Mycotoxins in food of animal origin: a review

Marco A. Jonker, Hans P. van Egmond and Rainer W. Stephany

CRL document 389002 095

December 20, 1999

CRL-European Union Community Reference Laboratory on Residues
RIVM-National Institute of Public Health & The Environment
P.O. Box 1, 3720 BA Bilthoven, The Netherlands

This study has been performed as a result from decisions 96/519/EG (CEG 1996) and 98/587/EC (CEC 1998 b) as a part of the CRL duties and laid down in EU Council Directive 96/23/EC (Council of the EU 1996 b).

Mailing list

European Commission DG Sanco

- Mr. A.M. Checchi Lang, Director of Directorate E: Public, animal & plant health
- Dr. E. Poudelet
- Mrs. S. Jülicher
- Mr. P.J. Wagstaffe

CRLs for residues and contaminants

- Dr. D. Behrendt, BgVV Berlin
- Dr. P. Maris, AFSSA (veterinary drugs) Paris
- Mrs. F. Janin, AFSSA (milk and milk products) Paris
- Dr. S. Caroli, ISS Rome
- Dr. A. Martinez, Marine Biotoxins, Madrid

NRLs

Who have been charged with investigations onto residues for substances of Council Directive 96/23/EC Annex I

- Group A4: resorcylic acid lactones including zeranol
- Group B3d: mycotoxins

Others

Chief Veterinary Officers for Public Health of EU memberstates

TABLE OF CONTENTS

Summary

Mycotoxins are an important category of natural toxins, occurring globally in food and feed. Various international organisations, including several Services of the European Commission pay attention to mycotoxins. As a consequence of *Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products*, EU memberstates must include investigations on residues of mycotoxins in primary animal products within their Annual National Plans. This CRL document, part of a continuing series of monographs, is primarily meant as a guidance for the laboratories charged with these investigations and also as a guidance for the EC Services involved in the evaluation of the results of the Annual National Plans of the 15 Member States and if appropriate the Annual National Plans of Non Memberstate countries exporting into the European Union.

As to current knowledge, the most significant mycotoxin, in terms of occurrence in animal products, is aflatoxin M_1. This toxin occurs in milk and milk products as a consequence of the consumption of feed contaminated with aflatoxin B_1 by dairy cattle. Aflatoxin M_1 has been classified as possibly carcinogenic to humans and since 1 January 1999 the EU regulates aflatoxin M_1 in milk at a maximum admissible level of 0.05 µg/kg. In the EU aflatoxin M_1 seems to be well under control. The analytical methodology is well-developed. Recently a method for aflatoxin M_1 has been validated in a joint EU/AOAC International collaborative study. The method employs extract cleanup by an immunoaffinity column, followed by HPLC separation and fluorescence detection. The method is capable for determining aflatoxin M_1 at the levels of interest in the EU. Several BCR milk powder reference materials, certified for their aflatoxin M_1 mass fraction, and a BCR reference aflatoxin M_1 calibrant solution are readily available. In the EU, laboratories can take part in proficiency tests for aflatoxin M_1 analysis, organised by the European Union CRL for Milk and Milk Products and by the UK-based FAPAS.
Residues of aflatoxins in meat and meat products are of minor importance, provided that the aflatoxin contents of animal feedstuffs fulfill the European regulations.

Residues of ochratoxin A in organs and meat contribute to approximately 5% of the total human exposure to ochratoxin A. Ochratoxin A is, like aflatoxin M_1, considered as a possible human carcinogen. Specific regulations for ochratoxin A in various products are currently proposed at Community level, however not for animal products. Various methods of analysis exist to determine ochratoxin A. Methods for cereals and coffee have recently been validated in joint EU/AOAC International collaborative studies, but formal validation studies for ochratoxin A in meat and meat products have not been carried out and they are not foreseen. BCR certified reference materials for ochratoxin A in wheat flour are available, but attempts to develop a reference material for ochratoxin A in pig kidney have failed.

Not much is known about the possible carry over of other mycotoxins from feed to animal products. The few studies carried out in this respect involve deoxynivalenol, cyclopiazonic acid, fumonisins, zearalenone and sterigmatocystin. The outcome of these studies do not show residue levels of significance to threat human health.

Monitoring of animal products for residues of mycotoxins should at least include aflatoxin M_1. Investigations for ochratoxin A are of a lower priority. Monitoring of animal products for other mycotoxins is currently not recommended.

1. Introduction

Council Directive 96/23/EC *of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC, and 86/469/EEC, and Decisions 89/187/EEC and 91/664/EEC* has come into force in the European Union in July 1997 (Council of the EU 1996 b). The scope of the Directive includes investigations by EU memberstate laboratories, within their Annual National Plans, on residues of mycotoxins in primary animal products. These concern bovine, ovine, caprine, porcine and equine animals, poultry, aquaculture animals and, milk and milk products. The aim of this report is to give a brief overview of those mycotoxins that belong to the scope of Council Directive 96/23/EC, and to provide information about their (potential) occurrence, toxicity and possibilities for their determination in food of animal origin. In addition, some information will be given on current and expected relevant regulations, both within the EU, as well as worldwide. This document is distributed to the National Reference Laboratories for residues in the European Union (CEC 1998 a), who have been charged with investigations onto residues of mycotoxins and resorcylic acid lactones (RALs) in food of animal origin.

Mycotoxins are metabolites of fungi which are able to produce harmful effects like acute toxic, carcinogenic, mutagenic, teratogenic, atherogenic and estrogenic effects on animals. Commonly, biological conversion products of mycotoxins are also referred to as mycotoxins. The term mycotoxin is derived from the Greek words "ΜΥΚΗΣ" (fungus) and "ΤΟΞΙΚΟΝ" (arrow-poison). Toxic syndromes resulting from the intake of mycotoxins by humans and animals are known as mycotoxicoses. Mycotoxicoses remained "neglected diseases" (Forgacs 1962) until the early 1960s, when "Turkey X disease" broke out in Great Britain. Within a few months more than 100,000 turkeys died (Asplin 1961). This catastrophy led to a multidisciplinary investigation, which found aflatoxins produced by certain *Aspergillus* species. Discovery of the aflatoxins greatly stimulated scientific interest in mycotoxins and mycotoxicoses. Since 1960, many thousands of publications and many books were published about the different aspects of this subject. Mycotoxin contamination of food and feed depends highly on environmental conditions that lead to mould growth and toxin production. Data about incidence and levels of contamination are limited by many factors, including the financial resources to conduct surveys, the availability of laboratory facilities to carry out analyses, the sampling procedures used, the reliability and limits of performance of the analytical methods used and the competences of the analysts. Probably no edible product can be regarded as absolutely free from mycotoxin contamination, considering that mycotoxin production can occur in the field, during harvest, shipment processing, and storage of a given commodity.

