requirements amounted for 84 to 127 μg/day/individual (Santoprete, 1997). There are no specific regulations on maximum levels of chromium in foods.

6.8. Aluminium

Aluminium is a major component of the earth's crust. It occurs ubiquitously in the environment in the form of silicates, oxides and hydroxides, combined with other elements such as sodium and fluoride and as complexes with organic matter. Aluminium is released to the environment both by natural processes and from anthropogenic sources and enters the aquatic and terrestrial food chain.

Concentrations of aluminium in plant tissues vary considerably (80-350 mg/kg in soybean, vicia, trifolium and rye-grass; Sparling and Lowe, 1996) depending on local geological conditions, pH of soil, presence or absence of complexing agents, species of plant and portion of plant (accumulation in roots) examined. Normally in pastures aluminium concentrations lower than 100 mg/kg dry matter are found. Under unfavorable conditions (soiled, high stocking rate, wet weather) values can be much more than 1000 mg/kg. Low aluminium concentrations could be found in grains. Reported concentrations varied between 5 and 68 mg/kg DM also in grain products (Vogt and Jaakola, 1978; Schenkel and Klüber, 1987). In potatoes and sugar beets concentrations were between 20 and 140 mg/kg DM, for sugarbeet pulp and mollasses Al concentrations of about 115 up to 550 mg/kg DM were reported. In different rations for dairy cattle, fattening bulls and sheep Al concentrations varied bewteeen 100 and 600 mg/kg DM (Schenkel and Klüber, 1987). In a horse diet Schryver *et al.* (1986) analyzed 336 mg/kg. Grazing animals ingest considerable amounts of soil, sometimes over 10 percent of their total dry matter intake. Intake of this could result in aluminium consumption as high as 1.5 percent of the diet dry matter. Very low concentration could be determined in milk products (< 10 mg/kg; DokumentationsstelleUniversität Hohenheim,

1985). Meat and bone meal contained bewteen 100 and 500 mg/kg DM and for fish meal values between 35 and 350 mg/kg were reported.

Higher amount of aluminium, up to 15000 mg/kg, could be found in soft phosphates and sometimes also in trace element coumpound used for supplemetation (Ammemann *et al.*, 1977; Schenkel and Klüber, 1987). Natural aluminium minerals, especially bentonite and zeolite, are used also as technological feed additives.

Absorption of aluminium via the gastrointestinal tract is usually less than 1% in the animals. The main factors influencing absorption are solubility, pH and chemical species. Organic complexing compounds, notably citrate, enhance absorption. Aluminium is distributed in most organs with the highest levels found in the brain, liver and kidney, while bioaccumulation occurs in bones in mammals and birds, in gills in fish. Excretion through the milk is very limited in the cow as well as in the human (WHO, 1989).

Aluminium was regarded as a non-toxic element for animals, but concerns about the safety of aluminium for the human have raised from the evidence it has neurotoxic effects in experimental animals and in humans on long term kidney dialysis. Morphological and biochemical modifications at the spinal cord, brainstem and selected areas of the hippocampus, as well as associated progressive encephalopathy and behavioural impairment have been observed following parenteral exposure. It has been hypothesized that aluminium in the drinking-water would be a risk factor for the development or acceleration of Alzheimer's disease as well as for impaired cognitive function in the elderly. There is considerable evidence that aluminium is neurotoxic in experimental animals, although there is considerable variation among species. However the morphological and biochemical modifications are different from those that occur in Alzheimer's disease (AD). There is no evidence to support a primary causative role of aluminium in AD, and aluminium does not induce AD pathology in vivo in any species, including humans (WHO, 1989). It has been indicated that monomeric inorganic Al^{3+}, $AlOH^{2+}$, and $Al(OH)_2^{+}$ are the most toxic forms, whereas Al-F and organic Al compunds show reduced toxicity.

In humans the intake of aluminium from food and beverages excluding water has been estimated to 2 to 6 mg/day in children and 6 to 14 mg/day in adults (results from several countries) (IPCS, 1997) with the lower values probably reflecting a lower use of aluminium-containing additives in the preparation of cereal grain products (bread, etc.) (UK MAFF, 1993). This represents 90 to 95% of the total aluminium intake per day. Drinking-water may contribute 0.2 to 0.4 mg/day. In some circumstances, such as occupational exposure and antacid drug use, the levels of exposure will be much greater, *e.g.* > 500 mg. Animal edible tissues and products (milk, egg) contain low amounts, *e.g.* pig muscle varied from 11.5 to 53 mg/kg fresh tissue (Vyaizenen *et al.,* 1997). Processing of plant (cereals) and animal products (milk) making use of aluminium-containing food additives (*e.g.* aluminium silicates) increases considerably their initial aluminium contents, and grain products (flour), processed cheese or infant formulae represent major dietary sources of aluminium (Pennington and Shoen, 1995).

The acute toxicity of metallic aluminium and aluminium compounds is low, the reported oral LD50 values being in the range of several hundreds to 1000 mg aluminium/kg body weight per day. The lowest-observed-adverse-effect level (LOAEL) for developmental effects (decreased ossification, increased incidence of vertebral and sternebrae terata and reduced fetal weight) was 13 mg aluminium nitrate/kg body mass while no effect was observed for much higher doses of aluminium hydroxide. There is no indication that aluminium is carcinogenic. No acute pathogenic effects in the general human population have been described after exposure to aluminium. A provisional tolerable weekly intake (PTWI) of 7.0 mg/kg bw has been established for aluminium (FAO/WHO, 1988). The highest total aluminium intake (14 mg/day) measured in adults represents only 23% of the PTWI (equivalent to 60 mg/day).

There are no specific regulations on maximum levels of aluminium in foods.

6.9. Nitrites

Natural occurrence of nitrites in the environment is a consequence of the nitrogen cycle, but usually nitrites are found in very low concentration. Nitrites are formed in nature by the action of nitrifying bacteria as an intermediate stage in the formation of nitrates. Conversely, microbiological conversion of nitrates to nitrites may also occur, for instance in the digestive tract.

The main anthropogenic sources result from the use of N-fertilizers but also from nitrates present in animal, municipal, industrial and transport wastes. Plants are also a significant dietary source of nitrates.

Nitrites are widely used in the processing and preservation of certain meat products. Concentrations found in cured meat ranged from 3-208 mg/kg (Ashton, 1970). Nitrites have been used in some countries for the preservation of fish meal submited to heat treatment, but are no longer permitted as they have been suspected to generate nitrosamines when reacting with higher amines present in fish.

Analysis of nitrite has not been a priority in recent years after its use in fishmeal was discontinued in the early 1990s in Europe. Only few data are available, but then suggest that the content in fishmeal produced by indirect drying techniques is below 2 mg/kg.

In monogastric animals, most nitrite is absorbed in the upper digestive tract. Any non-absorbed fraction is metabolized by the intestinal microflora to nitrates and other nitrogenous compounds such as nitrosamines. A reverse endogeneous metabolic conversion of nitrate to nitrite can also occur (Spiegelhalder *et al.*, 1976). In contrast, in ruminants, the rumen flora is able to reduce nitrite / nitrate to ammonia.

No data are available concerning either the endogeneous nitrite contents of animal products or the conversion and transfer rate of nitrates and nitrites through the animal food chain. However, the fast excretion in urine of the

absorbed nitrate and nitrite ions and their non-cumulative character allows the conclusion that no bioaccumulation occurs in animal tissues and products.

The formation of methaemoglobin particularly in the young animals appears to be the main issue of toxicological concern. However this effect is seen only when nitrates concentration are substantially higher than those currently allowed in water and feedingsuffs. Thus exposure of rats to 100, 1000, 2000 and 3000 mg nitrite/l drinking water for 24 months led to about 0 % (very slight, but reversible increase after 2 months), 5%, 12% and 22% increase of the methaemoglobin concentration, respectively, when compared to controls (tap water) (Shuval and Gruener, 1972).

At higher levels or with chronic exposure, other toxic effects can become evident. Sodium nitrite is mutagenic on bacterial systems such as *E. coli* and *S. typhimurium*, but no data are available on its eventual mutagenic action in mammalian systems. If high nitrite doses (1000 to 3000 mg/l drinking water) induced some pathological changes in the heart (small to degenerative foci of cells and fibrosis) and lung (dilated bronchi with lymphocyte infiltration, emphysema) following chronic exposure, but no carcinogenic effect was observed either in the rat or mice (Greenblatt and Mirvish, 1973; Taylor and Lijinsky, 1975). Neither embryotoxic nor teratogenic effects were observed in rats following the administration of 2000 or 3000 mg/l drinking water, but a pronounced dose-related increase of mortality of new-born rats was observed in the first 3-week period of life, possibly as a consequence of transplacentally induced methaemoglobinaemia (Shuval and Gruener, 1972).

Table 6: Proximate toxic doses of nitrates-nitrites in pigs (Wolter, 1982) expressed as mg/l or mg/kg feed.

Item	Drinking water	Feed	
Product	Nitrates	Nitrates	Nitrites
Tolerance	1300	3000	1000
Toxicity	-	30000	1000

Doses of up to 500 mg nitrate / l drinking water administered during two successive reproductive cycles in the rabbit does were not deleterious on reproductive performance, blood cell counts and haemoglobin level. Furthermore, no effect on performance and mortality rate of their progeny were reported (Kammerer and Siliart, 1993).

