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

(Adopted on 20 February 2003, updated on 25 April 2003)

1. BACKGROUND

Council Directive 1999/29/EC[1] 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		

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22 April 1999 on the undesirable substances and products in animal nutrition (E.C.O.J. n° L 115 of 04/05/1999, p. 32) repealed from 1rst August 2003 by the Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed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

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:

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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,
- other organic contaminants, and
- plants and natural plant products.

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.

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)[2], 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 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 animals is mostly caused by airborne deposition of lead on the surface of plants and on soil.

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[2] 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)

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 and 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 (95th 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 believed 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 amounts of aluminium, up to 15000 mg/kg, could be found in soft phosphates and sometimes also in trace element compounds 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, 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 nitrite/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[3]

[3] 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 and recommendations

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[4]

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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[4] 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[5] (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

[5] 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. Conclusion and recommendations

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[6] 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.

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[6] 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).

- 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.

- 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.

8.1. Introduction

Organic contaminants come from different anthropogenic sources. Their occurrence is often the direct or side- result of the use of substances in agriculture or in industry. A number of substances are listed in Council Directive 1999/29/EC as undesirable, most of them being pesticides used in the past in Europe or in some cases still in use in parts of the world.

8.2. Substances already listed: organochlorine compounds

8.2.1. Organochlorine pesticides

These compounds have been introduced in the 1940s as insecticides for plant (pre- and postharvest) and farm animal protection. As a consequence of serious concerns that arised in the 1970s concerning their toxicity but also their long persistence in the environment and ability to accumulate in the food chain, they have been banned from most applications in agriculture in Europe and the U.S.A. in the 1970s-1980s. Consequently in 1987 (Council Directive 87/519/EC[7]) organochlorines entered the Annex of Council Directive 74/63/EC[8] on undesirable substances in feedingstuffs and maximum contents in feedingstuffs and feed materials have been set for each organochlorine compound.

It is noteworthy that organochlorines continue to be used in some third countries.

The ten organochlorine pesticides currently listed share common features:

- they belong to four categories of chlorinated cyclic structures with low polarity (lipid soluble) and good resistance to physico-chemical degradation
- they do accumulate in fatty tissues of fish, birds and mammals, but also in plants (by transfer from soil)
- they are persistent in soil and sediments
- they are toxic for the animals depending on the compound
- they can be easily detected as single compounds as well as a family (multi-residue methods) at very low levels in adequetion with the maximum limits set
- their environmental load decreases progressively due to the implementation of the ban

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[7] E.C.O.J. n° L 304 of 27/10/1987, p.38

[8] E.C.O.J. n° L38 of 11/2/1974, p. 31

In view of the ban of the listed organochlorine pesticides with the exception of endosulfan in Europe and the resulting decreasing levels in feedingstuffs, the situation regarding exposure of animals and humans to such compounds through products of European origin appears to be satisfactorily managed. However, their inherent known toxicity together with the continuing use in third countries still justifies that their presence in imported feed be monitored.

8.2.2. Dioxins

Dioxins were already the subject of a detailed risk assessment made by SCAN in 2000[9] and are not further considered in this document. Their presence in the list of undesirable substance in feed remains justified.

8.3. Possible other undesirable substances

8.3.1. Other pesticides

In addition to organochlorinated compounds, other pesticides are used:

- as plant protection products (PPPs) and regulated by Council Directive 91/414/EEC[10]
- as veterinary products or biocides and covered by different Community Directives.

The evaluation of PPPs is a stepwise process taking into account their normal conditions of use. It checks the absence of unacceptable effects of PPP on crops or plant products, impact on human and animal health arising from the product itself (operator exposure) and from its residues in food and water (consumer exposure). Influence on the environment is also assessed, including both fate and distribution in the environment and impact on non target species.

Products in use are currently the subject of a reevaluation. Active substances included in products, which authorisation may be withdrawn as a consequence of the outcome of that scientific assessment, can be considered *de facto* as « undesirable substance ».

Should a banned substance still be found in feed commodities despite the fact it is non cumulative and has a very limited environmental persistence would, in the case of domestic products, certainly reflect a recent misuse. The case of cumulative and persistent substances such as organochlorines is different as a possible misuse may not be always distinguishable from the background contamination.

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[9] Opinion of the Scientific Committee on Animal Nutrition on the dioxin contamination of feedingstuffs and their contribution to the contamination of the food of animal origin, adopted on 6 November 2000, available at http://europa.eu.int/comm/food/fs/sc/scan/index_en.html

[10] E.C.O.J n° L230 of 19/8/1991, p. 1

To conclude that persistent substances should enter or not the Annex of Directive 1999/29/EC is typically a management issue that should be scientifically documented on a case by case analysis.

8.3.2. Polychlorinated biphenyls (PCBs)

Polychlorinated biphenyls (PCBs) are not known to occur naturally. They have been industrially produced (total approx. 1.6 million tons worldwide) as complex mixture of isomers (called congeners) until the late 1970s. They can also be inadvertently generated as by-products of other chemicals. The major part of the PCBs was used in capacitor and transformers, although a wide variety of other applications has been described (*e.g.* heat transfer systems, plasticizers in PVC, lubricating oils, paints).

PCBs may be considered now ubiquitous pollutants, most of them being found in soils and sediments. PCBs are persistent in the environment. Their environmental transport is complex and global, through air (the atmosphere pathway was estimated to contribute to 60-90 % of PCB input into the Great Lakes), water and biotic chain.

They have been found at low but measurable levels in nearly all aquatic biota and terrestrial species. PCBs are chemically relatively inert, highly lipophilic and quite resistant towards biotransformation. They accumulate in fatty tissues and levels increase through the food chain.

Bioaccumulation and biomagnification are congener-dependent, with a rough correlation between the extent of accumulation and the degree of chlorination.

The toxicology of PCBs has been extensively studied (review by Safe (1994)). Reproductive toxicity, inhibition of growth, porphyria, immunotoxicity, hepatotoxicity, neurotoxicity, dermal toxicity, carcinogenicity, enzyme induction, have been observed in various animal species. Recently attention was focused on the potential of PCBs to cause adverse effects through disruption or modulation of the endocrine system. The IARC has classified PCBs as probably carcinogenic to humans (class 2A).

The toxicological effects are structure-related and PCBs containing no or only a single ortho-chlorine substituent appear to be the most toxic congeners. These congeners exhibit a co-planar configuration similar to that of 2,3,7,8-tetrachlorodibenzo-p-dioxin and mimic the effect of dioxins in biological systems. Toxic effects of these congeners include developmental impact (teratogenicity and embryo toxicity), hepatoxicity and immunotoxicity (ATSDR, 2000). Coplanar PCBs exert extensive reproductive effects on both sexes , especially when the animals are exposed during early development (Safe, 1994 for review). This type of congener (or corresponding hydroxylated metabolites) has demonstrated anti-estrogenicity or anti-androgenicity, both *in vitro* and *in vivo.*

Non-coplanar congeners generally have much lower acute toxicities than co-planar PCBs but many are present in much higher concentrations in the food chain and in human tissues and exhibit a higher neurotoxicity. A number of non co-planar PCB congeners have estrogenic potential.

The Lowest Observed Adverse Effect Level (LOAEL) usually retained for PCBs risk assessment is based on decreased antibody (IgG and IgM) production in monkeys exposed to PCB mixtures. A very low values of 5 µg /kg bw/day has been established for Aroclor 1254 which is typical of moderately to heavily chlorinated PCB (coplanar and non-coplanar) mixtures. (Cogliano, 2001).

Although toxicity symptoms in accidental toxic outbreaks in animals are generally rather non specific, exposure to PCBs can lead to "chick oedema disease" (subcutaneous edema of the neck, ataxia, ascites) in poultry (Bernard *et al.*, 1999). However, no adverse effect would occur under background contamination conditions.

It can be assumed that the animal PCB body burden derives mainly from feeding. Data indicate the existence of a background PCB contamination in feedingstuffs. Fatty feed materials of animal origin (fish oil and fish meal, animal fats) are the greatest contributors. The SCAN already draw the attention on the fact that very limited data are available concerning the contamination of feed materials with dioxin-like PCBs (SCAN, 2000).

Non-accidental human exposure to dioxin-like PCBs (in conjunction with dioxins) has been examined by the SCF and mainly occurs through food (90%). Due to their accumulation across the food chain, food of animal origin contributes to 90% of the food exposure (SCF, 2000). Given their similar physico-chemical properties and metabolic fate it can be anticipated the same figures apply to non-coplanar PCBs. Routine analysis allows distinguishing congeners. However, in practice, because of the large number of congeners, seven "marker PCBs" are selected for monitoring purposes: PCB 28, 52, 101, 118, 138, 153 and 180 and are analysed by using multi-residue methods. The fact that several metabolites such as hydroxyl or methylsulfonyl PCB are also active compounds and may concentrate in livestock tissues should also be taken into account in a monitoring strategy.