The growing awareness that mycotoxins may play an etiological role or contribute to the occurrence of human diseases, led several international organisations to include the topic "mycotoxins" in their areas of interest and working programmes. Gradually, national and international regulations were developed, limiting the presence of mycotoxin residues in human food and animal feed. Hereafter are listed international organisations and programmes that structurally pay attention now to mycotoxins. Some of the tasks performed by these organisations are given, without the intention to be exhaustive:

WORLDWIDE ORGANISATIONS*		
NAME [abbreviated]	IMPORTANT ACTIVITIES IN RELATION TO MYCOTOXINS	FULL NAME and SEAT of HEAD QUARTERS
AOACI	Validation of methods of analysis through collaborative studies within the Methods Committee on Natural Toxins	The Association of Official Analytical Chemists International, Gaithersburg, US
CCFAC	Development of food standards and codes of practice for mycotoxins to facilitate international trade in foods	The Codex Alimentarius Commission through their Codex Committee on Food Additives and Contaminants, The Hague, NL
FAO	International conferences, e.g. jointly with WHO and UNEP in Tunis, March 1999; mycotoxin training programmes for developing countries; overview documents on worldwide mycotoxin regulations	The Food and Agriculture Organisation Rome, IT
IAEA	Co-ordinated research programme on evaluation of methods of analysis for determining mycotoxin contamination of food and feed, in particular for developing countries	The International Atomic Energy Agency through their Joint FAO/IAEA Division of Nuclear Techniques of Food and Agriculture, Vienna, AT
IUPAC	Organisation of international conferences on mycotoxins and phycotoxins, e.g. in Guarujá, Brazil, May 2000; joint research projects within the Commission on the Chemistry of Food, Edible Oils and Fats, and Derivatives	The International Union of Pure and Applied Chemistry, Research Triangle Park, US
WHO	Environmental Health Criteria documents on various mycotoxins, risk assessment of mycotoxins through the Joint Expert Committee on Food Additives and Contaminants (JECFA) in conjunction with FAO	The World Health Organisation (WHO), Geneva, CH

*) see also "Glossary of Abbreviations" (chapter 8)

EUROPEAN ORGANISATIONS)	
NAME	IMPORTANT ACTIVITIES IN RELATION TO MYCOTOXINS
ILSI Europe (The European Branch of the International Life Sciences Institute)	Organisation of workshops and review of analytical and toxicological aspects of specific mycotoxins, within the Task Force on Natural Toxins
CEN (The European Committee for Standardisation)	Harmonisation and standardisation of mycotoxin methodology within Technical Committee 275/working group 5: Biotoxins
EC-Directorate General Industry (formerly DG III)	Estimation of levels of exposure of EU inhabitants to certain mycotoxins [Scientific Co-operation on Questions relating to Food (SCOOP)]
EC-Directorate General Competition (formerly DG IV)	Pilot Programme for the testing of the quality of some important foodstuffs of organic and conventional agriculture on the consumer market in eight member states
EC-Directorate General Agriculture (formerly DG VI)	(EU) National Reference Laboratories (NRL) and the (EU) Community Reference Laboratory (CRL) on Residues at Bilthoven (NL) with tasks on mycotoxins
	(EU) National Reference Laboratories (NRL) and the (EU) Community Reference Laboratory (CRL) on Milk and Milk Products at Paris (FR)
EC-Directorate General Science, Research and Development (formerly DG XII)	Development and validation of analytical methods, and development of (certified) reference materials for various mycotoxins [Standards, Measurements & Testing (SMT)] COST Action 835: Agriculturally important fungi; working group "chemical and biological characterisation of toxic metabolites".
EC-Directorate General Consumer Policy and Consumer Health Protection (formerly DG XXIV)	Risk assessment of various mycotoxins [Scientific Committee on Food (SCF)]

*) Head Quarters of the European Organisations have their seats at Brussels, BE

Within Europe, the EC and CEN have structural cooperation. CEN advises the EC about methods performance criteria and methods of analysis for various mycotoxins, to be used for official purposes. After validation has been carried out according to internationally harmonised procedures, such methods (see also 5.2), can be incorporated into European legislation. In practice the advice of CEN in the mid-1990s on (lack of) suitable mycotoxin methodology has led to several on-going mycotoxin analytical research projects, funded by the SMT Programme. These are

directed towards improvement and validation of mycotoxin methods through international collaborative studies. CEN-developed performance criteria for aflatoxin methods have been already partly incorporated in Community Legislation (EC 1998 b). It is expected that CEN-developed as well as CRL developed performance criteria for other mycotoxin methods (see table VIII) will also be adopted in EU regulations for mycotoxins.

Specific regulations for mycotoxins have been implemented over the years in at least 77 countries worldwide (FAO 1997). Most of the mycotoxin regulations, existing in 1996, concern aflatoxins in food and feed. In fact all countries with mycotoxin regulations have at least tolerances for aflatoxin B_1 and/or aflatoxins B_2, G_1 and G_2 in foods and/or animal feedstuffs. Specific regulations also exist for aflatoxin M_1 in milk and milk products (for details of EU aflatoxin regulations, see chapter 4). Less frequently, regulations exist for other mycotoxins (i.e. patulin, ochratoxin A, deoxynivalenol, diacetoxyscirpenol, zearalenone (F2-toxin), T-2 toxin, chetomin, stachybotrytoxin, phomopsin and fumonisins B_1 and B_2) in various foods and feedstuffs. A review of the currently existing mycotoxins regulations around the world shows that they mostly relate to vegetable products, with the exception of aflatoxin M_1. Specific legislation for aflatoxin M_1 in milk(products) is found in harmonised EU and MERCOSUR (Argentina, Uruguay, Brazil, and Paraguay) legislation and in 14 other countries. Seven European countries have also specific regulations for aflatoxin M_1 in foods (based on milk products) for infants and young children. One country has a regulation for "all" mycotoxins in ice cream. Specific legislation for mycotoxins in other animal products is very scarce. Following are the exceptions:

- Ochratoxin A in pig kidneys (Denmark).
- Aflatoxin B_1 and aflatoxin M_1 in animal fat (Russia).
- Aflatoxin B_1 in milk products (Russia).
- Zearalenone, one of the RALs, in meat (EU)

In conclusion, from the legislative point of view, aflatoxin M_1 in milk and milk products seems the most relevant "mycotoxin/food of animal origin" combination, requiring attention nowadays.

In the following sections of this CRL document summarized information will be given on the occurrence, toxic properties and EU regulations of the mycotoxins, that are most relevant to Council Directive 96/23/EC. The analytical possibilities, (EU) developments in mycotoxin methodology, and availability of (certified) reference materials will also be discussed. Finally we will make some recommendations for the NRLs about the activities to be undertaken within the Annual National Plan of their memberstate for the coming years.

2 Occurrence in animal products

2.1 In general

Mycotoxins are produced by moulds and occur naturally on a wide range of commodities. Especially cereals are known food sources that can be contaminated with mycotoxins. The main routes of exposure to humans can be distinguished in primary contamination of food commodities, named-fermented foods and secondary routes of exposure. (Fink-Gremmels 1996).