6.10. Radionuclides

Radionuclides are potential contaminants of the feedingtuffs and are therefore considered as undesirable substances. However they have already been scientifically assessed for the establishment of a specific legislation[2]

[2] Council Regulation (Euratom) No 2218/89 of 18 July 1989 amending Regulation (Euratom) No 3954/87 laying down maximum permitted levels of radioactive contamination of foodstuffs and of feedingstuffs following a nuclear accident or any other case of radiological emergency - E.C.O.J. n° L 211 of 22/7/1989, p. 1.

6.11. Conclusion

6.11.1. The following ions and elements listed in Council Directive 1999/29/EC are commonly encountered substances with known toxicities.

In each case, the contribution of products of farm animal origin to the human exposure is limited and listing of these elements as undesirable substance in feed, although concomittantly contributing to an overall reduction of human exposure to toxic forms, is mainly justified by reasons of animal health.

A detailed risk assessment appears necessary for the elements listed under (1) and (2), in particular to address the following.

(1) Elements that should be retained in the list of undesirable substances

- Lead at the limit fixed in the current legislation may affect the health of sheep and possibly other ruminants. Consequently the maximum value in complete feed for this animal species should be reviewed.
- Methyl mercury is recognised as significantly more toxic than inorganic mercury, therefore the determination of total mercury in feed may not always accurately reflect the risk posed by the organic forms. As a consequence, a detailed assessment should address the risks related to the organic forms of mercury.
- Cadmium at the limit fixed in the current legislation may affect the health of pigs. Consequently the maximum value in complete feed for this animal species should be reconsidered.
- Arsenic in its organic forms has a limited toxicity, therefore the determination of total arsenic in feed may not always accurately reflect the risk posed by the inorganic forms. As a consequence, a detailed assessment should address the risks related to the inorganic forms of arsenic.
- Fluorine limits in the current legislation protect only the health of some species. For poultry and horse, limits fixed in feedingstuffs are above their tolerance. Consequently the maximum value in complete feed for these animal species should be reconsidered.

(2) Ions that could be removed from the list of undesirable substances

Nitrites are endogenous compounds naturally present in feed materials of plant and animal origin. Their natural levels in feedingstuffs have not been reported to cause intoxication of farm animals. As a consequence, retaining limit for nitrites in feedingstuffs appears to serve no practical purpose.

6.11.2. Additional elements that were considered by SCAN for possible inclusion in the list of undesirable substances

- Chromium is of no toxicological concern in animals and in low concentration may be of some benefit. In regard to human risk no problem of chromium excess is expected to occur due to the specific contribution of animal products. Therefore, chromium is not considered by SCAN to be an undesirable substance in feed.
- Aluminium toxicity has no practical relevance for animals. Aluminium does not accumulate in edible animal tissues and products (milk, egg), consequently their contribution to the overall exposure of the human consumer is very low and not affected by aluminium content in feedingstuffs. SCAN does not consider aluminium to be an undesirable substance in feed.
- Radionuclides are potential contaminants of the feedingtuffs and are therefore considered as undesirable substances. However they have already been scientifically assessed for the establishment of a specific legislation.

MYCOTOXINS

7.1. Introduction

Mycotoxins are toxic metabolites produced by filamentous fungi, especially saprophytes, growing on agricultural crops and products. It has been established that mycotoxins are responsible for a variety of animal and human diseases, and even death. Although mycotoxins have caused some dramatic epidemics in humans and animals, such outbreaks are very rare. Mycotoxicosis is essentially a chronic problem caused by an underlying contamination of crops, particularly cereals, with toxigenic fungi. Fungal toxins are estimated to affect as much as 25 per cent of the world's crops each year (Lawlor and Lynch, 2001). However, the variable production of mycotoxins together with ill-defined symptoms make it difficult to estimate the real incidence of mycotoxicosis (Prelusky *et al*., 1994).

The biological effects of mycotoxins are numerous (Betina, 1984). They can be acutely and/or chronically toxic, depending on their chemical structure and concentration, the extent of exposure of animal consuming contaminated feed and the health status (Charmley *et al*., 1995; Fink-Gremmels, 1999). In animals, targets for acute effects include liver, kidney, central nervous system, skin and reproductive system. Some mycotoxins are carcinogenic.

7.2. Occurence

Mycotoxin contamination of forages and cereals frequently occurs in the field following infection of plants with particular pathogenic fungi or with symbiotic endophytes. Production of mycotoxins by fungi can also occur during processing and storage of harvested feed materials when environmental conditions such as moisture and ambient temperature appropriate for development of spoilage fungi are met. It is conventional to subdivide toxigenic fungi into "field" or plant pathogenic and "storage" or saprophytic/spoilage organisms. *Fusarium* spp. are representatives of field fungi while strains of *Aspergillus* spp. and *Penicillium* spp. are common storage fungi.

Mycotoxigenic species may be further distinguished on the basis of geographical prevalence, due to the specific environmental requirements for growth and secondary metabolism: *Aspergillus flavus* and *Aspergillus ochraceus* proliferate under warm, humid conditions, while *Penicillium verrucosum* develops under temperate climate. Consequently *Aspergillus* mycotoxins predominate in plant products emanating from the tropics and other warm regions, while *Penicillium* mycotoxins occur widely in temperate countries. *Fusarium* species are more ubiquitous, but even within this genus some species are almost exclusively associated with cereals from warm countries.

Interactions of several factors operating simultaneously are usually more important than any single factor in controlling mycotoxin production (Moss, 1991). Visible fungal growth on the grains does not necessarily mean that they are contaminated with mycotoxins, and *vice versa* (Fink-Gemmels, 1999).

Although fungal growth may not be evident on the kernels, for example due to drying or to use of fungicides, high concentrations of mycotoxins may still be found.

It is important to recognise that two or more mycotoxins can be produced by the same species of fungus and that some mycotoxins are produced by more than one fungal species. Analysis of a single commodity often shows the presence of several mycotoxins.

Among the mycotoxins, the current European Community list of undesirable substances only includes aflatoxin B_1 and ergot.

7.3. Mycotoxins listed in Council Directive 1999/29/EC[3]

7.3.1. Aflatoxin B_1

Among the aflatoxins (B_1, B_2, G_1 and G_2), aflatoxin B_1 is the most toxic, both for humans and animals, and is a potent carcinogen. Its metabolite aflatoxin M_1 (4-hydroxyderivative of aflatoxin B_1) appears in milk and milk products as a direct result of intake of aflatoxin B_1–contaminated feed (Van Egmond, 1989). The excreted amount of aflatoxin M_1, as a percentage of aflatoxin B_1 intake, ranges from 1-6 %. Aflatoxin M_1 is of concern to humans consuming contaminated milk and dairy products. As aflatoxin B_1 is the most toxic of the aflatoxins, levels of other aflatoxins in feed are expressed as aflatoxin B_1 equivalents (Mount, 2001).

The European Community established regulations for the content of aflatoxin B_1 in animal feedingstuffs in 1976 and for the aflatoxins B_1, B_2, G_1, G_2 and M_1 in human food in 1998. As well, practically all candidate EU countries have specific regulations for aflatoxins in animal feed. The animal feed regulations in the EU set limits low enough to prevent noticeable adverse animal health effects and to avoid levels of aflatoxin M_1 in milk above the EU limit of 0.05 µg/kg.

The maximum permitted levels in the EU are among the lowest in the world, and are based on the ALARA (*As Low As Reasonably Achievable)* principle. This approach has led to a situation where levels of aflatoxin B_1 in animal feed are currently well under control. No harmful effects on livestock are to be expected. The aflatoxin M_1 levels in milk and dairy products exceed only in exceptional cases the regulatory limit. On average aflatoxin M_1 levels have varied from 0.01- 0.02 µg/kg over the last decade. Current EU regulations for aflatoxin B_1 in feedingstuffs are adequate in terms of protection of

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[3] Council Directive 1999/29/EC of 22 April 1999 on the undesirable substances and products in animal nutrition (E.C.O.J. n° L 115 of 04/05/1999, p. 32) repealed from 1rst August 2003 by the Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed (E.C.O.J. n° L 140 of 30/5/2002, p. 10).

both animal and human health against, respectively, aflatoxin B_1 and M_1.

7.3.2. Ergot

The term ergot refers to the dark sclerotia formed by several species of the genus *Claviceps*. Of these fungi, *Claviceps purpurea* is the most important in terms of frequency of occurrence. It is mainly found on rye, triticale and wheat, but also on other cereals and grasses. A number of alkaloids are formed in the sclerotia each containing an indol ring and chemically considered as derivatives of d-lysergic acid. The total alkaloid content of the sclerotia is quite variable, and may differ by a factor of ten (Wolff and Richter, 1989).

A possible carry over of ergot alkaloids or of their metabolites into food of animal origin has not yet been determined with the exception of milk where a carry over does not seem to occur (Wolff *et al.*, 1995). This needs further investigation.

The concentration of sclerotia in cereals intended for human and animal consumption is presently restricted to 500 mg (Commission Regulation (EEC) No 689/92 of 19 March 1992) and 1000 mg per kg (Council Directive 1999/29/EC of 22 April 1999), respectively. However, the validity of the weight of sclerotia as a criterion for regulation or legislation in general can be questioned for two reasons:

– the physical methods used to separate contaminated and non-contaminated grains on the basis of size can be inaccurate
– the relationship between the content of sclerotia and total alkaloids is highly variable.