According to the potency to induce dioxin-like toxicity, the WHO has established toxic-equivalency factors (TEFs) for 17 dioxin congeners and 12 dioxin-like PCB congeners (van den Berg *et al.*, 1998). The Tolerable Weekly Intake set by the SCF is 14 pg WHO TEQ /kg body weight that covers both dioxins and dioxin-like PCBs (SCF, 2001).

In conclusion, PCBs are ubiquitous contaminants of feed and food commodities. Acute toxicity in farm animals has only been observed after severe feed contaminations. Background feed material contamination exists and accumulation in animal fat occurs. Human

safety concerns exist due to the fact these substances are active at very low concentration and that different toxicological end-points have been identified in relation with the different PCB congeners.

8.3.3. Brominated flame retardants

Brominated flame retardants include polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD) and polybrominated biphenyls (PBBs).

The use of PBBs is banned or restricted in various countries, including EU member states (CEC, 1988). They are/were "high production volume" chemicals widely used in particular in synthetic fibres, plastics and coatings. They share physico-chemical properties with PCBs, are highly persistent, lipophilic and tend to bioaccumulate. Chemical analysis is not feasible on a routine basis.

Contamination of environmental compartments occurs mainly via waste (landfill) and industry emissions (air, wastewater) (IPCS, 1994). Uptake by plants is likely to be low. Fries and Jacobs, 1980 could not detect PBBs in the harvested forage grown on soil with residue levels up to 0.3 mg/kg. They are found in sewage sludge (Jansson, 1993; UBA, 1998). Brominated diphenyl ethers were found in nearly all analysed terrestrial wild animals and fishes.

The main toxic effects of PBBs are weight loss, liver damage, effects on immune system and reproduction. PBBs are weakly teratogenic in cattle and laboratory animals. Endocrine disrupting activity is also suspected. There remains however significant lack of toxicological data on exposure and effects. No ADI was established by FAO/UNEP (1992). PBB (mainly based on hexabromobiphenyl) is classified as a (non-genotoxic) rodent carcinogen producing liver tumors in mice and rats, and is considered possibly carcinogenic to humans (group 2B) (IARC, 1987). The lowest NOAEL was 0.15 mg/kg BW from a 2-year rat study (WHO, 1994).

Considerable information on direct exposure through feed, and indirect contamination of humans and environment has been obtained after the Michigan Accident in 1973, a case of accidental PBB contamination of feed in where PBBs were mistakenly (assumed to be a magnesium additive) mixed with feed; however, as such compounds do not have any application in the agricultural production of feed materials and plant uptake appears to be insignificant, feed materials are not expected to be directly contaminated. Nevertheless, in view of the similarity with PCBs, similar distribution may be expected.

The insufficient knowledge on the toxicological properties of polybrominated flame retardants does not allow SCAN to conclude on this class of compounds. However, the similarity of PBBs with PCBs, and their potential endocrine-disrupting activity, argue for further consideration on these flame retardants.

8.3.4. Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants widely occuring at trace levels in eco-systems such as soils, sediments, the atmosphere and plants (Baek *et al.*, 1991 ; Simonich and Hites, 1994). Apart from natural sources like vegetation fires, point-source pollution such as oil spills, abandoned industrial sites, fossil oil energy production, sewage sludge or vehicle exhausts have raised concern about the possible transfer of these compounds along the food chain (Jones *et al.*, 1989).

Several studies have shown that the atmospheric deposition of PAHs onto plants represents the major way of contamination of leafy vegetables, grains, fruits and derived oil. No significant transfer from contaminated soil to plants has been shown to occur (Sims and Overcash, 1983 for review). Another source of PAHs in feed/food includes thermal technological treatments such as drying by direct heating with air that contains combustion gases.

As far as the water food chain is concerned, dietary uptake by fish of PAH-contaminated sediments or water organisms indicate low uptake efficiency (Meador *et al.*, 1995 for review). However the same authors reported that the continuous exposure of fish to PAHs in coastal and estuary contaminated area leads to higher residual PAHs values than in the open sea.

Several PAHs are well documented carcinogens producing mainly local tumors in a variety of species after skin application, inhalation, subcutaneous or intramuscular administration (IARC, 1983). Studies showing carcinogenicity after oral administration have also been reported. Papillomas and carcinomas in the upper digestive tract (tongue, esophagus and forestomach) have been shown to occur in mice administered orally with benzo[a]pyrene or a coal tar mixture (also lung and liver tumors in that latter study) (Culp *et al.*, 1998) while in a rat study malignant tumours were observed in a variety of organs: mainly in liver and forestomach, but also in oral cavity, small intestine, auditory canal, and skin and appendages (Kroese *et al.*, 2001). Other *in vivo* toxicological end points such as reproduction toxicity and immunotoxicity, gave positive responses only for high oral concentrations (IPCS-Inchem report for review).

As far as fish are concerned, tumors (typically in the liver of bottom dwelling flatfish species) have frequently been described and associated with polluted sediment. This was the case in field, mesocosm and laboratory studies using PAH containing sediment or extract. Other observations support the causal relationship with PAHs and furthermore laboratory studies with single compounds such as benzo[a]pyrene or dimethylbenzanthracene confirm the carcinogenic potential of PAHs in fish (de Maagd and Vethaak, 1998).

For animal feed, contamination sources are likely to be generally the same and in the same order of magnitude as for human food. Therefore, at most, the risk for animals would be in the same order. However, for most livestock including farm fish, as lifetime (and therefore duration of exposure) is limited, no health risk is expected.

The metabolic fate of anthracene, pyrene and benzo[a]pyrene in the rat has shown that the orally dosed PAHs are largely absorbed by the gastrointestinal tract, metabolically activated to highly reactive electrophillic metabolites, subsequently metabolized and excreted as metabolites in the urine and feces (van Schooten *et al.*, 1997). Other authors have shown that benzo[a]pyrene, despite its lipophilic properties, is not stored in the adipose tissue of pig and fowl and also the human (Graf *et al.*, 1975). Similarly for fish, PAHs are actively metabolised and are not stored into tissues.

The human consumer exposure to PAHs has been evaluated in UK total diets (Dennis *et al.*, 1983). This study indicates that cereals and oil/fats (mainly from vegetable origin) contribute the major part (approximately one third each) of the contamination, while fruit, sugars and vegetable bring about a fourth of it. Milk and unprocessed meat and fish contributions (1.9, 4.8 and 1.8% respectively) are very limited. A similar study carried out in Dutch total diet samples (de Vos *et al.*, 1990) leads to similar conclusions: cereals and vegetable oil/fats appear as the main contributors to the dietary PAHs burden (12.4 and 55.2% respectively) while meat, fish and milk represent only 2.5, 1.8 and <1% respectively. Also in the assessment of the SCF the intrinsic levels from animal produce were not mentioned as a significant source of dietary PAHs although technological (combustion associated) processing would form a major risk factor for PAHs exposure (SCF, 2002). Therefore animal products do not contribute significantly to the human exposure to PAHs.

In the recent assessment of PAHs by the SCF (2002), it was concluded that "*the estimated maximum daily intake of benzo[a]pyrene from food is approximately 420 ng benzo[a]pyrene per person, equivalent to approximately 6 ng/kg bw/day for a person weighing 70 kg. This estimated maximum daily intake is about 5-6 orders of magnitude lower than the daily doses observed to induce tumours in experimental animals.*"

In conclusion, PAHs are ubiquitous contaminants of feed and food commodities. The major oral human exposure is by far due to contaminated plant derived products, unprocessed animal products contribution being marginal. Therefore PAHs background contamination of feedingstuffs is unlikely to represent a health hazard to farm animals, and to humans from the consumption of animal products.

8.4. Conclusion and recommendations

The current list of organic contaminants include organochlorine pesticides and dioxins.

- The organochlorine pesticides listed in Council Directive 1999/29/EC resulted in decreasing levels in feedingstuffs. Consequently, the situation regarding exposure of animals, and of humans to such compounds through products of European origin, appears to be satisfactorily managed. However, their inherent known toxicity together with the continuing use in some third countries still justifies that their presence in imported feed be monitored.

- Dioxins have been already the subject of a dedicated report of the SCAN in 2000 and the Committee's conclusions are still valid.

In addition, the Committeee considered other contaminants.

