EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Scientific Opinions
C2 - Management of scientific committees; scientific co-operation and networks

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

(Adopted on 20 February 2003)

1. BACKGROUND

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

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

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

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

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

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

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

2. TERMS OF REFERENCE

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

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

- those substances, products or botanical impurities of which the listing as undesirable substance has become completely obsolete

- those substances, products or botanical impurities which can be on the basis of their toxicological profile considered as priority for evaluation

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

The evaluation should comprise for each undesirable substance the

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

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

(c) impact on animal health

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

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

TABLE OF CONTENT

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 risk assessment but a general review of the substances already listed and of those that could also be considered for listing, on the basis of the current scientific knowledge.

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

- ions and elements,

- mycotoxins,

- organic chemical contaminants, and

- botanical impurities.

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

IONS AND ELEMENTS

Mycotoxins

6.1. 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.

6.2. 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.

6.3. Mycotoxins listed in Council Directive 1999/29/EC[1]

6.3.1. *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

---

[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 1[rst] 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).

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

6.3.2. *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.

6.4. 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.

6.4.1. 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[2] (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).

---

[2] 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.

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 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.

6.4.2. *Fusarium mycotoxins*

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

6.4.2.1. 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.

6.4.2.2.   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..

6.4.2.3. 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.

6.4.2.4.   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).

6.5. Other feed associated mycotoxins

6.5.1. 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.

6.5.2. 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.

6.6. Conclusions

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

- Current EU legislation[3] 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

---

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

validated method for determination of zearalenone and its metabolites and that these would have to be developed.

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

- 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.

7. ORGANIC CONTAMINANTS

8. BOTANICAL IMPURITIES

9. REFERENCES

9.1. Heavy metals

9.2. Mycotoxins

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

NTP (1982) Carcinogenesis bioassay of zearalenone in F344/N rats and F6C3F$_1$ mice. National Toxicology Program Technical Reprot Series No. 235, Department of Health and Human Services, Research Triangle Park, NC

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Voss, K.A., ChamberlainW.J., Bacon, C.W. , Herbert, R.A., Walters, D.B. and Norred, W.P. 1995. Subchronic feeding study of the mycotoxin Fumonisin B$_1$ in B6C3F1 mice and Fisher 344 rats. Fund Appl Toxicol24:102-110.

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

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

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

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

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

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

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

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

9.3. Organic contaminants

9.4. Botanical impurities