Therefore, only with knowledge of the content of the most important ergot alkaloids in feedingstuffs and diets will it be possible to evaluate the toxic potential of *Claviceps* more precisely (Bauer, 1988).

Specific limits for ergot alkaloids have not been established in the EU nor elsewhere. Setting limits would be scientifically justified as the toxic potential of ergot, and consequently its impact on animal health, vary depending on alkaloid content and composition. Published methods to determine the individual ergot alkaloids are usually based on liquid chromatography with fluorescence detection but there are currently no formally validated methods for the determination of ergot alkaloids in animal feed. If the approach taken by legislation is adapted to cover more specifically ergot alkaloids, then a validated reliable method for their determination in feedingstuffs would be necessary.

7.4. Other potentially undesirable mycotoxins

A large number of fungal secondary metabolites have been identified, many of which have been shown to be toxic for animals and humans. Novel

metabolites are constantly being identified and therefore this field needs to be regularly reviewed.

SCAN has selected on the basis of incidence and potential toxicity those it considers the most relevant at this point in time.

7.4.1. Ochratoxin A

Ochratoxins are secondary metabolites of some *Aspergillus* and *Penicillium* strains. Ochratoxin A and ochratoxin B are two forms that occur naturally as contaminants, with ochratoxin A being more ubiquitous, occurring predominantly in cereal grains and in the tissues of animals reared on contaminated feed. *Penicillium verrucosum* is the predominant ochratoxin A-producing fungus in Europe. Other ochratoxin A producing strains include members of the *Aspergillus ochraceus* and *Aspergillus niger* groups (Frisvad and Viuf, 1986).

Ochratoxin A is commonly found in cereals in Europe but concentrations are generally low. In Germany, approximately 70% of 2300 samples of cereals and related products were positive for ochratoxin A, but only 1.4% of the samples contained more than 0.003 mg/kg[4] (Wolff, 2000). Ochratoxin A concentrations were determined in 300 samples of farm-stored United Kingdom grown cereals. Ochratoxin A was detected in 22 (15%) of the wheat samples with a mean value of 0.0019 mg/kg for the positive samples, 35 (27%) of barley samples with a mean value of 0.0026 mg/kg and 0.006 (29%) of oat samples with a mean value of 0.0005 mg/kg (FSA, 1999). In France in samples of unprocessed maize, ochratoxin A levels ranged from <0.0001 (84%) to 0.0014 (1%) mg/kg (FSA, 1999). However hot spots can be found where concentrations greatly exceed these means. Peak concentrations in maize of 5125 µg/kg in Yugoslavia and 27500 µg/kg barley and oats in Denmark have been recorded (Krogh, 1980).

Ochratoxin A is partially absorbed from the gastrointestinal tract in monogastrics. Consequently ochratoxin A has been found in edible tissues and products of monogastric animals, particularly pork products in Europe (Krogh *et al.*, 1974). In ruminants, ochratoxin A is mainly metabolised by the rumen microbiota to ochratoxin α before absorption. This major metabolite appears less toxic than ochratoxin A (Creppy *et al*., 1983). The detection of ochratoxin α in milk is an indication of the presence of ochratoxin A in dairy cattle feed rations.

However, it has been estimated that, in the EU, the overall contribution of products of animal origin to human exposure is, on

[4] Limit fixed by Commission Regulation (EC) No 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs - E.C.O.J. n° L 77 of 16/3/2001, p. 1.

average, not more than 3 % of the total ochratoxin A burden (Miraglia and Brera, 2001).

Field cases of ochratoxicosis in farm animals (pigs, poultry) have been reported from several European countries, the primary manifestation being chronic nephropathy. The lesions include tubular atrophy, interstitial fibrosis and, at later stages, hyalinized glomeruli. It has produced nephrotoxic effects in all species of monogastric animals studied so far, even at the lowest level tested (200 µg/kg feed in rats and pigs). In slaughterhouses cases of mycotoxic porcine nephropathy studied by Hald and Krogh (1972), residues of unchanged ochratoxin A were found in all tissues investigated (kidney, liver and muscle) the highest level up to 0.067 mg/kg, occurring in the kidney.

Ochratoxin A is excreted in the urine and faeces. The relative contribution of each of these excretory routes in different species is influenced by the extent of enterohepatic recirculation of ochratoxin A and it's binding to serum macromolecules (WHO, 2002).

Ochratoxin A induced gene mutations in bacteria and in mammalian cells in genotoxicity studies. In mammalian cells, it induced DNA damage and chromosomal aberrations *in vitro* and *in vivo*. Ochratoxin A is thus considered genotoxic both *in vivo* and *in vitro* (WHO, 2002). There is currently inadequate evidence in humans for the carcinogenicity of ochratoxin A.

Ochratoxin A is a nephrotoxic and teratogenic compound and may also cause immunotoxic effects (Prelusky *et al.*, 1994). Ochratoxin A has been regarded as an important factor for human endemic nephropathy in the Balkan areas (Petkova-Bocharova and Castegnaro, 1991; Fuchs *et al*., 1991; Beardall and Miller, 1994), although the evidence is ambiguous (Plestina, 1996; Joint FAO/WHO Expert Committee on Food Additives, 2001).

Ochratoxin A contamination of crops is undesirable both because of its known adverse effects on animal health and its possible significance as a human carcinogen. At present there is no EU legislation regulating ochratoxin A in feedingstuffs, although some European countries have established local controls. Direct exposure of humans to ochratoxin A is controlled by Community legislation.

7.4.2. Fusarium mycotoxins

Fusarium species produce a variety of mycotoxins. Of particular interest are zearalenone, the trichothecenes, the fumonisins and moniliformin.

7.4.2.1. Zearalenone

Zearalenone is an estrogenic compound produced by several different species, primarily by *F. graminearum* (teleomorph *Gibberella zeae*)

and by *F. culmorum*. These fungi infect grains normally during blooming. Zearalenone is usually produced preharvest but can also be produced under extremely bad storage conditions (*e.g.* high moisture content).

Zearalenone occurs in a wide variety of cereals. Analyses of cereals done in various central and northern Member States show concentrations ranging from 0.002 to 0.174 mg/kg, peak concentration 2.0 mg/kg for the wheat was reported in Poland: (Placinta *et al.,* 1999).

Zearalenone is metabolised in pigs to α-zearalenol and β-zearalenol and in cattle to α-zearalenol, β-zearalenol, α-zearalanol and β-zearalanol. Zearalenone and its metabolites are capable of being transmitted to tissues and milk. In UK, zearalenone was detected in 3 percent of conventional retail milk samples at levels ranging from 0.0012 to 0.0055 mg/l (Smith *et al.* 1994).

Zearalenone induces estrogenic effects in mammals, including early maturity of mammary glands and reproductive organs and an increase in their size. At higher doses zearalenone interferes with conception, ovulation, implantation, fetal development and viability of newborn animals (Kennedy *et al.,* 1998). Estrogenic activity of zearalenone metabolites has also been reported. Pigs appear to be the most sensitive species. The NOEL in pigs is 0.040 mg/kg of bw per day (Creppy, 2002; Kuiper-Goodman *et al.*, 1987).

There is some evidence of precocious sexual developments in humans exposed to zearalenone, however these data primarily derive from Puerto Rico and were probably due to the use of a commercial animal growth promoter (Ralgro®) based on zearalenone metabolites and not a consequence of natural exposure (Saenz de Rodriguez *et al*. 1985)

There are no data at present which suggests any risk to consumers of products derived from animals exposed to natural levels of zearalenone.

The genotoxic potential of zearalenone and its metabolites has not been clarified. These substances are classified by IARC in Group 3 (not classifiable as to their carcinogenicity to humans) (NTP, 1982; IARC, 1999).

Due to its adverse effects on mammals, zearalenone is one of the most important mycotoxins from the animal health point of view. This has been recognised by two Member States (Germany, Austria) who have recommended maximum levels for zearalenone in feed. Other European countries (Cyprus, Estonia, Lithuania, Romania and Slovenia) have specific regulations setting limits in feed. It is noted that there is no standardised and internationally validated method for determination of zearalenone and its metabolites.

7.4.2.2. Trichothecenes

Four major groups (A-D) of trichothecenes classified by structure are commonly recognised. Groups A and B are the most important because they occur naturally in significant quantities in feed (FAO, 1997, Whitlow *et al*. 2000). Type A-trichothecenes are among the most toxic mycotoxins found in Europe and include the toxins T-2, HT-2, acetyl T-2, diacetoxyscirpenol, 15-acetoxyscirpenol and neosolaniol. The B trichothecenes, such as deoxynivalenol (DON), nivalenol, 3- and 15-acetyl-DON and fusarenon-X, are more commonly encountered but generally less toxic than those of group A.

(1) Deoxynivalenol (Vomitoxin)

Fusarium graminearum and *Fusarium culmorum*, two typical field fungi, are the most important sources of deoxynivalenol (DON). These species commonly contaminate cereal crops in Europe (Müller *et al.*, 1993).

In Norway 70% of the 5000 cereal samples collected in 1988-96 were contaminated with > 0.03 mg/kg DON, oats being the more frequently contaminated cereals (Langseth and Elen, 1997). DON was also the main toxin found in oats in 1987-1992 in Germany (Müller *et al*., 1998). In Finland, the concentration detected in feeds and grains ranged between 0.007 and 0.3 mg/kg and in oats from 1.3 to 2.6 mg/kg (Hintikka *et al*., 1988). In the Netherlands the concentration of DON was detected al levels ranging from 0.020 to 0.231 mg/kg for wheat; from 0.004 to 0.152 mg/kg for barley; from 0.056 to 0.147 mg/kg for oats and from 0.008 to 0.384 mg/kg for rye (Placinta *et al.*, 1999). The very widespread occurrence of DON in European cereal crops has led to the suggestion that it could be used as a marker of fungal contamination and the possible presence of other *Fusarium* mycotoxins (Lawlor and Lynch, 2001).