- When the outcome of a risk assessment leads to the withdrawal of a pesticide, that compound should *de facto* be considered as potentially undesirable in feed.

- Polychlorinated biphenyls (PCBs) are ubiquitous stable and lipophilic biocumulative contaminants that concentrate along the food chain. Their toxicity is known and varies depending on the congener. Analysis, determination and quantification is feasible but is not done in practice, except for some marker congeners that are selected for routine analysis. Their occurrence, together with toxicity, in particular for dioxin-like PCBs, justify that such compounds be considered for possible inclusion and that a detailed risk assessment be undertaken.

- The insufficient knowledge on the toxicological properties of polybrominated flame retardants does not allow SCAN to conclude on this class of compounds. However, the similarity of PBBs with PCBs, and their potential endocrine-disrupting activity, argue for further consideration of these flame retardants.

- PAHs are ubiquitous contaminants of feed and food commodities. The risk for animals are considered negligible when exposed to PAHs background contamination of feedingstuffs. The major oral human exposure is by far due to contaminated plant derived products, unprocessed animal products contribution being marginal. Therefore, the exposure to PAHs of consumers eating foods derived from animals given feeds containing background concentrations of PAHs will be minimal in comparison with their total dietary exposure to PAHs. Thus the inclusion in the list of undesirable substances in feed does not appear justifed.

9.1. Introduction

The quality of traded commodities used as ingredients in animal feed and, as a consequence, the quality of animal feedingstuffs has substantially improved over the last decade. This improvement has been supported and encouraged by the introduction and increasing use of quality assurance schemes initially by the manufacturers of animal feeds but latterly spreading to encompass those supplying raw materials. As a consequence it is now extremely rare for those responsible for monitoring trading standards to encounter a sample of either a feed ingredient or a compounded feedingstuff that fails to meet the limits imposed by the Undesirable Substances Directive. As an example, none of the approximately 1000 samples/year examined by the Danish Plant Directorate have fallen outside the specification of the Directive in the last ten years (see http://www.plantedir.dk). Other European laboratories responsible for the monitoring of feed quality report similar experiences.

Nonetheless, feed ingredients are inevitably contaminated with a variety of materials of botanical origin. Depending on the feed or feed ingredient, these may derive from weeds growing within the crop or at field margins at the time of harvest or from adventitious contamination during storage and/or transport. The vast majority of such contamination is innocuous and only rarely is noxious or potentially toxic material detected. Recent examples have included the detection of *Ricinius communis,* the castor oil seed, in a sample of Indian rapeseed and seeds of *Datura* spp. in samples of linseed. However the majority of the botanical material listed by name in the annex to the Undesirable Substances have not been seen for many years and appear to have been incorporated into Community legislation as a result of historical incidents involving one or more Member States.

Light microscopy is the principal means of detection used for botanical contaminants with quantitation dependent on the physical separation of the contaminating material. The successful identification of contaminants is dependent on several factors including the skill of the operator, the availability of reference material and the degree to which the sampled material has been processed. Commination and heat/pressure treatments can destroy much, or all, of the anatomical/histological feature on which identification is based.

At present 17 plant species and 5 natural products are named in the annex to the Directive with prescribed limits for their occurrence in feed materials. These are individually considered below.

9.2. Plant species listed by name in the annex to the Directive

9.2.1. Ricinus communis *L. (Castor Oil plant)*

The Castor Oil plant is a native of India and a member of the Euphorbiaceae. It is very variable in habit and appearance, occuring as a tree 10-12 m high in tropical latitudes, of slender growth (3-5 m) in Mediterranean countries. Further north, where it is cultivated as an ornamental plant, it is merely shrubby. The fruit is a three-lobed, green or red capsule with a soft, spiny exterior. One large, mottled seed develops in each lobe.

Plants are grown commercially for the non-volatile fatty oil (90% ricinoleic acid, 12-hydroxy *cis*-9-octadecaenoic acid) extracted from seeds by either pressing or solvent extraction. Seeds are produced for industrial purposes (1.05 mt in 2002) primarily in India and China and to a lesser extent Brazil. The oil has been used for many years as a purgative, but it has many other commercial uses including the manufacture of some plastics, textiles and textile finishing materials, paints and varnishes and cosmetics.

The plants are highly toxic because of the presence of ricin, a water-soluble glycoprotein concentrated in the seed endosperm but present in lower amounts in the rest of the plant and reputed to be one of the most poisonous of the natural occurring compounds. Ricin acts by translocating to the cytosol the enzymatically active toxin-A chain, which inactivates ribosomes (Wesche *et al*., 1999). A second lectin ricine, and a toxic alkaloid ricinine (Yuldashev, 2001) are also present in seeds.

There is great species variability in susceptibility to ricin, with humans and horses being most at risk. The lethal oral dose of ricin is approximately 0.1 g/kg body weight for horses, 1-2 g/kg for most other livestock, but 5.5 g/kg body weight for goats. All animals (livestock and pets) are vulnerable.

The seed is only toxic if the outer shell is broken or chewed open. Seeds swallowed intact usually pass without incident. Signs of toxicity may not manifest for 18 to 24 hours after ingestion. The animal first shows signs of depression and a mild increase in temperature. Later, gastrointestinal signs predominate, including vomiting, profuse diarrhoea, colic and abdominal pain. The affected animal may then go into convulsions, collapse and die, with death generally occurring within 36 hours of consumption. The toxic signs are the result of severe gastrointestinal irritation, anaphylaxis and shock.

Analytical methods exist for the direct determination of ricin and for the alkaloid ricinine, the later having been used as a marker for castor bean extracts in forensic samples (Darby *et al*., 2001).

9.2.2. Jatropha curcas *L. (Purghera, Physic nut)*

Jatropha curcas is a another member of the family Euphorbiaceae known for its toxicity. It is native to tropical America, but is now cultivated widely in tropical countries throughout the world. It grows as a shrub or tree to 6 metres in height. The fruit capsule it produces, when dry, splits into three valves, all or two of which commonly have a single oblong black seed.

The plant, including the seeds, is widely used for a variety of domestic purposes and in traditional systems of medicine. There has been limited interest in its potential as a commercial oilseed crop, as the oil has properties similar to palm oil (Openshaw, 2000).

Seeds contain curcin, a toxic glycoprotein with a 54% homology with the ricin A chain and with a similar mode of action (Lin *et al*., 2003). In humans, symptoms following ingestion are largely those associated with gastro-intestinal irritation. There is acute abdominal pain and a burning sensation in the throat about half an hour after ingestion of the seeds, followed by nausea, vomiting and diarrhoea. In severe intoxications dehydration and haemorrhagic gastroenteritis can occur. There may be CNS and cardiovascular depression and collapse. Two seeds are a strong purgative, while four to five seed are said to have caused death.

Poisoning from ingestion of the seeds of the *Jatropha* plant is well known in veterinary practice in areas where the plant is grown and autopsy findings include, severe gastroenteritis, nephritis, myocardial degeneration, haemagglutination, and sub-epicardial and subendocardial haemorrhages as well as renal sub-cortical and sub-pleural bleeding.

A study assessing the acute oral toxicity of *J. curcas* showed that different ruminants had different susceptibilities. Calves which received 0.25 or 1g/kg feed died within 19 hours of administration, whilst goats given a similar daily dose were either killed or died within 7-21 days. It was not established whether this species difference lies in direct cytotoxic action or in the capacity with which the active substances contained in *J. curcas* seed are converted to metabolites more or less toxic than the parent compounds. (Ahmed & Adam, 1979)

Feeding chicks boiled *J. curcas* seeds produced growth depression, hepatonephropathies, and haemorrhages. Increasing the concentration of boiled seed in the diet, reduced average feed intake, weight gain and protein efficiency and decreased gross blood features, serum total protein, albumin and globulin, while serum creatinine level was increased. Mortality was significantly increased with increasing dietary level of *Jatropha* seeds (Adeyemi *et al*., 2001).

9.2.3. Croton tiglium *L. (Croton)*

Native to tropical Asia from India to New Guinea and north into Indonesia and China where it occurs as a small shrub or tree up to 12 m tall. The fruit capsule is triangular, three-lobed; and produces three seeds per fruit.

Croton is also a member of the Euphorbiaceae, but somewhat less toxic than the other two genera listed in the annex. The major toxic agent, crotin, is a collective name for a group seed glycoproteins each with varying toxicity but with a similar mode of action to that of ricin (Sperti *et al.,* 1976). Phorbal 12,13 diesters are also present and probably account in part for the irritant and purgative action of the ingested seeds.