- The primary contamination of food commodities is prominently based on that of food crops. It is a historical and still a worldwide problem for public health of mankind (FAO 1997).
- Named-fermented foods like fermented grains and starchy roots in Africa, Asia and Latin America are produced by using moulds. These traditional fermentation processes are based mostly on availability of raw materials in the region and climatic conditions. The related moulds are often known producers of mycotoxins. In Europe, moulds are used in the production of certain cheeses and meat products. The moulds used in cheese production can produce mycotoxins like cyclopiazonic acid, roquefortines, mycophenolic acid, PR (*Penicillium roqueforti*) toxin and penicillinic acid. Meat products can be distinguished in traditionally mould-ripened meats and sausages with spontaneously obtained moulds. The mycotoxins brevianamid A, citreoviridin, ochratoxin A, rugulosin, fumitremorgen B, verruculogen, cyclopiazonic acid and griseofulvin B were found in salami-type sausages. In long-ripened country cured hams aflatoxins were detected.
- The secondary route of exposure of consumers is through the presence of residues of mycotoxins and their metabolites in primary animal products (edible tissues of slaughter animals, milk and eggs). Mycotoxins associated with this secondary exposure are within the scope of this CRL document. Among the foodstuffs of animal origin that may contain residues of mycotoxins, is the important group of the dairy products (Egmond 1989). The presence of mycotoxins in dairy products may be the result of the contamination of the feedstuffs consumed by dairy cattle. The major concern here is aflatoxin M_1. This toxin is secreted in the milk, after cows have ingested aflatoxin B_1 with their feed. Aflatoxin M_1 ends up in various dairy products and is stable (Yousef 1989).The carry over rate ranges from 1-6 %. The higher rates occur at lower concentrations of aflatoxin B_1 in the feed and for cows with a high milk yield. Apart from the dairy products, other foods of animal origin may contribute to human exposure to mycotoxins, e.g. by their direct consumption (meat, organs, eggs) or their indirect consumption through incorporation in meat products (blood). An example is the carry over of ochratoxin A from porcine feedstuff into organs and blood. Pig blood and plasma are used in the preparation of various sausages, thus meat products can become contaminated with ochratoxin A.

In the paragraphs hereafter specific attention will be given to residues of both aflatoxins and ochratoxin A. In a separate paragraph some (limited) information on carry over and residues of other mycotoxins onto animal products is given.

2.2 Aflatoxins

2.2.1 Aflatoxins in edible tissues of animal origin

Limited published information from recent years is available on the occurrence of aflatoxins in edible tissues of animal origin. In table I a summary is given of some relevant studies on aflatoxin B_1 (AFB_1) and aflatoxin M_1 (AFM_1).

Table I. Occurrence of aflatoxins in edible tissues of animal origin

Compound analysed	Product					References
	Species	Matrix	Samples analysed	Positive samples %	Range of levels (µg/kg)	
AFB_1	hare, pheasant, deer	liver	n.g.*	n.g.*	0.2 - 1.2	Bukovjan 1992
	hare, pheasant, deer	kidney	n.g.*	n.g.*	0.3 - 3.2	
AFB_1	calf	liver	4	100	2.8 - 5.6	Stoloff 1979
	pig	liver	4	25	<0.1 - 0.08	
AFM_1	calf	liver	4	100	1.9 - 4.9	
	pig	liver	4	0	<0.1	

*n.g.: not given

Some studies were conducted on the carry over of aflatoxins from animal feedstuffs into edible tissues.
One experimental study was made by feeding pigs, rats, rabbits and ducks with 25, 160 and 240 µg/kg body weight of aflatoxin B_1 for periods of 2-36 weeks. Residues of 20-80 µg/kg of aflatoxins B_1 and M_1 were found in the skeletal muscle, liver and kidney of the slaughtered animals (Maryamma 1989).
Others investigated the negative effects of a combination of aflatoxins B_1 and G_1 in contaminated feed given to pigs (having a slaughter weight of about 150 kg) on meat quality and the residues in the animal tissues. Three different groups of pigs got rations of 500, 600 and 800 µg/kg of the combination of these aflatoxins. Only residues of aflatoxin B_1 could be detected in the liver (1.10 - 1.80 µg/kg), in the kidney (0.25 - 0.65 µg/kg) and in muscle (0.15 – 0.25 µg/kg) (Bononi 1995).
Another study was made with steers (of about 250 kg living weight) to which feedstuffs contaminated with 0, 60, 300 and 600 µg/kg aflatoxin B_1 were given for 155 days. Thereafter, aflatoxin B_1 was withdrawn 2 weeks before slaughter. After 1 week liver, muscle and fat samples were collected and assayed for aflatoxin B1 and M_1. Even though there were subtle liver lesions in the steers with the 600 µg/kg ration, residues of both aflatoxins were not detected in these samples (Helferich 1986).
The studies described above were performed with extremely high values of aflatoxins in feedstuff. If the EU keeps the imports of feedstuffs and raw materials well under control, i.e. in agreement with EU tolerated limits for aflatoxin B_1 in feedstuffs and

raw materials (see chapter 4), there are no problems to be expected with residues of aflatoxins in edible tissues.

2.2.2 Aflatoxins in eggs

Chicken eggs of 35 establishments in the southern part of the United States were investigated on the occurrence of aflatoxin B_1 (Stoloff 1978). The highest aflatoxin contamination in the corn or cottonseed meal crop used as feedstuff was expected in this area of the USA. Only one of the 112 samples investigated was positive for aflatoxins and appeared to have a level of 0.06 μg/kg in the liquid egg white. This value is very low compared to current regulations for aflatoxin B_1 in food. This means that there will be no problem of aflatoxin B_1 contamination in eggs.

2.2.3 Aflatoxin M_1 in dairy products

The establisment and refinement of regulations in the late 1970s, the 1980s and the 1990s to control the aflatoxin contents of dairy rations led to the expectation that aflatoxin M_1 levels in milk would reduce. At the same time, newer developments would make detection possible at lower concentrations of aflatoxin M_1. Over the last decades a significant decrease in aflatoxin M_1 levels in milk and milk products is visible. In the 1990s aflatoxin M_1 in milk seems to be well under control in the EU, thanks to stringent regulations (see chapter 4) and the joint efforts of both the feedstuff and dairy industries. In table II an overview is given of some recent published European data about aflatoxin M_1 (AFM_1) in milk and milk products, that confirms the above observation.

Table II. Occurrence of Aflatoxins in milk and dairy products.

Compound analysed	Product				References
	matrix	samples analyzed	distribution of samples	range of levels (μg/kg)	
AFM_1	cheese	88 [1)]	73 15	< 0.01 0.01 – 0.06	Dragacci 1996 a
AFM_1	cheese [Grana Padano cheese]	223 [2)]	4 203 15 1	< 0.005 0.005 – 0.10 0.10 – 0.25 > 0.25	Pietri 1997
AFM_1	milk	284 [3)]	284	< 0.01	Anonymus 1997
AFM_1	milk products [infant foods]	34 [4)]	15 19	< 0.02 0.02 – 0.06	Sizoo 1997

[1)] French investigations from 1992 until medio 1995
[2)] Italian investigations from 1991 until 1995
[3)] German investigations in 1996
[4)] Dutch investigations in 1994

Taking account of the EU limit of 0.05 μg/kg milk (and proportionally higher limits for milk products, depending on the percentage of dry matter), there is currently no reason for concern. Nevertheless we recommend to continue monitoring milk and milk products for aflatoxin M_1.