The DON undergoes rapid metabolism and elimination in livestock species, and is transferred only in trace amounts into milk, meat or eggs. (D´Mello *et al.*, 1997). Therefore, the contribution of feed contaminated with DON to contamination of food of animal origin can be considered as low.

DON, amongst the trichothecenes, has been shown to have the greatest adverse impact on animal health (Miller *et al*. 2001). Pigs are the most sensitive species. Chronic exposure to DON causes decreased body weight gain, depressed feed intake (Rotter *et al*. 1994), liver damage, decreases humoral and cell-mediated immunity and reduces host resistance (Pestka, 1994). Poultry and to a greater extent ruminants are more resistant, whereas fish have been found susceptible.

There are gaps in the available data concerning the combined effects of trichothecenes in animals. Reproductive problems due to the

concomitant presence of DON and zearalenone in the same ration may occur (Böhm, 2000).

DON has been implicated in human mycotoxicosis, singly and in combination with T-2 toxin and other trichothecenes, but this is a very rare event. DON has also been reported as immunosuppressive at concentrations which are encountered naturally. Recent findings indicate some genotoxic effects of trichothecenes including DON in human cell lines (Ehrlich, 2002).

In recognition of the economic losses caused by DON in animal feeds a number of countries have established advisory levels in cereals. In the USA the Food and Drug Administration advises that cereal and cereal by-products intended for non-ruminants should not contain more than 5 mg DON/kg, and for ruminants 10 mg/kg. Similar advice is given in some EU Member States (The Netherlands, Germany, Austria) and other European countries (Cyprus, Estonia, Lithuania, Slovenia).

An intercomparison of trichothecenes analysis performed between European countries (Pettersson and Langseth, 2002) showed the need for further methods development and improvement, and subsequent validation.

(2) T-2 toxin

Group A trichothecenes are typically produced by *Fusarium sporotrichioides, Fusarium poae* and *Fusarium equiseti*. The field contamination of cereals with these fungi occurs sporadically and relatively infrequently compared to *F. graminearum* and *F. culmorum*, the major sources of DON. T-2 and HT-2 toxins have been detected at levels ranging form 0.003 to 0.250 mg/kg and 0.003 to 0.020 mg/kg, respectively, but these mycotoxins only occurred in combination with DON and zearalenone (Placinta *et al*., 1999).

T-2 toxin was one of the first trichothecenes to be identified and is known to be amongst the most potent mycotoxins. It has been associated with a major outbreak of Alimentary Toxic Aleukia in humans in Russia in 1944 following consumption of contaminated grain (Joffe, 1978).

In animals it has been reported to have extremely toxic effects on skin and mucous surfaces and can induce lesions on the mucosa of the mouth and oesophageal region in poultry and pigs. Non-ruminants seem to be more sensitive than ruminants (Placinta *et al.*, 1999). Reduced feed intake and body weight gain, buccal-oral ulceration and plaque formation were observed in chicks exposed to T-2 contaminated grain (WHO, 2002).

One of the significant effects of T-2 toxin is its immunosuppressive activity (Corrier and Ziprin 1986), probably linked to the inhibitory effect of this toxin on the biosynthesis of macromolecules (Bunner

and Morris, 1988). There is evidence that T-2 toxin may be carcinogenic in animals (D´Mello and Macdonald, 1997).

Despite its toxic effects, only few countries (Russia, Israel) have set limits for T-2 toxin in feed (0.1 mg/kg feed) or food..

7.4.2.3. Fumonisins

The fumonisins are synthesized, mainly by strains of *Fusarium verticillioides* (syn. *Fusarium moniliforme)* and *F. proliferatum*. At least 12 fumonisin analogues are known, the most important being the B series (fumonisins B_1, B_2 and B_3) which often occur together in maize (Placinta *et al*., 1999). The most significant crop, in which fumonisins occur, is maize, particularly that grown in warmer regions of the world. However, sorghum and rice are occasionally affected (FAO/WHO, 2001, Moss, 2001, Creppy, 2002). In maize, even healthy looking kernels can frequently contain fumonisin levels of about 0.001 mg/kg (FAO/WHO, 2001). In heavily infested maize, levels of up to 37 mg/kg of fumonisins have been reported (Pittet, 1998). In Italy the concentrations of fumonisin B_1 ranged between 0.01 to 2.33 mg/kg and in Portugal, from 0.09 to 3.37 mg/kg. The highest values for Fumonisin B_1 co-occurred with aflatoxins in 48 percent of samples (Placinta *et al*., 1999). Fumonisin contaminated feed is a safety issue for animals, the exposure to humans by residues in animal products being apparently neglible. While the sensitivities of different animal species differ (horse being one of the most sensitive), the concentrations occurring in imported, infected maize could reach the range where toxic effects might be possible.

Few studies on fumonisin residues in animal products apparently have been done, and when found, the residues have been mainly been associated with liver and kidney (Prelusky, 1994). No fumonisins were detected in the milk of two cows fed with experimentally contaminated feed (*F. proliferatum* culture material) resulting in exposure of the animals to 3 mg fumonisin B_1 per kg body weight per day (Richard *et al.*, 1996). Carry-over to eggs was not found (Prelusky, 1994). Consequently, human exposure to fumonisin results almost totally from consumption of contaminated maize.

In animals fumonisins (particularly B_1) are known to cause a wide range of different illnesses, such as equine leuko-encephalomalacia (ELEM) in horses and porcine pulmonary edema (PPE). The exposure levels resulting in ELEM within weeks range between 8 – 22 mg/kg feed, while levels ranging from 44 to 200 mg/kg result in liver damage (Wilson *et al.,* 1992). The experimental oral dose leading to PPE in less than 5 days in swine was 20 mg/kg body weight per day (Gumprecht *et al.*, 2001), while a dose of 0.4 mg/kg body weight per day was sufficient to cause mild PPE in piglets in four weeks (Zomborszky *et al*, 2000). The biochemical target appears to be membrane sphingolipid metabolism (Voss *et al*. 1995).

In long-term studies fumonisin B_1 has been shown to be carcinogenic in rodents causing both liver and kidney tumours. On the basis of renal toxicity a provisional maximum tolerable daily intake (PMTDI) has been defined as 2 µg/kg of body weight (for fumonisins B_1, B_2 and B_3, alone or in combination) (WHO, 2001). There is also epidemiological evidence linking fumonisin exposure to oesophageal cancer in human populations consuming beer made from contaminated maize (Rheeder *et al.*, 1992).

At present there are no regulatory or advisory limits for fumonisins in crops intended for feed use.

7.4.2.4. Moniliformin

Moniliformin is produced by some 30 different *Fusarium* species, of which *F. proliferatum* and *F. subglutinans* are the most important.

Moniliformin has been detected in maize, wheat, rye, triticale, oats and rice, and co-occurrence with fumonisins has been reported (Gutema *et al.*, 2000). Published data on occurrence of moniliformin in Europe are rather scarce. They are restricted mainly to maize and maize products in Poland and the UK, with levels in the UK varying from 0.015-0.135 mg/kg. Because of the ubiquitous occurrence of *Fusarium* species in Europe, the toxin might occur more generally in agricultural commodities in EU Member States, but data are lacking to confirm this.

Moniliformin is toxic to animals (rats, mice and at higher levels to poultry), with effects that include haemorrhages in the gastrointestinal tract, and damage to liver and heart. No effects on growth and carcass parameters and on meat quality of poultry were seen at levels up to 16 mg/kg feed (Allen *et al.*, 1981). However, at levels of approx. 100 mg/kg feed adverse effects, such as reduced weight gain and increase of relative heart weight were recorded (Harvey *et al*., 1997).

The acute and long-term toxicity of moniliformin for humans is not known and a Tolerable Daily Intake has not been established. It is not known whether there is carry-over of moniliformin into animal products and there are no published data on residues of moniliformin in animal products.

Worldwide there are currently no known regulations for moniliformin in food or feed. Analytical methodology to determine moniliformin in maize (-products) is readily available (Munimbazi and Bullerman, 2000).

7.5. Other feed associated mycotoxins

7.5.1. Mycophenolic acid

Mycophenolic acid is produced by species of different fungal genera such as *Penicillium*, *Paecilomyces*, *Septoria* or *Verticicladella*. *Penicillium roqueforti* is one of the most important sources of mycophenolic acid and occurs frequently in silages. An examination of 233 silage samples showed that mycophenolic acid was present in 32 % of the samples at concentrations ranging from 0.02 to 35 (mean 1.4) mg/kg (Bauer *et al.*, 2001). Other data are not available.

Mycophenolic acid blocks the conversion of inosine-5-phosphate and xanthine-5-phosphate to guanosine-5-phosphate. As T and B-lymphocytes rely primarily on the *de novo* biosynthesis of purine rather than on the purine salvage pathway, mycophenolic acid blocks their proliferative response and inhibits both antibody formation and the production of cytotoxic T cells (Allison and Eugui, 2000; Mele and Halloran, 2000). This is the reason why mycophenolic acid is used as an immunosuppressant after organ transplantation.