Croton oil is produced in limited commercial amounts in India and Europe, with most of the commercial supply of seed being obtained from Sri Lanka and India. Otherwise the plant has some medicinal properties and its use is recorded in many traditional systems of medicine. It is grown in Europe and elsewhere as an ornamental plant.

9.2.4. Crotalaria *spp*.

Crotalaria are annual or perennial herbs with a pea-like fruits with many seeds which, when dry, rattle giving rise to the common names Rattlebox or Rattleweed.

In the early 20th century, several African species of *Crotalaria* were brought to the United States for use as soil improvement plants. However its use for this purpose was soon discontinued. The drawbacks to the cultivation of *Crotalaria* were the presence of alkaloids poisonous to livestock or poultry (Mattocks, 1986) and the hard seeds that tended to germinate and come up years later as volunteers in other crops. Consequently *Crotalaria spp.* are now treated as a noxious weed in at least some States in the USA. The potential for their contamination of soybean/soybean meal imported into Europe from the US appears to be reason for the inclusion of *Crotalaria* in the annex to the Directive. *Crotalaria* spp. do not occur in Europe except as a result of cultivation.

Six or seven species of crotalaria have been identified as toxic to animals. The two most prevalent species found in the south-eastern United States are *Crotalaria giant striata*, and *Crotalaria spectabilis* Roth, with the latter being most harmful to poultry. The herbage and seeds are considered equally toxic as a hepatotoxic pyrrolizidine alkaloid monocrotaline (Copple *et al*., 2002) is present in the entire plant. The alkaloids, fulvine and cristpatine, also have been isolated and identified as macrocyclic esters of retorsin.

Consumption of *C. spectabilis* by laying hens has been reported to cause a rapid decrease of egg production and increased mortality.

During post-mortem examination the birds were found to have ruptured livers with internal haemorrhage, and a fluid collection in the body cavity. Growing birds exhibited droopiness, huddling, mortality, marked abdominal fluid accumulation, and surface haemorrhages on the liver followed by tissue deterioration.

Feeding trials using day-old chicks and ground *C. spectabilis* demonstrated that the adverse-effect level lay between 0.01 and 0.1% of the diet. Concentrations in excess of 0.3% were fatal within 18 days. Levels of *C. spectabilis* above 0.05% severely reduced egg production over six weeks. Hens on those levels were very emaciated due to feed rejection, and birds on 0.2% ate very little feed after the first week. Turkey studies have demonstrated that *C. spectabilis* was toxic to the liver when given to four week old birds in quantities greater than 0.125% of the diet. Cirrhosis developed when 0.25% or more was fed. *C. spectabilis* studies with Bobwhite quail have indicated that the seeds were poisonous but that under ordinary conditions they would not be eaten by birds when there was a choice of feedingstuffs. No successful treatment for crotalaria poisoning has been reported.

9.2.5. Camelina sativa *(L.) Crantz. (false flax, linseed dodder, gold-of-pleasure)*

Camelina is a spring-sown brassica that has been cultivated in Europe since the Bronze Age for its oil seed and fibre content. It was widely grown up to the early 1940's, but commercial production ceased with the introduction of oilseed rape. It is considered by some as an under exploited oilseed crop. This renewal of interest in the species is mainly due to the demand for alternative low-input oilseed crops with the potential for a non-food utilisation of the seed oil (Seehuber 1984; Putnam *et al*., 1993). Camelina oil has a unique fatty acid pattern characterised by a linolenic acid (C18:3) content of 30% to 40% and an eicosenic acid (C20:1) content of around 15%, with less than 4% erucic acid (Seehuber 1984; Marquard and Kuhlmann 1986; Budin *et al.,* 1995), which suggests applications similar to linseed oil (Luehs and Friedt, 1993). A relatively low content of glucosinolates is found in camelina compared to other *Brassica* species (Lange *et al.*, 1995), which makes the utilisation of the seed meal by livestock easier.

It is difficult to see why this species was included in the annex to the Undesirable Substances Directive. Possibly it was because, when flax (*Linum usitatissimum*) was grown on a large scale in Europe, this plant was a common weed in the crop. It was also known as a weed of grain crops and consequently considered undesirable. The decline of flax as a crop has been associated with a parallel decline, virtually to extinction, of *Camelina*. Other than the presence of glucosinolates, which are found in many other *Brassica* species, there appears to be no other recorded toxicants. It is very unlikely that either the seeds of this plant or any other plant part would have

adverse effects on livestock when present in the low levels associated with contamination.

9.2.6. Mustard seeds

The taxonomy of the plant species identified as "mustard" in the annex has undergone some revision since the Directive came into force. In particular the distinction between the various sub-species of *Brassica juncea* is no longer upheld and Indian, Brown and Chinese mustards are considered simply to be varieties of *Brassica juncea*. "Sareptian" mustard now appears to be a term that has an existence only with the context of this Community Directive.

- *Brassica juncea* (L.) Czern. et Coss. (Indian, Chinese or Brown mustard)

A Perennial herb, usually grown as an annual or biennial, up to 1 m or more tall, which originated from the hybridisation of *Brassica nigra* with *Brassica campestris.* This probably happened in South Western Asia and India where the natural distribution of the two species overlaps. Like *Brassica nigra*, the black mustard (q.v.), it has been grown for oilseed, greens and as a spice. Prior to the 1940's, *B. juncea* was considered to be inferior to *B. nigra* in the making of mustard but in the 1940's a new yellow-seeded variety of *B.juncea* was imported into the USA from China and became widely cultivated. This was because, unlike *B. nigra,* the new variety could be mechanically harvested.

The most important toxic constituent in mustard is sinigrin (2-propenylglucosinolate) which, upon hydrolysis by the enzyme myrosinase (EC 3.2.3.1), yields allyl isothiocyanate, a volatile liquid known as Oil of Mustard. Sinigrin is found throughout the plant and in the vegetative parts its concentration decreases from seedling to early flowering stage, increases in late flowering then decreases again during seed formation and maturation. In seeds, where the highest concentrations of sinigrin are located, levels increased during maturation (Rangkadilok *et al*., 2002). The yield of allyl isothiocyanate from *B. juncea* is 0.25 - 1.4% (usually ca 0.9%). Other minor volatile components also released by enzymatic hydrolysis include methyl, isopropyl, *sec*-butyl, butyl, 3-butenyl, 4-pentenyl, phenyl, 3-methylthiopropyl, benzyl, and phenylethyl isothiocyanates.

Allyl isothiocyanate is an irritant, rubefacient and vesicant. It is one of the most toxic of the essential oils. Isothiocyanates such as those present in mustard have been implicated in endemic goitre (hypothyroidism with thyroid enlargement). They also have been reported to produce goitre in experimental animals. Allyl isothiocyanate was found to be more than one thousand times more cytotoxic in a Chinese hamster ovary cell line than the glucosinolate, showing significant cytotoxic activity at concentrations below 1

μg/ml. Allyl isothiocyanate was unable to induce either chromosome aberrations or sister chromatid exchanges even at highly cytotoxic doses unlike sinigrin, which induced aberrations *in vitro* (Musk *et al*., 1995).

In one of the few studies on the direct effects of mustard oil on livestock, triggered by reports of reduced feed intake in lambs consuming forage brassicas, allyl isothiocyanate (10 mmol/day) was administered to sheep offered dry grass pellets *ad libitum*. Food intake was significantly depressed in the test group and elevated plasma γ-glutamyl transpeptidase activity indicated possible liver damage (Duncan and Milne, 1993).

Methods for the detection and quantification of sinigrin and allyl isothiocyanate either singly, or simultaneously (Tsao *et al*., 2002) or after complete hydrolysis (Tsiafoulis *et al.*, 2003) are well developed. While these may be expected to work well with contaminants in a matrix that does not produce isothiocyanates, problems may arise in mixed feedingstuffs containing other *Brassica* species, notable canola, which also contain 2-propenylglucosinolate.

- *Brassica nigra* (L.) Koch (Black mustard)

A much-branched, aromatic, fast-growing annual herb, to 4 m tall, which produces dark reddish-brown to black seeds about 1 mm in diameter. The origin of the species is unknown, but some believe it to be from a Mediterranean centre with a secondary centre in the Near East. It is now widespread in Central and South Europe, and other areas with a temperate climate. It is also a frequent weed of waste places and cultivated fields.

Black mustard seed contains approximately one-third by weight oil, of which 40% is erucic acid (Basu *et al*., 1973). Like the other mustards, the major toxic factor is allyl isothiocyanate derived from sinigrin. The seeds also contain a novel and potent thermostable protein inhibitor of trypsin and subtilisin (Genov *et al*., 1997).