Annual Associate Referee reports on aflatoxin M_1 of AOAC International confirm also that in more recent years (from 1996 to 1999) aflatoxin M_1 is "well under control" in the EU (Egmond 1996-1999).

2.3 Ochratoxins

2.3.1 Ochratoxins in edible tissues of animal origin

Several studies have been undertaken on residues of ochratoxin A in animal products. Ochratoxin A has been detected in a variety of organs and meat products. In table III an impression is given about what has been found in some published studies.

Table III Occurrence of ochratoxin A in edible tissues of animal origin

Species	Matrix	Product Samples analysed	Positive samples %	Range of levels (µg/kg)	References
pig	blood sausages	125	16	0.1 - 3.4	Gareis 1996
pig	liver-type sausages	100	19	0.1 - 1.7	
pig	Bologna-type sausages	100	19	0.1 - 3.2	
pig	kidney	148	34	2 - 104	Scott 1978
poultry	muscular tissue	14	36	4 - 29	
pig	kidney	401	31	2 - 104	Krogh 1992
pig	kidney [1)]	10403	39	0.1 - 196	Kuiper-Goodman 1989
pig	sausage [2)]	337	18	0.1 - 3.5	
pig	liver [3)]	76	5	< 21	
poultry	meat	5	0	none detected	Top 1991
calf	sausage	1	0	none detected	
pig	chopped pork	1	0	none detected	
not given	meat products	20	0	none detected	

[1)] This range of levels of ochratoxin A in pig kidneys is obtained from the results of investigations in European countries [AT, BE, CZ, DK, FI, DE, HU, NO, PL,CH, GB, YU).
[2)] Results of investigation in DE and CH
[3)] Results of investigation in YU

The values given are indicative only, as not much is known about sample representativity and the reliability of the analytical methodology used. In a recent position paper on ochratoxin A for the meeting of March 1999 of the Codex Alimentarius Commission on Food Additives and Contaminants (Codex Alimentarius Commission 1998) it is estimated that pork and poultry contribute to approximately 5

% of the human exposure to ochratoxin A. If this estimation is correct, it appears that animal products are not a major contributor to dietary exposure to ochratoxin A. This is something that monitoring agencies have to keep in mind, when setting up monitoring activities, targeted to assess the occurrence of ochratoxin A in food.

2.4 Other mycotoxins

Besides the above mentioned aflatoxins and ochratoxin A there are few literature sources that are dealing with the carry over of other mycotoxins from feedstuffs to food of animal origin. They are briefly summarized hereafter.

2.4.1 Deoxynivalenol in milk

Holstein cows were fed a diet with 0.59, 42 and 104 mg daily intake of deoxynivalenol during a 10-wk lactation study. No transfer of deoxynivalenol or its metabolite, de-epoxydeoxynivalenol, to milk was observed. Concentrations were below detectable limits (1 µg/ml) using HPLC-Mass Spectrometry (Charmley 1993).

2.4.2 Cyclopiazonic acid in poultry meat

A method for the determination of cyclopiazonic acid in poultry meat was developed (Norred 1987). In an experiment, chickens were orally dosed with 10 mg cyclopiazonic acid /kg body weight. It appeared that 14.5 % of the dose was in muscle 48 hours after administration.

2.4.3 Fumonisins

2.4.3.1 Fumonisins in milk

^{14}C-fumonisin B_1 was ingested by dairy cows (Prelusky 1994) and the pharmacokinetics of residues examined between the time of administration and the time the animal(products) reach the human food supply. Diary cows (445-630 kg) were dosed orally (1.0 or 5.0 mg fumonisin B_1 /kg body weight) and intravenously (0.05 or 0.20 mg fumonisin B_1/kg body weight) with fumonisin B_1. Detection of fumonisin B_1 residues in milk was negligible down to 5-6 ng/ml.
Fungal culture material with fumonisins B_1, B_2 and B_3 was mixed into the total diet and fed to two lactating cows (Richard 1996). The two cows obtained an average of 3 mg fumonisin B_1/ kg body weight per day during 14 days. In this period milk samples were collected and investigated for fumonisins. Two laboratories applied analytical techniques with a limit of detection of 5 ng/ml. Fumonisins were not detected. The investigators concluded that the carry over from feed to milk in dairy cows is not significant and not a hazard for human health.
In another study, three lactating cows were subjected to a carry over study (Hammer 1996). An intravenous dosis of 30 mg fumonisin B_1 was applied to the cows. The milk was investigated on the occurrence of fumonisin B_1 by ELISA with a lowest detection level of 200 ng fumonisin B_1 /kg of milk.The carry over rate reached a maximum of 0.11% of fumonisin B_1. The conclusion was that the carry over of fumonisin B_1 is negligible from the point of view of consumer protection.
The overall conclusion of these experiments is that the carry over of fumonisin B_1 from diary cows into the milk is not expected to cause a consumer health problem.

2.4.3.2 Fumonisins in meat and eggs

^{14}C-fumonisin B_1 was ingested by laying hens and swine (Prelusky 1994) and the pharmacokinetics of residues examined between the time of administration and the time the animal (products) reach the human food supply.
Laying hens of 30 weeks old (1.30-1.68 kg) received an intravenous (2.0 mg 0.64 μCi/kg body weight) or an oral dose (2.0 mg 0.64 μCi/kg body weight) of ^{14}C-fumonisin B_1. Measurements showed that almost all of the radioactivity was recovered in the excreta of the hens and that its level in the different organs and tissues was negligible (detection limit of 10-15 ng fumonisin B_1 metabolites per g tissue), except for some localization in the liver and kidney. Moreover, eggs were collected and separated into yolk, albumin and shell. The residue levels in these separate parts of eggs were negligible. Even feeding laying hens 4 mg/kg body weight daily during 28 days didn't lead to adverse effects.
Barrows of 10-14 weeks old (15-20 kg) obtained an intravenous (0.50 mg 0.35 μCi/kg body weight) or an intragastric (0.40 mg 0.25 μCi/kg body weight) dose of ^{14}C-fumonisin B_1. The oral bioavailability of fumonisin B_1 in swine is very poor, although the small remaining fraction is extensively distributed and persisted for a long time in special tissues like the liver. The pharmacokinetic data of a subsequent study with 2-3 mg/kg fumonisin B_1 in diets of swine during a longer period of time resulted in an accumulation of residue levels in especially the liver and kidney. Application of clean feed during 9 days cared for a quick decrease of tissue residue concentrations to near negligible levels (< 10 μg/kg).