Consequently, mycophenolic acid is a toxin of possible concern in silage (Schneweis *et al.*, 2000), but lack of data on immunotoxicity in farm animals, on occurrence and on its carry-over into animal products makes it impossible to evaluate its significance to animal and human health.

7.5.2. Cyclopiazonic acid

Cyclopiazonic acid (CPA) is produced by a number of fungal species of the genera *Penicillum* and *Aspergillus*, but its importance for the feed industry is its production by *Aspergillus flavus*, a major contaminant of maize. The toxic effects of CPA in poultry, pigs and sheep are well documented (Bryden, 1991). They include weight loss and diarrhea, and histological examinations of CPA exposed animals have shown alimentary tract hyperemia, hemorrhage and focal ulceration (Cullen *et al*., 1988). CPA also has the ability to chelate metal ions and this may be an important mechanism of CPA toxicity (Bryden, 1991).

As for mycophenolic acid, the lack of European data on occurrence and concentration in maize crops and on its carry-over into animal products makes it impossible to evaluate the significance of CPA to animal and human health.

7.6. Conclusions

Among the mycotoxins and products of microorganisms, the current European Community list of undesirable substances includes only aflatoxin B_1 and ergot.

- Current EU legislation[5] on aflatoxin B_1 in feed is stringent, detailed and effective in terms of human and animal health protection. There are no scientific reasons for its revision.

- For feed containing cereals, the current EU regulation limits the occurrence of ergot on the basis of weight of sclerotia present. Separation of contaminated and non-contaminated grains on the basis of size can be inaccurate. In addition, the toxic potential of ergot and consequently its impact on animal health is dependent on its alkaloid content and composition. This should be reflected in the legislation and therefore specific limits for individual ergot alkaloids rather than for ergot sclerotia would be preferable.

  For the ergot alkaloids, analytical methods exist. Their performance would need to be validated and standardised for feedingstuffs, according to internationally accepted programmes (CEN).

Apart from the substances already considered in the legislation, other mycotoxins can be identified in feedingstuffs, which may pose a sufficient risk for animals or humans to require regulation. The following were considered by SCAN for a possible full risk assessment before listing as undesirable substances.

- Ochratoxin A contamination of crops is undesirable both because of its known adverse effects on animal health and its possible significance as a human carcinogen. Therefore SCAN recommends that, as a priority, it be considered for inclusion in the list of undesirable substances in feed and that a full risk assessment should be undertaken.

- Zearalenone has a potent estrogenic effect and consequently causes physiological disturbances and fertility problems in mammals. Its control in feedingstuffs appears desirable and therefore SCAN recommends that it also should be considered for inclusion in the list of undesirable substances. It is noted that there is no standardised and internationally validated method for determination of zearalenone and its metabolites and that these would have to be developed.

- Deoxynivalenol (DON) is found in the majority of European cereal crops destined for animal feed. Although not a problem for consumer health chronic exposure of susceptible livestock (particularly pigs) can lead to problems of animal health and is a cause of significant economic loss. Consequently SCAN recommends that it also be considered for inclusion in the list of undesirable substances. Further consideration should also be given to the analytical methods required for its detection.

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[5] Council Directive 1999/29/EC of 22 April 1999 on the undesirable substances and products in animal nutrition (E.C.O.J. n° L 115 of 04/05/1999, p. 32) repealed from 1rst August 2003 by the Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed (E.C.O.J. n° L 140 of 30/5/2002, p. 10).

- T-2 toxin, although a potent toxin, is of lesser concern due to its apparently limited occurrence and low concentration in feedstuffs. SCAN does not currently consider it necessary to include this mycotoxin in the list of undesirable substances, but recommends that some monitoring of European crops is undertaken and that this position is reviewed periodically.

- Fumonisins can be responsible for serious adverse health effects in horses and pigs, but only when present at concentrations in feedingstuffs that normally are not found in Europe. Present data suggest that human exposure to fumonisins via animal products is negligible. Therefore, SCAN suggests that setting limits for fumonisins and introducing control measures is at present unnecessary. Given the high concentrations of fumonisins that may be found in maize imported from warm regions, routine inspection would be desirable.

- Moniliformin is a toxin of possible concern in animal feedingstuffs (especially maize-based), but the lack of data on occurrence and its carry-over into animal products make it impossible to evaluate its significance to animal and human health. Further studies on moniliformin would be needed to allow a more detailed risk assessment.

- Mycotoxins such as mycophenolic acid and cyclopiazonic acid may represent emerging risks, although scientific knowledge to qualify and quantify this risk is presently unavailable. Further studies should be encouraged to allow a complete risk assessment.

ORGANIC CONTAMINANTS

BOTANICAL IMPURITIES

10. REFERENCES

10.1. Heavy metals

Adams, M.A., Bolger, P.M., Gunderson, E.L. (1994): Dietary intake and hazards of arsenic. In: Chappell, W.R., Abernathy, C.O., Cothern, C.R. (eds.): Arsenic: Exposure and Health. Northwood U.K., pp. 41-49.

Ashton, M.R. (1970): The occurrence of nitrates and nitrites in foods. BFMIRA lit. Surv. N°7, pp. 32.

Australia-New Zealand Food Authority (1998): The Australia Market Basket Survey, Melbourn: Information Australia.

Ballin, U., Kruse, R., Rüssel, H.A. (1994): Determination of total arsenic and speciation of arseno-betaine in marine fish by means of reaction-headspace gas chromatography utilizing flame-ionization detection and element specific spectrometric detection. Fresenius J. Anal. Chem. 350, 54-61.

Berntssen, M.H.G., Lundebye, A.-K. (2001): Energetics in Atlantic salmon (*Salmo salar* L.) parr fed elevated dietary cadmium. Comp. Biochem. Physiol. 128C: 311-323.

Berntssen, M.H.G., Aspholm, O.Ø., Hylland, K., Wendelaar Bonga, S.E. , Lundebye, A.-K. (2001): Tissue metallothionein, apoptosis and cell proliferation responses in Atlantic salmon (*Salmo salar* L.) parr fed elevated dietary cadmium. Comp. Biochem. Physiol. C, 128, 299-310.

Berntssen M.H.G., Lundebye A.-K., Julshamn K., Solli B., Hylland K., Waagbø, R. (2003): Upper limits of organic and inorganic mercury in feed to Atlantic salmon (*Salmo salar* L.) parr. Aquaculture Nutrition, submitted.

Bloom, N.S. (1992): On the chemical form of mercury in edible fish and marine invertebrate tissue. Can. J. Fish. Aqaut. Sci. 49, 1010-7.

Bunce, H.W.F. (1985): Fluoride in air, grass, and cattle. J. Dairy Sci. 68, 1706-1711.

Bundesministerium für Verbraucherschutz, Ernährung und Landwirtschaft (2000): Nationales Kotrollprogramm Futtermittelsicherheit.

Carter, D.E. (1995): Oxido-reduction reactions of metal ions. Environ. Health Perspect. 103, suppl. 1, 17-19.

Chiou WenShing, Chen KouoLung, Yu Bi (1997): Effect of roxarsone on performance, toxicity, tissue acculmulation and residues of eggs and excreta in laying hens. J. Sci. Food Agri. 74, 229-236.

Clarkson, T.W. (1993): Mercury - major issues in environmental-health. Environ. Health Pers. 100, 31-8.

Cockell, K.A., Hilton, J.W. (1988): Preliminary investigations on the comparative chronic toxicity of four dietary arsenicals to juvenile rainbow trout (*Salmo gairdneri* R.). Aquatic Toxicol. 12: 73-82.

Cockell, K.A., Hilton, J.W., Bettger, W.J. (1992): Hepatobiliary and hematological effects of dietary disodium arsenate heptahydrate in juvenile rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol. 103C: 453-458.

Crössman, G. (1984): Cadmium in Futtermitteln. Schriftenreihe des Bundesministeriums für Ernährung, Landwirtwschaft und Forsten. Angew. Wissenschaft 335, 18-29.

CSTEE (2001): EU Scientific Committee on Toxicity, Ecotoxicity and the Environment. Opinion on: Position paper on Ambient AIR Pollution by Arsenic Compounds.Final version, Opinion expressed at the 24th CSTEE Plenary, Brussels, June 2001.

Dokumentationsstelle Universität Hohenheim (1985): Mineralstoffe und Spurenelemente in Futtermitteln. Not published.

Dudka, S., Miller, P. (1999): Accumulation of potentially toxic elements in plants and their transfer to the human food chain. J. Environ. Sci. Health B 34, 681-708.

Evers, U., Schlipköter, H.-W. (1991): Lead. In: Merian, E. (ed.): Metals and their compounds in the environment. VCH, Weinheim pp. 971-1014.

FAO/WHO (1988): Aluminium. In: Evaluation of certain food additives contaminants. 33rd report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series no. 776), pp. 28-36.

Francesconi, K.A., Edmonds, J.S. (1989): Accumulation of arsenic in yelloweye mullet (*Aldrichetta forsteri*) following oral administration of organoarsenic compounds and arsenate. Sci. Tot. Environ. 79, 59-67.

Frost, D.V. (1967): Arsenicals in biology – Retrospect and prospect. Fed. Proc. 26,194-208.

Grave, H. (1981): Fluoride content of salmonids fed on Antartic krill. Aquaculture 24, 191-196.