- *Brassica carinata* A.Braun (Ethiopean mustard)

Brassica carinata, a natural hybrid of female *B. nigra* and male *B. oleracea*, originated in and largely restricted to Ethiopia and where it is used both as a leaf vegetable and as an oilseed. It is known to be highly heat and drought tolerant. Like the other mustards the seed in particular contains significant amounts of glucosinolate.

There has been some interest both in Europe and in Canada in commercial production as a source of industrial non-food use oil. (see for example EU FAIR CT96 1946). Naturally occurring lines have been found in which the erucic cid content of the seed oil varies between 0 and 50+%.

9.2.7. Madhuca longifolia *(L.) Machr. (Mahua, Mowrah, Bassia, Madhuca and many others)*

Madhuca longifolia is a large evergreen or semi-evergreen tree with a dense spreading crown extensively cultivated in warm climates for its oil-containing seeds. The distinction made in the annex to the Directive between *Madhuca longifolia* and *Madhuca indica* Gmelin is no longer supported and the species are considered synonymous.

The seed oil, which is a common ingredient of hydrogenated fat in India, contains oleic (46.3%) and linoleic (17.9%) acids as the major unsaturated fatty acids and the saturated fatty acids palmitic (17.8%) and stearic (14.0%) acids. Defatted seed meal contains 29.4 % protein and 9.8% saponins which are toxic at this level causing injury to the mucosa of the digestive tract and haemolysis of the blood. Detoxification by heat treatment has been attempted, but the treated cakes have a very low in digestibility. However, the levels of saponins could be reduced by treatment with isopropanol. Detoxified seed flour appears to be a good source of protein for food and feed products (Singh and Singh, 1991).

The saponin fraction of is composed of several oleanane-type triterpene glycosides of which madlongisides A-D appear unique to *Madhuca longifolia* (Yoshikawa *et al*., 2000). Also detected are mimusopside A, Mi-saponins A, B, and C, and 3-O-β-D-glucopyranosyl protobassic acid. (Li *et al*., 1994; Yoshikawa *et al*., 2000).

Inclusion of unprocessed seed-cake at up to 20% of the total dry matter in concentrate mixtures for lactating cows did not adversely affect the dry-matter intake, nutrient digestibility, milk production, milk composition or the economics of feeding. (Talpada *et al*., 1995). The unprocessed seed meal also appeared well tolerated by calves (Tiwari *et al*., 1996). There appears no data on the feeding of the intact seed to any livestock species or the seed meal to livestock other than ruminants. However, while the presence of toxic saponins is undesirable, the presence of *Madhuca longifolia* seed at the concentrations associated with adventitious contamination would be expected to be tolerated by non-ruminants and have no detectable effects on ruminants.

9.2.8. Prunus armeniaca *(Apricot) and* Prunus dulcis *var amara (Bitter almond)*

The Rosaceae includes many common fruit trees, including apricot and almond (sweet and bitter). Commercial production world-wide in 2002 was 1.4 mt of almonds and 2.7 mt of apricots. Seeds, leaves and roots of this family contain a cyanogenic glycosides, of which amygdalin (D-mandelonitrile-gentiobioside) is the most commonly encountered and quantitatively the most important. Amygdalin derived from apricot has been referred to as laetrile. The glycoside

itself has only a low toxicity. It is only when seeds are crushed and moistened that the enzymes responsible for its breakdown are released and cyanide (hydrocyanic acid) produced. Amygdalin is first hydrolysed by a highly specific β-glycosidase (EC 3.2.1.118) to prunasin (D-mandelonitrile-D-glucoside), and then by second β-glycosidase (EC 3.2.1.118) specific to prunasin to the aglycone and glucose. The aglycone (mandelonitrile) is then further metabolised by hydroxynitrile lyase to cyanide and 4-hydroxybenzaldehyde. It has been estimated that bitter almonds can produce up to 250mg HCN/100g seed.

Although JECFA in their latest consideration (JECFA, 1992) were unable to estimate a safe level for the ingestion of cyanogenic glycosides because of a lack of quantitative data, the toxicity of cyanide itself has been extensively studied. Essentially, cyanide causes a decrease in the utilisation of oxygen in the tissues, producing a state of histotoxic anoxia. This occurs through inactivation of tissue cytochrome oxidase by cyanide, which combines with Fe^{3+}/Fe^{2+} contained in the enzyme.

All mammals are susceptible to cyanide poisoning, the minimum lethal dose for most species being approximately 2mg/kg body weight. Ruminants are more susceptible than monogastric animals.

Apricot seeds may be available as by-products of the preparation of dried fruit, canning or jam-making and seed meal from almonds from the production of the "oil of bitter almond". Almond hulls, have also been used in cattle and sheep rations in fresh and ensiled form. However, nutritionists and feed manufacturers are well aware of the risk posed by the presence of cyanogenic glycosides and present day use of such by-products is unusual.

9.2.9. Fagus silvatica - *unhusked beech mast*

Mast is defined as the fruit of forest trees such as beech or oak used as food for pigs and derives from Old High German *Mast* meaning food. As the name implies the seed of beech and oak have been traditionally used as a feed for pigs either as a meal or directly grazed, for the fattening of domestic poultry and for park deer. The residues left after oil extraction of beech seeds were used to make cake for animal feed and reported cases of poisoning seem to derive from the use of cake which contained significant amounts of husk. The cause of this intoxication is not known but may have been due to the presence of saponins. Horses are said to be particularly sensitive, although most reported cases seem to have involved cattle (Cooper and Johnson, 1984 and references therein).

9.2.10. Weed seeds and unground and uncrushed fruits

This catch-all category included in the annex to the present Directory provides some flexibility to the listing of undesirable material of botanical origin but is open to a number of interpretations. Taken

literally most, if not all, common feed ingredients would be considered undesirable as the occurrence of alkaloids of various types and other toxic substances is commonplace and all vascular land plants contain "glucosides". Emphasis is also given to intact material as a pragmatic response to the practical needs of microscopy, the principal means of detection.

Weed seeds are universally present in harvested crops and consequently in feedingstuffs. Most laboratories responsible for quality control have lists of those weed species considered innocuous whose presence are rarely, if ever, reported and of other weed species which may present a hazard. The list used in one such laboratory (RIKILT, Wageningen, The Netherlands), drawn up in 2000 is shown in Table 7 and gives some indication of the range of contaminating weed seeds that may be encountered.

Table 7. List of seeds of weeds recognised by microscopic examination of feed ingredients and feedingstuffs. Seeds considered to pose a hazard when found as a contaminant

Aethusa cynapium L	*Fumaria officinalis.*	*Polygonum aviculare* L.
Agrostemma githago L.	*Galeopsis tetrahit* L.	*Polygonum convolvulus* L.
Cannabis sativa	*Hyoscyamus niger* L.	*Ranunculus arvensis*
Chrysantemum segetum L	*Lepidium sativum* L.	*Ricinus communis*
Conium maculatum L	*Some Lolium* spp.	*Rumex crispus*
Coronilla varia L.	*Melandrium album* (Mill.)	*Sebania* spp.
Colchicum autumnale L.	*Melampyrum arvense* L.	*Saponaria officinalis* L.
Crotalaria spectabilis	*Melilotus albus Med.*	*Sherardia arvensis* L.
Datura spp.	*Nigella arvensis* L	*Stellaria media* (L.) Vill.
Delphinium consolida L.	*Papaver rhoeas* L.	*Thlaspi arvense* L.
Diplotaxus tenuifolia L.DC	*Plantago lanceolata* L.	*Vaccaria pyramidata* Med.
Echium vulgare L.	*Plantago major* L.	*Vicia hirsuta* (L.)S.F. Gray
Eruca sativa Miller L.	*Plantago media* L.	*Xanthium* sp.

In contrast to the list in table 7, only three examples of toxic weeds are included in the annex, largely because their toxic properties are at least partialy understood and because there have been specific incidents of contamination detected. The three are two *Lolium* species (*Lolium temulentum* and *Lolium remotum*) and *Datura stramonium*.

- *Lolium temulentum* L. (Darnel)

Darnel poisoning has been recognised since the 19th century in livestock and also in humans as a result of the contamination of flours and meals. Extracts of seeds in acute and oral and intraperitoneal toxicity tests in mice and rats produced symptoms of a gradual progressive depression of the central nervous system ending in coma and death of lethally intoxicated animals by respiratory failure (Hammouda *et al*., 1988). These effects have been attributed to the presence of alkaloids.