2.4.4 Zearalenone

2.4.4.1 Zearalenone in milk

Lactating cows were fed with zearalenone (Prelusky 1990). A dose of 544.5 mg zearalenone was administered daily during 21 days to a single cow. Maximum concentrations of 2.5 ng/ml of zearalenone and 3.0 ng of its metabolite α-zearalenol were found in the milk. Administered doses of 1.8 g and 6.0 g zearalenone during a one day feeding period delivered maximum milk levels of 4.0 and 6.1 ng /ml of zearalenone, 1.5 and 4.0 ng/ml α-zearalenol, and 4.1 and 6.6 ng/ml β-zearalenol respectively. It is concluded that in spite of feeding lactating cows with very high oral doses of zearalenone the milk would not normally pose a human health hazard.

2.4.4.2 Zearalenone and zeranol in meat.

Zearalenone is rapidly metabolised in animals and eliminated mainly as water soluble glucuronides. Studies about transmission of zearalenone residues to edible tissues are performed with unnatural amounts in the feedstuffs. Distribution and residue determination of zearalenone was studied in broilers (Mirocha 1982). The broilers were fed a diet of 100 mg/kg of zearalenone during 8 days. Then, [3H]zearalenone was intubated into the crops of the broiler chickens and its distribution monitored from 0 to 48 hours. In muscle, the retention of zearalenone was 23 to 25 μg/kg at 0.5 hour to 4 μg/kg at 48 hours. The muscle contained no zearalenol. In liver, zearalenone and α- and β-zearalenol were found with higher concentrations. It was concluded, that the residues of zearalenone in meat do not appear to be a problem.

However, a difficult problem was discovered some years ago in ruminants by the conversion of zearalenone to zeranol (fig. 1). Zeranol is known as an anabolic growth promotor and in the European Union it is banned since 1988 for the health protection of consumers. Since 1996 the wide group of Resorcylic Acid Lactones (RALs) belongs to the banned substances (Council Directives 96/22/EEC and 96/23/EEC 1996: references Council of the EU 1996 a and 1996 b respectively).

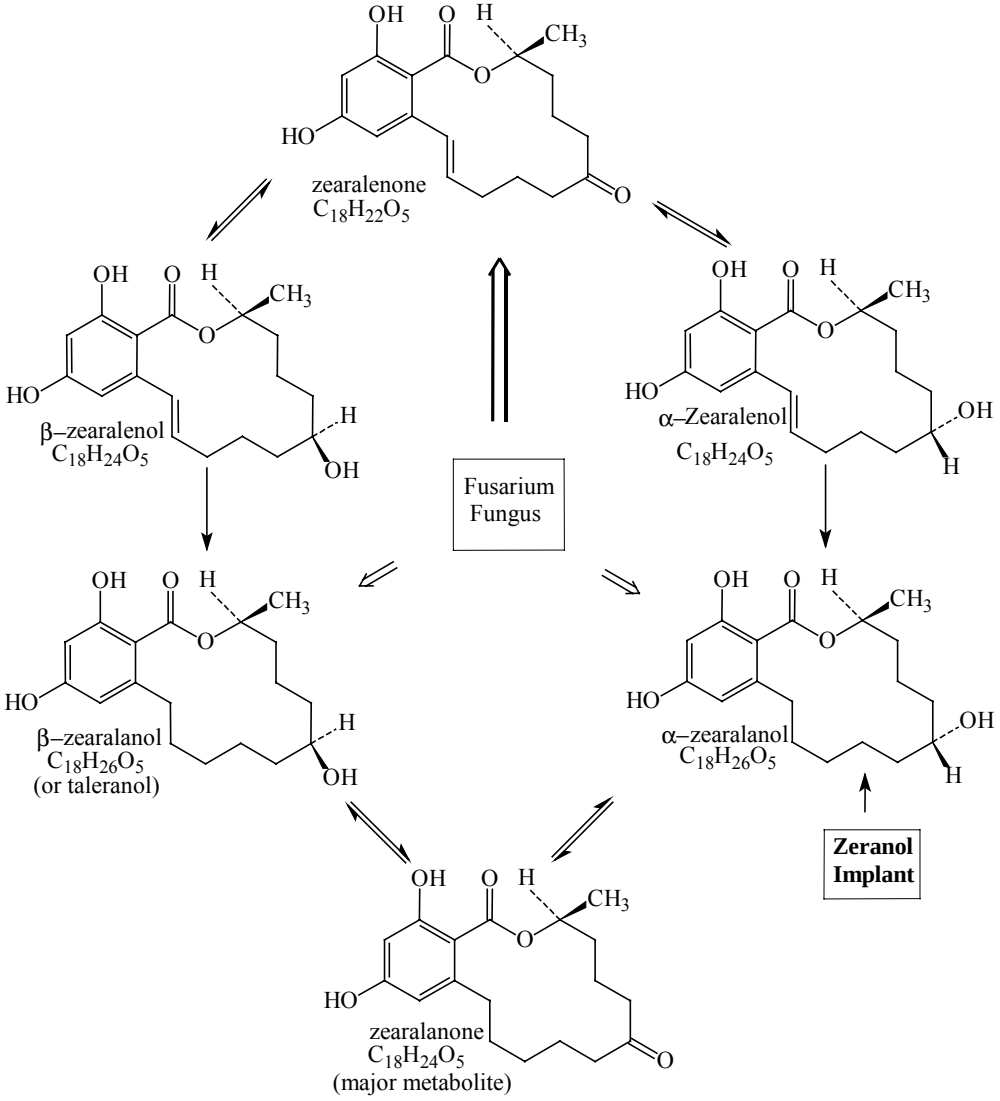

Fig. 1. Proposed routes for conversion of zearalenone into other RAL components.

A metabolic study of zearalenone in pasture-fed sheep showed the excretion of zeranol in their urine (Erasmuson 1994). A further investigation of the quantities of zeranol found in the different species were up to 2 ng/ml for sheep and 13 ng/ml in cattle. The experience of Erasmuson, that zeranol was found in the urine of pasture-fed animals, was confirmed by other investigators (Miles 1996, Ramazza 1998). Equal positive values for zeranol were obtained by the investigation of the bile of pasture-fed cattle. Values up to 2 ng/ml zeranol (Hewitt 1996) and up to 10 ng/ml zeranol (Kennedy 1998) were found in bile in respectively castrated male cattle and in slaughter cattle. The last author suggested that zeranol may be formed by the *in vivo* metabolism of naturally occurring *Fusarium* spp. toxins. The conversion of zearalenone into zeranol is suggested to happen in the rumen by hydrogenation of the aliphatic carbon-carbon double bond of α-zearalenol, the reduced hydroxy form of

zearalenone. It is an irreversible reaction and takes only place by the conversion of α-zearalenol into zeranol.