Greenblatt, M., Mirvish, S.S. (1973): Dose-response studies with concurrent administration of piperazine and sodium nitrite to strain A mice. J. Natl. Cancer Inst. 50, 119-124.

Gueguen, L., Pointillart, A. (1986): Alimentation minérale. In: Perez, J.M., Monet, P., Rérat, A. (eds.): Le Porc et son Elevage. Bases scientifiques et techniques. Maloine, Paris, pp. 297-322.

Hansen, L.G., Hinesly, T.D. (1979): Cadmium from soils amended with sewage sludge: effects and residues in swine. Environment. Health Perspect. 28, 51-57.

Hapke, H.-J. (1988): Toxikologie für Veterinärmediziner. 2nd ed., Eenke, Stuttgart, pp. 109, 189.

Holl, W., Hampp, R. (1975): Lead and plants. Residue Rev., 54, 79- 111.

Holeman, A., Malovrh, S., Knez, V. (2001): The effect of diet containing arsenic(III) oxide on the traits of eggs. Zbornik Biotehniske Fakultate jubljani, Zootehnica 78, 211-218.Horvat, M. (2001): Mercury - do we

know enough? In: Ebdon, L., Pitt, L., Cornelis, R., Crew, H., Donard, O.F.X., Quevauviller, P. (eds.): Trace element speciation for environment and health. The Royal Society of Chemistry, Cambridge, p. 127-141.

IPCS (1988): Chromium. International Programme on Chemical Safety. Evironmental Health Criteria 61.

IPCS (1997): Aluminium. International Programme on Chemical Safety. Evironmental Health Criteria 194.

IPCS (2000): International Programme on Chemical Safety. Evironmental Health Criteria 277.JECFA (1978): Evaluation of certain food additives and contaminants. Twenty-second report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series no. 631.JECFA (1989): Evaluation of certain food additives and contaminants. Thirty-third report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series no. 776.

JECFA (2000): Evaluation of certain food additives and contaminants. Fifty-third report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series no. 896.

JECFA (2001): Evaluation of certain food additives and contaminants. Fifty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series no. 901.

Jonnalagada, S.B., Prasada-Rao, P.V.V. (1993): Toxicity, bioavailability and metal speciation. Comp. Biochem. Physiol. 106C, 585-95.

Julshamn, K., Kjellevold Malde, M., Bjorvatn, K., Krogedal, P. (2003): Fluoride retention of Atlantic salmon (*Salmo salar*) fed krill meal. Aquaculture Nutrition, submitted.

Julshamn, K., Lundebye Haldorsen, A.-K., Berntssen, M.H.G., Bøe, B. (2002): Norsk oppdrettslaks er trygg mat. Norsk Fiskeoppdrett nr. 9, p. 40-41.

Kammerer M., (1993): Toxicité à moyen terme des nitrates: évaluation Siliart B. expérimentale des effets sur les fonctions de reproduction chez le lapin. Ann. Rech. Vet. 24, 434-444.

KTBL (2003): Erfassung von Schwermetallströmen in landwirtschaftlichen Tierproduktionsbetrieben. In preparation. Leach, R.M., Wei-Li-Wang, K., Baker, D.E. (1979): Cadmium and the feed dose: the effect of dietary cadmimum on tissue composition in chicks and laying hens J. Nutr. 109, 437-443.

Lock, R.A.C. (1975): Uptake of methylmercury by aquatic organisms from water and food. In: Koeman, J.H., Strik, J.J.T.W.A.: Sublethal effects of toxic chemicals on aquatic animals. Elsevier, Amsterdam, pp. 61-79.

McSheehy, S., Pohl, P., Velez, D., Szpunar, J. (2002): Multidimensional liquid chromato-graphy with parallel ICP MS and eletrospray MS/MS detection as a tool for the characterization of arsenic species in algae. Anal. Bioanal. Chem. 372, 457-466.

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

Mikac, N., Branica,M., Harrison, R.W. (2001): Total and organic lead distribution in water, sediment and organisms from the eastern adriatic coast. Chem. Spec. Bioavail. 13, 1221-128.

Mordenti A., Piva G. (1997): Cr contentChromium in animal nutrition and possible effects on human health. In: Canale S., Tittarelli F., Sequi P.: Chromium environmental issues. Franco Angeli, Milano, pp. 131-151.

Morrison, L.L., Chavez, E.R. (1983): Selenium-arsenic interaction in the weanling pig. Canad. J. Anim. Sci. 63, 239-246.Mount, D.R, Barth, A.K., Garrison, T.D., Barten, K.A., Hockett, J.R. (1994): Dietary and waterborne exposure of rainbow trout (*Oncorhynchus mykiss*) to copper, cadmium, lead and zinc using a live diet. Environ. Toxicol. Chem. 13, 2031-2041.

Müller, L. (2002): Vorkommen im Wasser un d Bedeutung von Quecksilber im Trinkwasser. In.Grohmann,A., Hässelbarth,U., Schwerdtfeger, W. (eds.) Die Trinkwasserverord-nung. 4. neu bearbeitete Aufl., E. Schmidt, Berlin, pp. 357 -370.

Nasreddine L. and Parent-Massin D. (2002). Food contamination by metals and pesticides in the European Union. Should we worry? Toxicology Letters, 127, pp.29-41.

Neathery, M.W., Miller, W.J., (1975): Metabolism and toxicity of cadmium, mercury and lead in animals: a review. J. Dairy Sci. 52, 1767-1781.

Neff, J.M. (1997): Ecotoxicology of arsenic in the marine environment. Environ. Toxicol. Chem. 16, 917-927.

Noël, L., Leblanc, J.-C., Guérin, T. (2003): Determination of several elements in duplicate meals from catering establishments using closed vessel microwave digestion with inductively coupled plasma mass spectrophotometry detection: estimation of daily dietary intake. Food Add. Contam 20, 44 –56.

NRC (1980): Mineral tolerance of domestic animals. Washington

OyaOhta, Y., Kaise, T., Ochi, T. (1996): Induction of chromosomal aberrations in cultured human fibroblasts by inorganic and organic arsenic compounds and the different roles of glutathione in such induction. Mutation research-fundamental and molecular mechanisms of mutagenesis 357, 123-129.

Pedlar, R.M., Ptashynski, M.D., Wautier, K.G., Evans, R.E., Baron, C.L., Klaverkamp, J.F. (2002a): The accumulation, distribution and toxicological effects of dietary arsenic exposure in lake whitefish (*Coregonus clupeaformis*) and lake trout (*Salvelinus namaycush*). Comp. Biochem. Physiol. 131C, 73-91.

Pedlar, R.M., Ptashynski, M.D., Evans, R.E., Klaverkamp, J.F. (2002b): Toxicological effects of dietary arsenic exposure in lake whitefish (*Coregonus clupeaformis*). Aquat. Toxicol. 57, 167-189.

Prinbilincova, J., Marettova, E. (1996): The effect of cadmium on reproductive performance of laying hens and egg quality. Zivocisna Vyroba 41, 57-62.

Puls, R. (1994): Mineral levels in animal health. Sherpa International, Clearbrook, Canada)

Robberecht, H., Cauwenbergh, R. van, Boscher, D., Cornelis, R., Deelstra, H., Cauwenbergh, R. van (2002): Daily dietary total arsenic intake in belgium using duplicate portion sampling and elemental content of various foodfuffs. Europ. Food Res. Technol. 214, 27-32.

Santerre, C.R., Bush, P.B., Xu, D.H., Lewis, G.W., Davis, J.T., Grodner, R.M., Ingram, R., Wei, C.I., Hinshaw, J.M. (2001): Metal residues in farm-raised channel catfish, rainbow trout, and red swamp crayfish from the Southern U.S. Journal of Food Science, 66, 270-273.

Santoprete G. (1997): CrTotal chromium content in foodstuffs and evaluation of the average amount of chromium uptake. In: Canale S., Tittarelli F., Sequi P.: Chromium environmental issues. Franco Angeli, Milano, pp. 153-179.

Schenkel, H., Klüber, J. (1987): Mögliche Auswirkungen einer erhöhten Aluminiumauf-nahme. Übers. Tierernährung 15, 273-300.

Schryver, H.F., Millis, D.L., Soderholm, L.V., Williams, J.W., Hintz, H.F. (1986): Metabolism of some essential minerals in ponies fed high levels of aluminiuim. Cornell Vet. 76, 353-360.

Shupe, J.L. (1980): Clinico pathologic features of fluoride toxicosis in cattle. J. Anim. Sci. 51,746-758.

Shuval, H.I., Gruener, N. (1972): Epidemiological and toxicological aspects of nitrates and nitrites in the environment. Am. J. Public Health 62, 1045-1052.

Smith, R. M. (1986): Effects of long-term, low-level oral cadmium on performance, blood parameters, and tissue and milk mineral concentrations of dairy cattle through first gestation and subsequent lactation. Ph.D. dissertation, Pennsylvania State University.

Sulivan, T.W., Douglas, J.H., Gonzales, N.J. (1994): Level of various elements of concern in feed phosphates of domestic and foreign origin. J. Poultry Sci. 73, 520-528.

Sparling, D.W., Lowe, T.P. (1996): Environmental hazards of aluminium to plants, invertebrates, fish and widlife. Rev. Environ. Contam. Toxicol., 145, 1-127. Review.

Taylor, H.W., Lijinsky, W. (1975): Tumour induction in rats by feeding heptamethyleneimine and nitrite in water. Cancer Res. 35, 812-815.