Seeds of *Lolium temulentum* contain the pyrrolizidine alkaloids loline, 6-methyl loline and lolinine (Dannhardt and Steindl, 1985; Hammouda *et al*., 1988) but not, as might be supposed, temuline which is now coincided an artefact of isolation. The aerial vegetative parts of the also contain loline and an alkaloid with a dizaphenanthrene nucleus, perloline. The origin of these alkaloids was for sometime uncertain but it was recognised that lolines, insecticidal alkaloids, were invariably produced in symbioses of certain *Epichloe* (anamorph *Neotyphodium*) species (fungal endophytes) with grasses, particularly of the genera *Lolium* and *Festuca*. It has now been shown that *Neotyphodium uncinatum*, the common endophyte of meadow fescue will produce loline, N-acetylnorloline, and N-formylloline when grown in the defined minimal media at in the absence of plant material (Blankenship *et al*., 2001). It can only be assumed that fungal endophytes are the source of the pyrrolizidine alkaloids in *Lolium temulentum* and are not produced by the plant in response to infection.

Various studies of the toxicity of the individual alkaloids has shown them to be only mildly toxic and individually not to reproduce the effects on the CNS seen with total extracts (Dannhardt and Steindl, 1985; Hammouda *et al*., 1988; Abdel-Fatah *et al*., 1991). Consequently the basis and extent of infected *L. temulentum* toxicity is unclear.

- *Lolium remotum* Schrenk

The inclusion of *Lolium remotum* in the annex appears to follow from the annual ryegrass toxicity, a predominately neurological disorder of livestock, that occurs in South and Western Australia. This is seen only after ingestion of galled seed heads of *L. remotum* infected by a nematode (*Anguina agrostis*) and a species of bacteria initially described as a strain of *Corynebacterium* but now recognised as *Clavibacter toxicus*. A total of eight toxic glycolipids have been isolated from infected seed heads collectively referred to as corynetoxins (Vogel *et al*., 1981). These glycolipids given in isolation produced the clinical symptoms and brain lesions in lambs consistent with annual ryegrass toxicity. Doses below those producing clinical symptoms can also affect production in sheep, particularly wool growth (Davis *et al*., 1996). The relevance of the Australian experience to Europe is questionable. *Lolium remotum* in the absence of galled seed heads would not be considered a toxic material.

- *Datura stramonium* (Jimsonweed, Loco weed, Angel's trumpet, Devil's trumpet)

The common traditional name for this American species is said to result from the death of British soldiers who consumed the plant in the colony of Jamestown, USA in the late 17th century. No doubt they were tempted to experiment because of its hallucinogenic

properties long used for ceremonial and spiritual purposes by the indigenous population. Most reported cases of poisoning are in humans as a result of abuse (Birmes *et al*., 2002).

The primary psychoactive substances are the tropane alkaloids atropine, hyoscyamine and scopolamine. Maximum contents are found in the stems and leaves of young plants, with hyoscyamine as the predominant component (Miraldi *et al*., 2001). Lesser amounts are found in seeds. The tropane belladonna alkaloids act as competitive antagonists to acetylcholine at peripheral and central muscarinic receptors at a common binding site. The peripheral receptors are on exocrine glands that affect sweating, salivation, and smooth and cardiac muscle. Poisoning results in widespread paralysis of parasympathetic innervated organs. As tertiary amines they also have central nervous system absorption, inhibit CNS receptors and result in a central anticholinergic syndrome of acute psychosis or delirium. These toxins are easily absorbed from mucous membranes and the gastro-intestinal tract. The half-life of atropine is approximately four hours. Metabolism occurs in the liver by hydrolysis, which eliminates approximately half the drug. The remainder is excreted unchanged in the urine.

D. stramonium is poisonous to animals although because of its unpleasant taste livestock rarely eat enough to be fatally poisoned. In cattle early signs of intoxication include excitability, tremors and bloating. In smaller ruminants animals like goat and sheep, stymptoms may also include drowsiness and the inability to stand. However, most cases of livestock poisoning come not from grazing the plant but occur in animals who have consumed hay or other harvested feed materials containing the weed (El Dirdiri *et al*., 1981; Nelson *et al*., 1982; Schulman and Bolton, 1998).

D. stramonium occurs naturally in North America and Central Europe and is occasionally detected as a contaminant of soybean originating from the USA. As the growth of soybean for export grew in South America, samples of soybean meal contaminated with the *Datura* species common to the region (*Datura ferox L.)* have been reported. Like *D. stramonium, D. ferox* also produces tropane alkaloids (Padula *et al*., 1976).

9.3. Relationship of botanical contaminants with the natural plant products listed in the annex

Many of the categories of toxic agents produced by botanical contaminants of feedingstuffs are also produced by the feedingstuffs themselves. For example, cyanogenic glycosides are found in a several common feed ingredients including sorghum (dhurrin), cassava (linamarin) and a number of forage legume species. Permitted maximum concentrations for some toxicants (including cyanide) are included in the annex to the Directive to limit the level of inclusion in complete feeds of any feed ingredient containing or capable of producing the toxicant. The same maximum values could, in principle,

incorporate and control toxicants introduced by contamination. However, if the maximum concentrations of specific toxicants permitted in feedingstuffs continue to be prescribed then:

- limits should be set following a full risk assessment
- analytical methods should be appropriate and relevant
- measures used should reflect the maximum amount of toxicant capable of being produced.

Some toxicants, such as cyanide and isothiocyanates, are produced from essentially non-toxic parent compounds by enzyme action only when the biological material is disrupted and in the presence of moisture. Consequently, concentrations of the toxic compound(s) can alter with time. Methods should be used that ensure full conversion to the toxic form before analysis to reflect the maximum possible concentration.

Many toxicants are alkaloids. However, alkaloids represent the most structurally-diverse groups of plant secondary metabolites, being defined simply as nitrogen-containing compounds not otherwise classified (*e.g* as peptides, purines etc.). Consequently no single analytical method can exists for their measure and no prescribed limits have been set in the legislation. The more toxic alkaloids occurring in potential contaminants are limited to a few specific groups such as the tropane or pyrrolizidine alkaloids for which established analytical schemes exist and for which statutory limits could be developed.

9.4. Natural plant products

9.4.1. Hydrocyanic acid

Linseed contains the glycoside linamarin which delivers hydrocyanic acid by the effect of an enzyme (linamarase). Lipid extraction does not destroy the heat labile enzyme. Fresh linseed meal may contain 0.25 – 0.6 g hydrocyanic acid/kg, which decreases during storage. Under normal feeding practice hydrocyanic acid is released slowly and will not become toxic. If the feed is consumed rapidly the probability of an intoxication increases.

Linseed is mostly used because of its dietetic (mucilaginous) effects.

9.4.2. Free gossypol

Gossypol, a yellow pigment, is a polyphenolic compound that occurs naturally in the seed, foliage and roots of most cotton plants, and is relatively heat stable. Gossypol is a natural defence compound produced by plants against insect pests and diseases. Gossypol can be found in “free” or bound (attached to a protein) forms with the bound form being less toxic than the “free” form. In the seeds, almost all the gossypol is found in the free form. Heat and moisture processing converts the free form into the less toxic, bound form. Free gossypol

is responsible for the toxicity of cotton products to non-ruminants and young ruminants. Adult ruminants are more tolerant of gossypol.

Cotton seeds may contain from 0.3-20 g/kg DM of gossypol, and concentrations of 4-17 g/kg DM have been quoted for the kernels. Screw pressed materials have 200-500 mg free gossypol/kg, pre-pressed solvent extracted meals 200-700 and solvent extracted 1000-5000 mg/kg.

Gossypol acts as an antioxidant and polymerisation inhibitor and is toxic to monogastric animals: pigs and rabbits are the most sensitive, whereas poultry are more tolerant. The general symptoms of gossypol toxicity are constipation, depressed appetite and loss of weight; death usually results from circulatory failure. Although acute toxicity is low, ingestion of small amounts over a prolonged period can be lethal. It is important to distinguish between free (soluble in 70-30 v/v aqueous acetone) and bound gossypol since only the former is considered to be physiologically active.

It is generally considered that pig and poultry diets should not contain more than 100 mg free gossypol/kg and that inclusions of cottonseed meal should be between 50-100 kg/t of feed. Particular care is required with laying hens since comparatively low levels of the meal may cause an olive green discoloration of the yolk in storage. An associated pink discoloration of the albumen is now considered to be due to cyclopropenoids and not gossypol as was once thought. Treatment with ferrous sulphate can ameliorate the biological effects of gossypol; the amount of iron to be added is largely empirical. To reduce the effects of gossypol on growth, the following proportions of iron to free gossypol have been used: for cattle 1:1, for broilers 2:1, for layers 4:l and for pigs 1:1.