Some authors tried to judge the natural occurrence of zeranol from the determination of the relative ratios of six closely related RAL (Resorcylic Acid Lactone) substances, namely zearalenone, α-zearalenol, β-zearalenol, zeranol, taleranol (the major bovine and ovine zeranol metabolite) and zearalanone. The obtained square root relationship between urinary concentrations of zeranol and the *Fusarium* toxins (Erasmuson 1994) could not be confirmed by others. Ramazza et al. (Ramazza 1998) conclude there is a ZEL/zeranol ratio >10 that should reveal the natural presence of zeranol in urine (in which ZEL means the combined contents of zearalenone, α-zearalenol and β-zearalenol). Likewise, the biliary concentrations of α-zearalenol always exceeded that of zeranol by a factor of at least 5:1 (Hewitt 1996). Maybe this can distinguish abuse (<5:1) from contamination (>5:1). To reveal the difference in illicit use and contamination it is nowadays preferred to direct the sampling and analytical procedure to all of the 6 RAL compounds.
In all above mentioned investigations there were no indications of illicit use of zeranol. Presumably contaminated feed in the form of ensilaged pasture caused the zeranol in the urine and bile of sheep and cattle. This conclusion had been justified because the urines of calves, not fed with pasture, were not positive to zeranol.
In the future, it may be desirable to regulate the zearalenone content of feedstuffs by EU regulations, as is the case for aflatoxin B_1 (see chapter 4). The problem of the occurrence of naturally zeranol in edible tissues is then at least under control. The "zeranol problem" is currently being investigated in a FAIR project, contract FAIR5-pl 973443, which started in june 1998 and goes on until june 2001.

2.4.4.3 Zearalenone in eggs

Radiolabeled zearalenone (Dailey 1980) was applied to investigate the carry over of zearalenone into eggs. A single dose of 10 mg [14C]-zearalenone/kg body weight was administered to laying hens. About 94 % of a single dose had been excreted in 72 h after administration. There was no major retention of zearalenone in edible tissues but residues of up to 2000 μg/kg in eggs (mainly in egg yolk) were found within 72 hr after dosing. Approximately 1% of the administered zearalenone was transmitted to eggs during initial 72 hr after dosing. The authors suggest that a prolonged exposure time might accumulate the lipophilic metabolite(s) of zearalenone in egg yolk.

2.4.5. Sterigmatocystin

2.4.5.1 Sterigmatocystin in milk

In a period of two weeks two cows were fed cattle cakes which were artificially contaminated with sterigmatocystin (Egmond 1978). The daily dosis was 10 mg sterigmatocysin for a cow. In the period of administration sterigmatocystin was not found in the cow's milk. In relation to the detection limit of the analytical method used it can be concluded, that a possible carry over of sterigmatocystin had to be smaller than 0.2 - 0.4 %. Because of the very low contamination of sterigmatocystin in feedstuffs it is likely that the presence of sterigmatocystin is negligible in the Dutch consumer milk. However, it could not be ruled out that sterigmatocystin might be converted into toxic metabolites that were present in the milk but not detectable by the method used.

2.5 Conclusions

As to current knowledge, the most significant mycotoxin, in terms of occurrence as a residue in animal products, is aflatoxin M_1 in milk. In the EU, aflatoxin M_1 seems to be well under control. Residues of ochratoxin A in organs and meat contribute to a minor extent to human exposure. Not much is known about the possible carry over of other mycotoxins from feed to animal products. The outcome of the few studies carried out in this respect do not show residue levels of significance.

3 Toxic properties

3.1 In general

Among the toxic properties of mycotoxins, the potential carcinogenicity to humans is of greatest concern to health authorities and regulating bodies. Since 1970, the International Agency for Research on Cancer (IARC) is working on a programme of the evaluation of the carcinogenicity of mycotoxins to animals and humans. The developments in establishing the criteria and their regular revisions of the risk assessment of mycotoxins have recently been reviewed (Castegnaro 1998). The recent IARC evaluations classified by group are given in Table IV.

Table IV. IARC classification of carcinogenicity for agents, mixtures or exposures.

Group	Definition
1	The agent (mixture) is carcinogenic to humans. The exposure circumstance entails exposures that are carcinogenic to humans
2A	The agent (mixture) is probably carcinogenic to humans. The exposure circumstance entails exposures that are probably carcinogenic to humans
2B	The agent (mixture) is possibly carcinogenic to humans. The exposure circumstance entails exposures that are possibly carcinogenic to humans
3	The agent (mixture, or exposure circumstance) is unclassifiable as to carcinogenicity in humans.
4	The agent (mixture, exposure circumstance) is probably not carcinogenic to humans.

The classification of mycotoxins according to the IARC evaluations of carcinogenicity to humans can be consulted on Internet (IARC 1999). The lists of IARC evaluations contain references to the individual Monographs Volumes 1-73 concerning mycotoxins among other agents, mixtures and exposures. The mycotoxins listed are summarized in table V. From this list it appears that, currently, the aflatoxins, ochratoxin A, sterigmatocystin and the fumonisins (with their structures given in fig. 2 and fig. 3) have been classified as either carcinogenic, or possibly carcinogenic to humans. Given the fact that occurrences of sterigmatocystin and the fumonisins are insignificant (see 2.5), the aflatoxins and ochratoxin A remain as mycotoxins, of primary toxicological concern in products of animal origin. Consequently hereafter, specific attention will be given to the specific toxic properties of the aflatoxins and ochratoxin A.

Table V. Summary of IARC evaluations of selected mycotoxins.

Mycotoxin	CAS Registration number	Degree of evidence of carcinogenicity		Overall evaluation of carcinogenicity to humans (explanation in table IV)
		Human	Animal	
Aflatoxins, naturally occurring mixtures of	1402-68-2	S	S	1
Aflatoxin B_1	1162-65-8	S	S	
Aflatoxin B_2	7220-81-7		L	
Aflatoxin G_1	1165-39-5		S	
Aflatoxin G_2	7241-98-7		I	
Aflatoxin M_1	6795-23-9	I	S	2B
Ochratoxin A	303-47-9	I	S	2B
Patulin	149-29-11		I	3
Sterigmatocystin	10048-13-2		S	2B
Toxins derived from Fusarium graminearum, F. culmorum and F. crookwellense		I		3
Zearalenone	17924-92-4		L	
Deoxynivalenol	51481-10-8		I	
Nivalenol	23282-20-4		I	
Toxins derived from Fusarium moniliforme		I	S	2B
Fumonisin B_1	116355-83-0		L	
Fumonisin B_2	116355-84-1		I	
Toxins derived from Fusarium sporotrichioides		I		3
T-2 toxin	21259-20-1		L	

Meaning of symbols:
S sufficient evidence; L limited evidence; I inadequate evidence