Tiews, K., Manthey, M., Koops, H. (1982): The carry-over of fluoride from krill meal pellets into rainbow trout (*Salmo gairdneri*). Arch. FischWiss. 32, 39-42.

UK MAAF (1993): Aluminium in food - 39th report of the Steering Group on Chemical Aspects of Food Surveillance. London, UK Ministery of Agriculture, Fisheries and Food, 52 pp.

Underwood, E., Suttle, N. (1999): The mineral nutrition of livestock. 3rd Ed. CABI Publ., Wallingford.

Vogt, P., Jaakola, A. (1978): The effect of mineral elements added to finish soils on the moineral contents of cereal, potato, and hay crops. II. Aluminium, boron, molybdenum, strontium, chromium, cobalt, lead, nickel. Acta agric. Scand., Suppl. 20, 69-79.

Vyaizenen, G., Savin, V., Tokar, A., Gulyaev, V., Zinkevich, V., Kusuetsova, I., Chugunova, Yu, Nikitina, Yu, Fedotov, A., Marinets, R. (1997): Reduction of the concentration of heavy metals in pork. Srinovodsto 1, 18-22.

Weigert, P. (1988): Schwermetalle. In: Der Rat von Sachverständigen für Umweltfragen. Umweltgutachten 1987. Kohlhammer, Stuttgart und Mainz, p.61.

WHO (1972): Evaluation of mercury, lead, cadmium and food additives amaranth, diethylpyrocarbonate and octylgallate.WHO Food Additives Series, n°4, FAO Nutrition Meeting Report Series, n°51a.

WHO (1977): Lead. Environmental Health Criteria 3. WHO, Geneva.

WHO (1981): Arsenic. Environmental Health Criteria 18. WHO, Geneva. 200 pp.

WHO (1989): Mercury - Environmental aspects. Environmental Health Criteria 86. WHO, Geneva, p. 13.

WHO (1989): Aluminium. Toxicological evaluation of certain food additives and contaminants. 30th JECFA meeting. WHO Food Additives Series N° 24.

WHO (1990): Methylmercury. Environmental Health Criteria 101, 144

WHO (1993a): Cadmium. Environmental Health Criteria 1, 44.

Wolter, R., 1982. Pathologie d'origine aluimentaire. In: Mornet, P, Tournut, J., Toma, B. (ed.): Le Porc et ses maladies. Maloine, Paris, pp. 373-390.

Wright, F., Palmer, J., Riner, J., Haufler, M., Miller, J., McBeth, C. (1977): Effects of feeding on organocadmium to cattle and sheep. J. Ag. Food Chem. 25, 293-297.

Yost, L.J., Schoof, R.A., Aucoin, R. (1998): Intake of inorganic arsenic in the North American diet. Hum. Ecol. Risk Assess. 4**,** 137-152.

Ysart, G., Miller, Croasdale, M., Crews, H., Robb, Baxter, M., Lárgy, C. de, Harrison, N. (2000): 1997 UK total diet study – dietary exposures to aluminium, arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, tin and zinc. Food Add.. Contam. 17, 775- 786.

10.2. Mycotoxins

Allen N. K., Burmeister, HR, Weaver, GA, Mirocha, CJ (1981) Toxicity of dietary and intravenously administered moniliformin to broiler chickens. Poultry Science 60: 1415-1417

Allison A. C., Eugui E. M. (2000) Mycophenolate mofetil and its mechanism of action. Immunopharmacology, 47: 85-118

Bauer J. (1988) Krankheit und Leistungsdepression in der Schweinehaltung durch Mykotoxine. Tierärztliche Praxis Suppl. 3 : 40-47.

Bauer, J. Schneweis, I and Meyer, K. 2001. Roquefortine C and mycophenolic acid in silages: significance for animal health. Magyar Allatorvosok Lapja 123: 679-685.

Beardall, J. and Miller, J.D. (1994). Natural occurrence of mycotoxin other than aflatoxin in Africa, Asia and South America. Mycotoxin Res. 10: 21-40.

Betina, V. (1984). Biological effects of mycotoxins. In Mycotoxins-production, isolation, Separation and Purification, Betina, V (ed.) p. 25-36, Elsevier Science pulishers B.V. Amsterdam.

Bryden, W.L. 1991. Occurrence and biological effects of cyclopiazonic acid. Pp. 127-147. In Mixe, K. and Richard, J.L. (Eds.) Emerging problem resulting from microbiological contamination. National Institute of Hygienic Science, Tokyo.

Bunner, D.L. and Morris, E.R. 1988. Alteration of multiple cell membrane functions in L-6 myoplasts by T-2 toxin : An important mechanism of action. Toxicol. Appl. Pharmacol. 92:113-121.

Böhm, J. (2000): Fusarientoxine und ihre Bedeutung in der Tierernährung. Übers. Tierernährg. 28: 95 – 132.

Charmley, L.L., Tremholm, H.L., Prelusky, D.B. and Rosenberg, A. (1995). Economic losses and decontamination. Nat. Toxins 3: 199-203.

Codex Alimentarius Commission, Position Paper on Fumonisins, Food and Agriculture Organization of the United Nations/ World Health Organization, January 2001

Corrier, D.E. and Ziprin, R.L. 1986. Immunotoxic effects of T-2 toxin on cell-mediated immunity to listeriosis in mice: Comparison with cyclophosphamide. Am J Vet Res 47: 1956-1960.

Creppy E.E., Stormer F.O., Roschenthaler R., Dirheimer G. (1983), Effects of two metabolites of ochratoxin A (4R)-4-hydroxyochratoxin A and ochratoxin alpha, on immune response in mice. Infect. Immun. 39: 1015-1018.

Creppy EE (2002) Update of survey, regulation and toxic effects of mycotoxins in Europe. Toxicol. Letters 127:19-28.

Cullen, J.M., Wilson, M.E., Hagler, W.M.Jr., Ort, J.F. and Cole, R.J. (1988). Histologic lesions in broiler chicks given cyclopiazonic acid orally. Am J Vet Res 49:728-731.

Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. Official J. European Commun. L140 (2002) 10-21.

D´Mello, J.P.F. and Macdonald, A.M.C. (1997). Mycotoxins. Anim. Feed Sci. Tech,. 69: 155-166.

D´Mello, J.P.F., Porter, J.K., Macdonald A.M.C. and Placinta, C.M. (1997). *Fusarium* mycotoxins. In: Handbook of Plant and Fungal Toxicants, D´Mello, J.F.P. (ed.) p: 287-291, CRC Press, Fl.

Ehrlich, V.A. (2002). Untersuchung der gentoxischen Effekte von nahrungsrelevanten Mykotoxinen in Humanzellen. Diplomarbeit, Institut für Krebsforschung der Univ. Wien.

Fink-Gremmels, J. (1999). Mycotoxins : their implications for human and animal health. Vet. Quart. 21: 115-120.

Food and Agriculture Organization (1997). Worldwide Regulations for mycotoxins 1995. A compendium. FAO. Food and Nutrition Paper 64, FAO, Rome, pp. 43.

Food Standard Agency (1999). Food surveillance information sheet number 192.

Frisvad JC, Viuf BT (1986). Comparison of direct and dilution plating for detecting *Penicillium viridicatum* in barley containing ochratoxin. In Methods for the Mycological Examination Foods. eds. A.D. King, J.l. Pitt, L.R. Beuchat and J.E.L. Corry, pp. 45-47. New York, Plenum Press.

Fuchs, R., Radic, B., Ceociv, S., Sostaric, B. and Hilt, K. (1991). Human exposure to ochratoxin A. In : Mycotoxins, endemic nephropathy and urinary tract tumours, IARC Sc. Pub. Nº 115. WHO. P. 131-135, Oxford University Press, New York.

Gumprecht LA, Smith GW, Constable PC & Haschek WM (2001) Species and organ specificity of fumonisin-induced endothelial alterations: Potential role in porcine pulmonary edema. Toxicology 160:71-79.

Gutema T., C. Munimbazi and L.B. Bullerman (2000). Occurrence of fumonisins and moniliformin in corn and corn-based food products of US origin. Journal of Food Protection 63: 1732-1737.

Hald, B, Krogh P. (1972). Ochratoxin residues in bacon pigs. Proceedings of the IUPAC Symposium: Control of Mycotoxins, Kungäl, Sweden, pg. 18.

Harvey, RB, Kubena, LB, Rottinghaus GE, Turk JR, Casper HH, Buckley SA (1997). Moniliformin from *Fusarium fujikuroi* culture material and deoxynivalenol from naturally contaminated wheat incorporated into diet of broiler chicks. Avian Dis. 41: 957-963.

Hintikka, E.L., Westerling, B., Saari, L., Berg, S. and Rizzo, A. (1988). Occurrence of trichothecens in feed and grain- trichothecenes poisoning in farmed rainbow trout. Microbiol. Aliment. Nutri. 6: 259-261.

IARC (1999) Overall evaluations of carcinogenicity to humans. IARC monographs, 76: 1-36. International Dairy Federation (1997). Monograph on Residues and Contaminants in Milk and Milk Products, Special Issue 9701: 79-88.

Joint FAO/WHO expert Committee on Food Additives (2001). February 2001. Summary and conclusions.