Cottonseed meal can be used safely in pig rations. Rations containing up to 0.01% free gossypol (about 20 % in the diet for a good screw-press or prepress solvent meal, but only 5 % for direct solvent-extracted meal) can be used without the addition of iron salts. Over these levels, iron must be added in a 1:1 weight ratio to free gossypol.

But Chinese research demonstrated with diets containing gossypol 76 and 100 mg/kg and supplemented with ferrous iron at 5 times free gossypol level, the performance of pigs was not improved Gao *et al*. (1989).

Cottonseed meal can also be used in rations for growing chickens if the free gossypol does not exceed 0.03%. Below this level, iron (2:1 iron to free gossypol) will completely overcome the depressing effect. Prepress solvent-extracted meal may be used in layer feed if the gossypol has been inactivated with iron (4:1 iron to free gossypol), if the free gossypol content is not above 0.4%. Ferrous sulphate may routinely be added to poultry diets containing cottonseed meal. For instance, for layers 0.05% iron (corresponding to 0.25% ferrous

sulphate septahydrate) can routinely be included in diets containing up to 10% cottonseed meal (and up to 0.16% iron would compensate adverse effects in rations containing greater amounts).

Experimental results showed that laying hens could tolerate free gossypol at up to 440 mg/kg diet without any significant adverse effect on egg production, egg weight, feed intake and feed conversion efficiency. Supplementation of diets containing free gossypol with FeSO4 did not affect egg production, egg weight and feed conversion efficiency. Free gossypol at 140 mg/kg feed had no effect on colour of egg yolk or egg white in fresh eggs but caused discoloration in eggs stored for 1 month or longer. FeSO4 at 120 or 240 mg/kg feed increased the tolerance to free gossypol to 280 mg/kg feed, and slightly decreased the occurrence of discoloration in stored eggs (Pourreza and Keshavarz, 1982a). The same authors (Pourreza and Keshavarz, 1982b) concluded from broiler trials that growing chicken can tolerate free gossypol at up to 590 mg/kg without any significant adverse effect on bodyweight gain, feed intake or feed conversion efficiency.

However, gossypol in cottonseed also limits its use in ruminant feeding. Ruminant animals have the ability to detoxify gossypol to some extent during the fermentation process. Signs of acute gossypol poisoning are difficult breathing, violent laboured respiration, weakness and death. These signs may appear suddenly after a stressful event and may resemble acute shipping fever. Adult cattle may have decreased milk production, go off feed, have difficulty breathing, weakness and diarrhoea (Kirk and Higginbotham, 1999).

Clear guidelines regarding maximum tolerable levels of gossypol for cattle are not available. Maximum levels for feeding whole cottonseed generally should not exceed 15 – 20 % of the total diet (Davis). Gossypol toxicity problems have not been reported in any of the studies with cattle carefully fed the recommended levels (Poore and Rogers, 1995). Although recommendations have been made for up to 10 percent whole cottonseed in the diet for developing young bulls, no recommendation is made by Davis because research indicates potential reduced fertility in young developing bulls. Arana *et al.* (1999) are of the opinion that the effects of gossypol intake on milk production and animal health have not been well established in California or elsewhere and deserve further investigation.

Kirk and Higginbotham (1999) suggest the maximum level of feeding for cattle less than one year of age is 0.05% to 0.1% of free gossypol. Adult cattle should have less than 0.1% to 0.2% of free gossypol in the total ration. This amounts to about 2 to 4 kg cottonseed per day

In Europe, the use of cottonseed meal is not very common (estimated to be 10,000-12,000 t/year) and is generally only recommended for ruminant feeding.

9.4.3. Theobromine

Theobromine (3,7-Dihydro-3,7-dimethyl-1*H*-purine-2,6-dione) belongs to a class of alkaloid molecules known as methylxanthines. Methylxanthines naturally occur in as many as sixty different plant species and include caffeine (the primary methlyxanthine in coffee) and theophylline (the primary methylxanthine in tea). Theobromine is the primary methylxanthine found in products of the cocoa tree (*Theobroma cacao)*, beans and shells.

Cocoa beans naturally contain approximately 10.6 – 42.3 g theobromine/kg. Different types of chocolate contain different amounts of theobromine (dark chocolates approximately 10 g/kg; milk chocolates 1-5 g/kg).

Theobromine affects humans similarly to caffeine, but on a much smaller scale. The heart, central nervous system, and kidneys are affected. Theobromine is mildly diuretic (increases urine production), is a mild stimulant, and relaxes the smooth muscles of the bronchi in the lungs. Theobromine has been used as a drug for its diuretic effect, particularly in cases where cardiac failure has resulted in an accumulation of body fluid.

Cocoa shells, beans and oilcake all have high nutritive values and could serve as feedstuffs for livestock except for their theobromine content.

Cocoa and chocolate products may be toxic or lethal to dogs (nausea and vomiting, restlessness, diarrhea, muscle tremors, and increased urination or incontinence, cardiac arrhythmias) and other domestic animals such as horses, pigs and poultry. This is because these animals metabolize theobromine more slowly than humans **(**half life for theobromine in dogs: 17.5 h, in humans: 2-3 h). The toxic dose of theobromine for dogs is 100 - 150 mg per kilogram of body weight.

Ruminants are obviously less susceptible, 60 % dried cocoa husk in a diet for sheep did not lead to any signs of theobromine poisoning (Otchere *et al.*, 1983).

9.4.4. Glucosinolates

Volatile mustard oils belong to the glucosinolates which are a class of ca. 100 secondary plant compounds (Kjaer and Skrydstrup, 1987) generally found in *Brassicaceae*. These compounds have a similar structure characterised by a common part containing a β-thioglucose coupled with a sulfonated oxime group and by a variable organic side chain constituted by alkyl, alcenyl, hydroxy-alcenyl, aryl, indolyl, sulfinyl, sulfonyl or thio residues. They are present in glycosides and released after hydrolysis (by enzymes in the presence of moisture). Present in high or low level (00 varieties) in rapeseed and in rapeseed meals, these molecules are easily broken down during animal digestion, leading to compounds with detrimental and antinutritional

characteristics (Astwood *et al.*, 1949). In this way, the 2-hydroxybut-3-enylglucosinolate or progoitrin forms the vinyl-thiooxazolidone (5-vinyl-1,3-oxazolidine-2-thione, 5-VOT) which is known to interfere with the thyroid and to damage vital organs (Fenwick *et al.*, 1983).

Another two experiments were conducted by Paquay *et al.* (1999) in order to optimize the incorporation of rapeseed meal (RPM) in diets for growing and fattening of bulls. In a first experiment, the effects of 20% of a low glucosinolate (LG)-RPM (3.14 mmoles/kg DM of concentrate in which 51% of progoitrin) were studied. In a second experiment, various proportions (0, 10, 20, 34 %) of an industrial LG-RPM were tested in order to determine the optimal level in diet of young bulls. Thirty-six young Belgian White Blue bulls were used. In the two experiments, large amount of LG-RPM (20 - 34%) did not reduce animal performance whatever the parameter considered (liveweight, daily weight gains, food intake, feed conversion, carcass weight, dressing percentages). Values did not also vary with the level of LG-RPM in the diet. Any effects were observed on the thyroid weight and the size of thyroid follicles. The secretion of thyroid hormones was not affected by RPM except for a decrease ($P < 0.05$) in the production of thyroxin by thyroid tissue of bulls that received 34% of RPM in Experiment 2. Results concerning plasma testosterone and cortisol were inconsistent between the two experiments with negative effects or no influence of LG-RPM. It was concluded that levels between 20 - 34% of LG-RPM in diet may be used for fattening bulls but investigations are still needed to outline the long-term effects on steroids and reproductive performance.

Jensen (1999) studied the improvement of the nutritive value of rapeseed by selecting varieties with very low glucosinolate content in pigs and broilers. He included 25% rapeseed in diets for broilers and piglets showing total glucosinolate contents (sum of progoitrin, gluconapin, glucobrassicanapin, 4-hydroxyglucobrassicin and glucobrassicin) between 4 and 36 mmol/kg rapeseed meal. Feed consumption and body weight gain of broilers were significantly depressed by 36 mmol total glucosinolate as well as body weight gain and feed conversion of piglets. High total glucosinolate tended to increase relative liver weight of piglets, increased sigificantly relative thyroid weight of broilers and piglets and depressed T_3 production in broilers. The study shows that meal from rapeseed varieties with a glucosinolate content less than 10 mmol glucosinolate/kg seed is of a higher nutritive value for broilers and pigs than meal from rapeseed varieties with 15-20 mmol glucosinolate/kg seed.