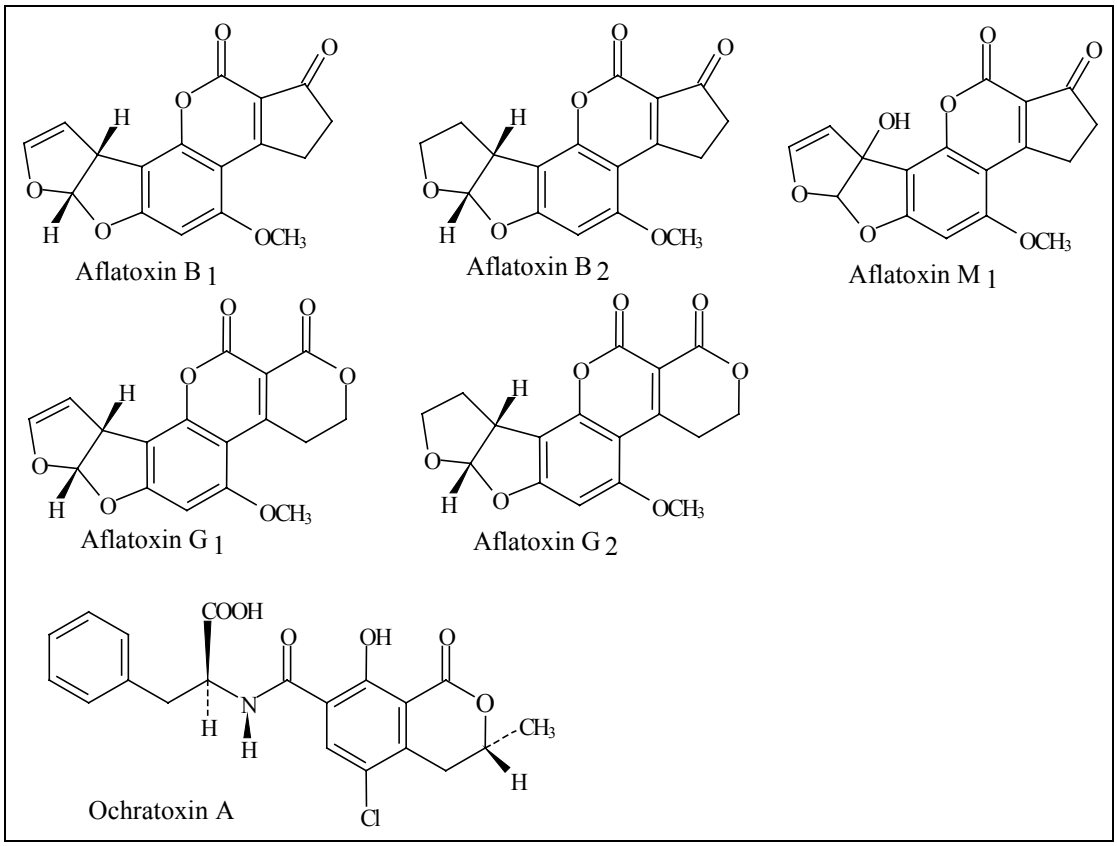

Fig. 2 Structures of the mycotoxins of Table V that showed significant contaminants of food of animal origin.

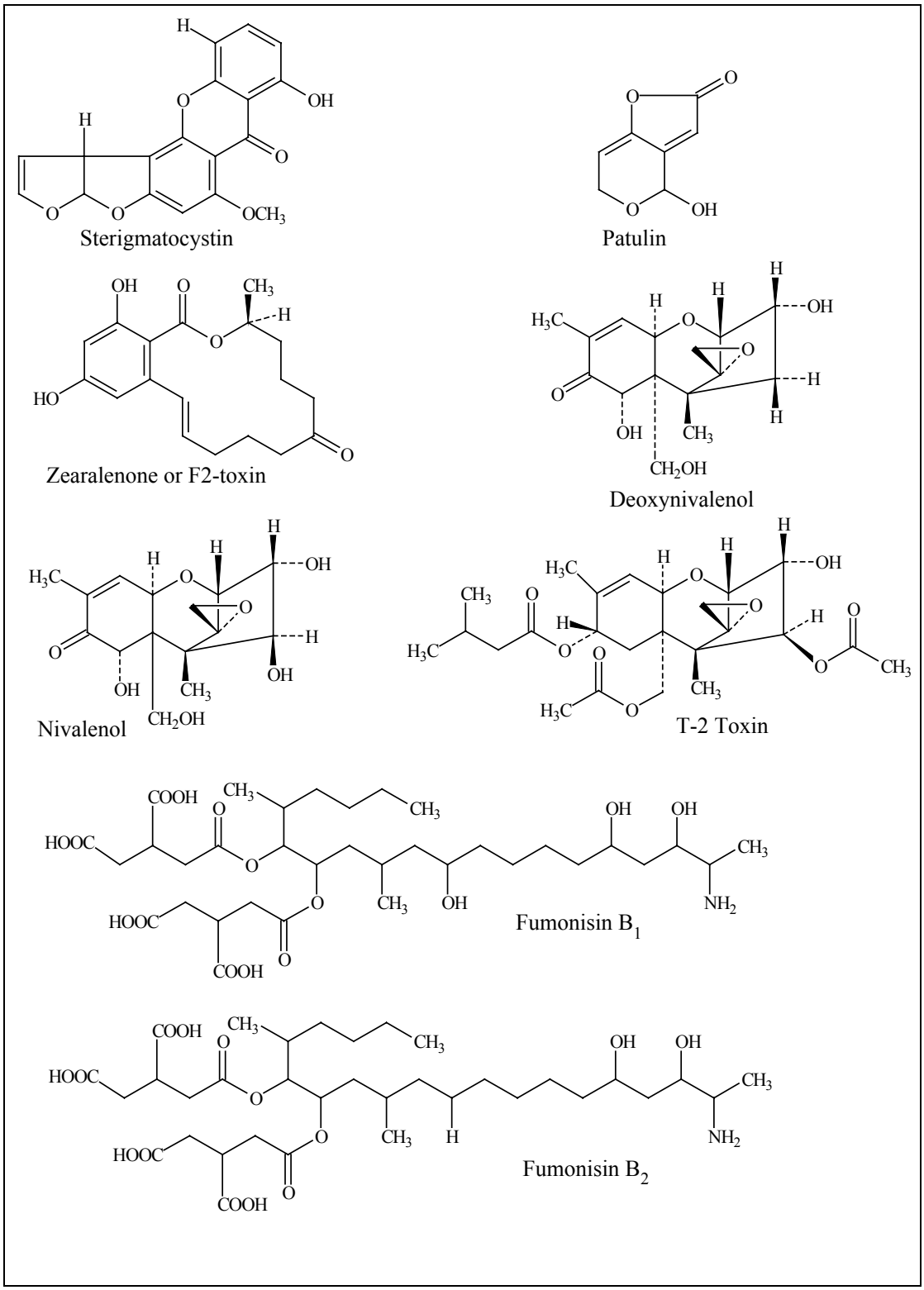

Fig. 3. Structures of the mycotoxins of Table V that showed generally negligible contamination of food of animal origin.

3.2 Aflatoxins

3.2.1 Aflatoxins B_1, B_2, G_1, G_2

Among the naturally occurring aflatoxins, the toxic properties of aflatoxin B_1 are the most widely studied. Aflatoxins have acute toxic effects (hepatotoxic in humans and animals and immunosuppressive in animals). The major concern, however, is that the aflatoxins are among the most potent mutagenic and carcinogenic substances known. The aflatoxins appeared to be extremely potent carcinogens in animal experiments. They are potent in all species investigated, i.e. mice, rats, hamsters, fish, duck, tree shrews and monkeys, and in several organs, of which the liver is the primary target (CEC 1996) Epidemiological studies suggest that aflatoxins can be responsible for cancer of the liver in humans in different parts of Africa and Asia, albeit in combination with hepatitis B virus (Rensburg 1986). The IARC concluded in 1993 that there is sufficient evidence in humans for the carcinogenicity of naturally occurring mixtures of aflatoxins, and for the carcinogenicity of aflatoxin B_1. This led to their group I classification (see table V). The knowledge on the toxicology of the aflatoxins, including analytical identification, agricultural and veterinary implications, toxicology and carcinogenesis in humans, and economic and regulatory problems associated with aflatoxin contamination and control, has been extensively summarized in a book (Eaton 1994).