Joffe, AZ. 1978. Fusarium poae and Fusarium sporotrichioides as principal causal agents of alimentary toxic aleukia. P. 21-86. In Mycotoxic Fungi, Mycotoxins, Mycotoxicoses: An Encyclopedic Handbook. Vol 3. Marcel Dekker, Inc., NY

Kennedy, D.G., Hewitt, S.A:, McEvoy, J.D., Currie, J.W., Cannavan, A., Blanchflower, W.J. and Elliot., C.T. (1998). Zeranol formed from *Fusarium* spp. Toxins in cattle *in vivo*. Food adit. Contam. 15: 393-400.

Krogh P. (1980). Ochratoxins: occurrence, biological affects and causal role indisease. In: Eaker D. & Wadstrom T. ed. Natural toxins, Oxford, Pergamon Press, pp.673-680

Krogh P., Axelson N.H. Elling F., Gyrd-Hansen N,., Hald B., Hyldgaard-Jensen J., Larsen A.E., Madsen A., Mortensen H.P., Moller T., Petersen O.K., Ravskov U., Rostgaard M., Aaulund O. (1974) Experimental porcine nephropathy: change of renal functions and structure induced by ochratoxin A-contaminated feed. Acta Pathol. Microbial. Scand. A246. 21.

Kuiper-Goodman, T., PM Scott, Watanabe H. (1987) Risk assessment of the mycotoxin zearalenone. Reg. Toxicol. Pharmacol. 7: 253-306.

Langseth, W. and Elen, O. (1997). The occurrence of deoxynivalenol in Norwegian cereals- differences between years and districts 1988-1996. Acta Agric. Scand. Sect. B. Soil and Plant Sci. 47: 176-184.

Lawlor, P.G. and Lynch, P.B. (2001). Mycotoxins in pig feeds 1: source of toxins, prevention and management of mycotoxicosis. Peer review 54(3): 117-120.

Mele, T, PF Halloran (2000) The use of mycophenolate mofetil in transplant recipients. Immunopharmacology 47: 215-245.

Miller, J.D., ApSimon, J.W., Blackwell, R., Greenhalgh and Taylor, A. 2001. Deoxynivalenol: A 25 year perspective on a tricothecene of agricultural importance. Pp. 310-320. In Summerel, B.A., Leslie, J.F., Blackhouse, D., Bryden, W.L. and Burgess, L.W. (Eds.) *Fusarium*: Paul E. Nelson Memorial Symposium. APS Press, St. Paul, Minnesota.

Miraglia, M, Brera, C (2001) Task 2.3.7. Assessment of dietary intake of ochratoxin A by the population of EU member states. Draft report, Rome, Italy.

Moss, M.O. (1991). The environmental factors controlling mycotoxin formation. In Mycotoxins and Animal Foods, Smith, J.E. and Henderson, R.S. (Eds.) pp. 37-56. CRC Press, inc. Fl.

Moss, MO. (2001). Chemical hazards and their control: Toxins. In Adams MR & Nout MJR (Eds) Fermentation and Food Safety. Aspen Publishers, Inc. Gaithersburg, Maryland pp.101-118.

Mount, M.E. (2001). Mycotoxins. PHR 150: 1-9.

Müller, H.M., and J., Schumacher (1993). A survey of the natural occurrence of *Fusarium* toxins in wheat grown in a south western area of Germany. Mycopathologia 121: 115-121.

Müller, H.M.,Reimann, J., Schumacher, U., Schwadorf, K. (1998): Natural occurrence of *Fusarium* toxins in oats harvested during five years in an area of southwest Germany. Food Addit. Contam. 15: 801-806

Munimbazi C, . Bullerman LB (2000). Chromatographic Method for Fumonisins in Corn. In: Mycotoxin Protocols, M.W. Trucksess and A.E. Pohland (Eds.). Methods in Molecular Biology 157. Humana Press, Totowa, New Jersey, USA, 131-145.

NTP (1982) Carcinogenesis bioassay of zearalenone in F344/N rats and F6C3F_1 mice. National Toxicology Program Technical Reprot Series No. 235, Department of Health and Human Services, Research Triangle Park, NC

Pestka J.J., (1994): Application of Immunology to the Analysis and Toxicity Assessment of Mycotoxins. Food and Agricultural Immunology. 6, 219 – 234

Pettersson H. and Langseth, W. (2002). Methods for trichothecene analysis- a status report. In. 6th European *Fusarium* Seminar & Third COST 835 worshop of Agriculturally Important Toxigenic Fungi, Nirenberg, H.I. (ed.), Berlin. pp.: 1186-117

Petkova-Bocharova, T. and Castegnaro M. (1991). Ochratoxin A in human blood in relation to Balkan endemic nephropathy and urinary system tumours in Bulgaria. In Mycotoxins, endemic nephropathy and urinary tract tumours IARC Scinetific Pub. nº 115, WHO, Castegnaro *et al*. (Eds.) p. 135-137. Oxford University Press, New York

Pittet A. (1998) Natural occurrence of mycotoxins in foods and feeds – an updated review. Revue Méd. Vét. 149: 479 – 492

Placinta, C.M., D´Mello, J.P.F. and Macdonald, A.M.C. (1999). A reviewed of worldwide contamination of cereal grains and animal feed with *Fusarium* mycotoxins. Anim. Feed Sci. Technol. 78: 21-37.

Plestina, R. (1996). Nephrotoxicity of ochratoxin A. Food. Adit. Contam. 13: 19-50 supp.

Prelusky, D.B., Rotter, B.A., and Rotter, R.G. (1994). Toxicology of mycotoxins. In: Mycotoxins in Grain, Compounds other than aflatoxin. Miller, an Trenholm (ed.).pp 359-403. Eagan Press, St. Paul., MN.

Prelusky D (1994). Residues in meat, milk and eggs. In: The Toxicology Forum, Special Meeting on Mycotoxin Health Risk, Conrol and Regulation, February 23-24, 1994. The Capital Hilton, Washington DC, US: 173-185 ARODOC 19220

Prelusky DB, Scott PM, Trenholm HL, Lawrence GA (1990). Minimal transmission of zearalenone to milk of dairy cows.J Environ Sci Health B, 25: 87-103.

Rheeder JP, Marasas, WFO, Thiel, PG, Sydenham, EW, Shephard, GS & Van Schalkwyk, D.J. (1992) *Fusarium moniliforme* and fumonisins in corn in relation to human esophagal cancer in Transkei. Phytopathology 82: 353-357.

Richard JL, Meerdink G, Maragos CM, Tumbleson M, Bordson G, Rice LG & Ross PF (1996) Absence of detectable fumonisins in the milk of cows fed *Fusarium proliferatum* (Matshushima) Nirenberg culture material. Mycopathologia 133:123-126.

Rotter, B.A., Thompson, BK, Lessard, M, Trenholm, HL and Tryphonas, H. (1994) Influence of low-level exposure to *Fusarium* mycotoxins on selected immunological and hematological parameters in young swine. Fund Appl Toxicol 23:117-124.

Saenz de Rodriguez, C.A., Bongiovanni A.M. and Conde de Borrego L., 1985. An epidemic of precocious development in Puerto Rican children. J. Pediatr. 107, 393-396.

Schneweis, I, K Meyer, G Engelhardt, J. Bauer (2002) Occurrence of zearalenone-4-beta-D-glucopyranoside in wheat. J. Agric. Food Chem. 50: 1736-1738.

Smith, J.E., Lewis, C.W., Anderson, J.G. and Solomon, G.L. (1994). Mycotoxins in human nutrition and health. EUR 16048 EN.

Van Egmond H.P. (1989). Mycotoxins in Dairy Products. Elsevier Applied Science Publishers Ltd, London, pp. 272.

Voss, K.A., ChamberlainW.J., Bacon, C.W. , Herbert, R.A., Walters, D.B. and Norred, W.P. 1995. Subchronic feeding study of the mycotoxin Fumonisin B_1 in B6C3F1 mice and Fisher 344 rats. Fund Appl Toxicol24:102-110.

Whitlow, L. W., Hagler W.M. Jr., Hopkins, B.A. and Díaz, D.E. (2000). Mycotoxins in feeds and their effects on dairy cattle. Feed Facts 10 (3): 1-5.

Wilson TM, Ross PE, Owens DL, Rice LG, Green SA, Jekins SJ & Nelson HA (1992) Experimental reproduction of ELEM – a study to determine the minimum toxic dose in ponies. Mycopathologia 117: 115 – 120.

Wolff, J. and Richter, W. (1989). ChemischeUntersuchunger an Mutterkorn. Getreide, Mehl und Brot 43: 103-108.

Wolff, J. (2000). Ochratoxin A in cereals and cereal products. Arch. Lebensmittelhyg. 51: 85-88.

Wolff, J., Richter, W.I.F. and Spann, B. (1995) Mutterkornalkaloide in der Milch? VDLUFA-Schriftenreihe 40, Kongreb band, 521-524.

World Health Organization (2001). Safety evaluation of certain mycotoxins in food. WHO Food Additives Series 47, WHO, Geneva, pp. 701.

World Health Organization (2002). Evaluation of certain mycotoxins in food. WHO Technical Report Series 906. WHO Geneva, pp. 62.

Zomborszky, M.K., Vetesi, F., Repa, I., kovacs, F., Bata, A., Horn, P. Toth, A, Romvari, R. (2000). Experiment to determine limits of tolerance for fumonisin B_1 in weaned piglets. J. Vet. Med. B, 47: 277-286.

10.3. Organic contaminants

10.4. Botanical impurities