The studies show that glucosinolates have adverse effects on the performance only at higher inclusion rates of rapeseed meal with higher concentration of glucosinolates. These effects are well known by feed producing companies and the farmers. For that reason rapeseed meal was used only at limited extent in former timers. Nowadays only rapeseed meal from the newer, so-called double zero

(00) varieties are used, which contains only small amounts of glucosinolates (<10 mmol/kg).

- Volatile mustard oil

  Allyl isothiocyanate (common: volatile mustard oil) is present among others in horseradish, rapeseed and some mustard species The volatile oil of these mustard species contains glycosides called sinigrine or sinalbine which are degraded by the enzyme myrosine to allylthiocyanate (and glucose and potassium bisulfate).

  The clinical symptoms are an acute gastroenteritis (colic, diarrhoea, foaming at the mouth), respiratory disturbances and probably photosensibility.

- Vinylthiooxazolidone

  Glucosinolates, a family of compounds including isothiocyanates (ITC) and vinylthiooxazolidone (VTO) are common in cruciferous, in plant and seeds, and consequently in rapeseed meal. VTO decreases the availability of iodine for thyroxine formation, growth depression and struma are the results. The 5-VOT is responsible for the reduction of the zootechnic performances (Maheshwari *et al.*, 1979). It is transferred to serum, milk, muscular tissues and to certain organs: liver, lung, kidney and mainly thyroid. The 2-hydroxybut-3-enylglucosinolate or progoitrin, is still found in moderate proportion in most of the "00" varieties of rapeseed and remains in meal after oil extraction. Therefore, despite the decrease of the glucosinolates quantity in rapeseed meal in Europe, their consumption can decrease yield and presents potential damages for the quality of animal growing. Mabon *et al.* (1999) studied the antinutritional factor 5-VOT in order to better understand its impact in the target organs and to correlate these results with the zootechnic performances. Lambs (80) allocated to eight groups received *ad libitum* concentrates containing 0 % (control), 5%, 10%, 15%, 20%, 25%, 30% and 40 % of rapeseed meal. The lambs were weaned at 50 ± 13 day of age and were slaughtered when their fattening state was estimated to be satisfying. The 32 bulls included in the study were allocated to four groups and received *ad libitum* concentrates containing 0 % (control), 10%, 20% and 34 % of rapeseed meal. The bulls were allotted at 300 ± 30 kg of body weight and were similarly slaughtered when their fattening state was estimated to be satisfactory. The concentrations of 5-VOT were measured in samples of liver, kidney, lung, muscle, thyroid, and plasma from the lambs and bulls with additional urine samples taken from the bulls.

  In lambs as well as in bulls 5-VOT was present exclusively with the ingestion of rapeseed meal. The amount of SCN^- ions and 5-VOT found in the target organs were proportional to the amount of

rapeseed meal introduced in the diets. The rapeseed meal induced a 5-VOT level significantly ($P < 0.05$) higher in the target organs such as lung and thyroid than the control. This difference is once again due to the glucosinolate concentrations between the diets. The results obtained with lambs show that the ingestion of high amount of rapeseed meal induces an accumulation of 5-VOT in lung and mainly in thyroid but in very low levels in muscle, liver, kidney and plasma. The 5-VOT concentration was significantly ($P < 0.05$) higher in thyroid than in lung. It is interesting to notice that 5-VOT has a very high affinity for thyroid. This behaviour can perhaps explain the morphological and the physiological changes in this organ. No difference was observed for the animal performances and carcass quality, apart from an hyperthyroidism. A VTO intake potentially equivalent to 15 mg of 5-vinyl oxazolidine-2 thione/ person/day is of concern as the latter compound has been suggested to be a major diet related cause of endemic goitre as a result of its presence in milk following the grazing of cattle and goats on cruciferous forages. But no evidence for any dietary (brassica) related thyroid problem has been observed in the UK population.

At regular inclusion rates of rapeseed meal in feed no adverse effects in animals are expected. The target organ of 5-VOT is the thyroid gland and to a much lesser extent the liver. The residues in edible tissues even in the liver are considered to be not of any risk for human health.

The consumption of cooked Brussels sprouts (30-75 mg of glucosinolate precursor of 5-vinylthiooxazolidone per day for 28 days leads to no effect on thyroid function in man (Heaney and Fenwick, 1985). But high proportion of 0 rapeseed meal (low in erucic acid) in the diet of the growing pig (15-20 %) lead to the enlargement of the thyroid gland and of the liver. But for newly introduced rapeseed 00 varieties (low in erucic acid and VTO), glucosinolates will not be a major problem.

In the view of SCAN vinyl-thiooxazolidone should no longer be included in the Directive as undesirable substances and volatile mustard oil retained only if used to control contamination.

9.5. Conclusion

Contamination of animal feedingstuffs with plant material is almost universal. Although most such contaminants are innocuous, the occasional detection of botanical material with the potential to damage animal health still occurs.

Insufficient data are available to consider a full risk assessment of botanical contaminants and given the low degree of risk associated with such contamination it is unlikely that such data will become available. However, assessments could be made for some of the compounds presumed responsible for their toxicity (HCN, allyl isothiocyanates).

Since the majority of the present botanical species listed in the annex to the Undesirable Substances Directive are of historical interest only or present no real hazard, the present list should be substantially revised (see 9.6).

Any revision of the annex should make allowance for the changing patterns of agriculture. For example, the increasing variety of plants used for the production of both fine pharmaceutical products and bulk chemicals (particularly industrial oils) may present problems of cross-contamination with food crops not currently encountered.

The ease of microscopic detection of botanical contaminants is inversely related to the degree of processing, particularly commination, of feedingstuffs. It would be advantageous if the physical detection of the presence of a potentially toxic contaminant could be supported or replaced by a quantitative chemical analysis of the specific compound(s) responsible for the toxicity and maximum limits set accordingly. The inclusion in the present annex to the Directive of isothiocyanates or hydrocyanic acid is not sufficient for this purpose and would need extension.

The organic compounds hydrocyanic acid, free gossypol, volatile mustard oil (allyl isothiocyanate), vinylthiooxazolidone and theobromine listed in annex to Directive 1999/29/EC are natural constituents (or derive from natural constituents) of plants used for feed purposes. Above certain concentrations, they affect the health of domestic animals while they are without effects on the human consumer of products derived thereof. The toxic potential of these plant constituents are recognised by feed manufacturers who take into consideration their adverse effects when formulating a ration. Least-cost programmes used to develop feed formulations contain restrictions that limit inclusion levels to ensure the safety of the target species.

9.6. Recommendations

In the view of SCAN, the annex to the Directive does not represent the present taxonomic status of potential botanical contaminants or the real risk represented by such impurities in modern animal feeding practice. Recognising that data necessary for a full evaluation of each plant species currently listed is not available or likely to be produced, SCAN recommends that the present directive be simplified and a single category adopted to address the risks associated with botanical impurities and to replace the existing entries (B_8-B_{10}; C_1-C_{12})

"*Plant material (seeds, vegetative plant parts) known to contain potentially harmful concentrations of alkaloids, other toxicants or compounds able to generate toxic derivates (e.g. cyanogenic glucosides, glucosinolates) singly or in combination*."

Because of its flexibility and capacity to deal with novel problems, microscopy should remain the primary method for the detection of botanical contamination. However, where possible, maximum limits for botanical contaminants of particular concern should be set on the basis of their known toxicants. Thus, for mustard seeds (to include the sum of *Brassica juncea*, *B. nigra* and *B. carinata*), their presence should be allowed provided that the limit set for allyl isothiocyanates from whatever source, is not exceeded.

It is recognised that limits cannot be set for all known toxicants in feed/feed contaminants. SCAN recommends that priority should be given to ricin (from *Ricinus communis*) or a marker of ricin. Until this limit is established, any detection of *Ricinus communis* should lead to rejection of the feed material.

Limits for other toxicants should be established as and when feed microscopy indicates a developing problem.

Finally gossypol, theobromine and vinyl thiooxazolidone which occur as natural constituents of feed ingredients but which are not relevant to the control of contamination should be excluded from the list of undesirable substances. Any risk posed by feed ingredients containing these compounds is contained by modern methods of feed formulation.

10. GENERAL RECOMMENDATION

The Scientific Committee on Animal Nutrition recommends to restucture the annex to the Directive on the undesirable substances in animal feedingstuffs into

A. Ions / elements

B. Mycotoxins

C. Other organic contaminants

D. Plants and natural plant products

The Committee recommends that the annex should be routinely reassessed at regular intervals, *e.g.* five years.

The annex should better reflect the actual emerging situation (by deleting certain undesired substances if their risk becomes negligible because of low exposure, and introducing new entries for substances with a harmful potential).

11. REFERENCES

11.1. Ions and elements

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