3.2.2 Aflatoxin M_1

Thus far only a limited number of animal studies have been carried out to determine the toxic properties of aflatoxin M_1, primarily because of the difficulty in obtaining sufficient quantities of the pure compound necessary for extensive toxicity testing. The few studies that were completed tend to come to the same qualitative conclusion: Aflatoxin M_1 has hepatotoxic and carcinogenic properties. Quantitatively, the (sub)acute toxicity of aflatoxin M_1 in ducklings and rats seem to be similar to or slightly less than that of aflatoxin B_1. The carcinogenicity is probably one to two orders of magnitude less than that of the highly carcinogenic aflatoxin B_1. The current classification of the IARC is 2B (possibly carcinogenic to humans, see table V). The fact that aflatoxin B_1 is among the most potent carcinogens known, warrants concern about aflatoxin M_1 in dairy products. The knowledge about the toxicity and carcinogenicity of aflatoxin M_1 has been summarized (Egmond 1989).

3.3 Ochratoxin A

Ochratoxin A causes a number of toxic effects in laboratory animals, primarily of a teratogenic, immunological, nephrotoxic and carcinogenic (mainly urinary tract tumours) nature. The most sensitive and notable effects are the nephrotoxicity and the kidney tumours (CEC 1996). In addition, ochratoxin A possibly plays a role in the etiology of Balkan Endemic Nephropathy and the occurrence of urinary tract tumours in man, human diseases occurring in rural areas in the Balkan. According to IARC, there is sufficient evidence in animals for carcinogenicity of ochratoxin A and inadequate evidence in humans for carcinogenicity. The overall conclusion of IARC is that ochratoxin A is possibly carcinogenic to humans (Group 2B, see table V). A special issue of the journal “Food Additives and Contaminants” on occurrence and significance of ochratoxin A in food appeared, as a result of a workshop organized by ILSI Europe in 1996 (ILSI Europe 1996).

4. EU regulations for mycotoxins

In the EU, Community regulations and official methods of analysis for aflatoxin B_1 in various animal feedstuffs are already in force since 1976. Over the years these limits were tightened and now they are among the lowest in the world (CEC 1991). In table VI the current EU regulations for feedstuffs are summarized. Validated analytical HPLC methodology is in place to make enforcement of the regulations possible (CEC 1992). For specific feedstuffs for dairy cattle a further reduction of the limit of aflatoxin B_1 is foreseen, in view of newer data on the relatively high carry over rate of aflatoxin B_1 from feed to aflatoxin M_1 in milk (see 2.1). An AOAC International collaborative study of an analytical method suitable to determine aflatoxin B_1 in feedstuffs at the µg/kg level is in the evaluation stage.

Table VI Maximum tolerated*) levels for aflatoxin B_1 in animal feedstuffs in the European Union (1994/1995 survey) (FAO 1997).

Kind of feedstuffs	Aflatoxin B_1 level (µg/kg)
straight	50
straight: groundnut, copra, palmnut, cottonseed, babassu, maize and their products	20
complete	10
complete for pigs and poultry (except young animals)	20
complete for cattle/ sheep/ goats except calves/ lambs/ kids	50
complete for calves and lambs	10
complementary	5
complementary for pigs and poultry (except young animals)	30
complementary for cattle/ sheep/ goats except diary animals/ calves/ lambs/ kids	50
raw materials: groundnut, copra, palmnut, cottonseed, babassu, maize and their products	200

*) all EU tolerances refer to a commodity content of 12 %

The European Union is currently also in the process of harmonising the limits and regulations for various mycotoxins in human foods. Since 1 January 1999 harmonized limits are in force for the aflatoxins B_1, B_2, G_1 and G_2 in certain foodstuffs of vegetable origin, and for aflatoxin M_1 in milk. Animal products other than milk are currently not being regulated. (EC 1998 a) (see table VII). Similar as for the feedstuff regulations, the maximum levels for the aflatoxins in human foods are quite stringent, as compared to limits set by countries outside the EU. It is expected that Community limits will soon be established for ochratoxin A in grains coffee, wine and beer, for patulin in fruit products, and for deoxynivalenol in grains. There are some thoughts to establish specific Community regulations for aflatoxins and ochratoxin A in baby food. In the longer term, specific Community regulations for food may as well be developed for the fumonisins, for some of the trichothecenes other than deoxynivalenol, and for zearalenone.

Table VII. Commission regulation for maximum admissible levels of aflatoxins in food products (EC 1998 a; EC 1999)

Products	**Aflatoxins maximum admissible levels (µg/kg) *)**		
	B_1	**$B_1 + B_2 + G_1 + G_2$**	**M_1**
groundnuts, nuts and dried fruit			
-groundnuts, nuts and dried fruit and processed products thereof, intended for direct human consumption or as an ingredient in foodstuffs	**2** [a)]	**4** [a)]	**–**
-groundnuts to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs	**8** [a)]	**15** [a)]	**–**
-nuts and dried fruit to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs	**5** [a,b)]	**10** [a,b)]	**–**
cereals (including buckwheat, *Fagopyrum sp*.)			
-cereals and processed products thereof intended for direct human consumption or as an ingredient in foodstuffs	**2**	**4**	**–**
-cereals to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs	**–** [c)]	**–** [c)]	**–**
milk (raw milk, milk for the manufacture of milk-based products and heat-treated milk as defined by Council Directive 92/46/EEC of 16 june 1992 laying down the health rules for the production and placing on the market of raw milk, heat-treated milk and milk-based products)	**–**	**–**	**0.05**

[a)] The maximum limits apply to the edible part of groundnuts, nuts and dried fruits. If nuts "in shell" are analysed, it is assumed when calculating the aflatoxin content, all the contamination is on the edible part.

[b)] The maximum limits shall be reconsidered before 1 July 2001 according to the progress of scientific and technological knowledge.

[c)] In as far as no specific limit will be fixed before 1 July 2001, the limits laid down in the table will apply thereafter to the cereals referred to.

[*)] Methods of sampling and reference analysis are described in Commission Directive 98/53/ EC (EC 1998 b).

5 Analytical methodology

5.1 In general

The basic steps in mycotoxin determination are outlined in Figure 4. The first step representative sampling, is of utmost importance, but it may cause tremendous problems. Many mycotoxins may be very inhomogeneously distributed in the commodities to be inspected, which makes it very difficult to draw a representative sample. In the EU detailed sampling plans are prescribed at Community level (EC 1998 b). Samples are further reduced in size to obtain test portions that usually vary in weight from approximately 20 to 100 g, a range resulting from a compromise between homogeneity requirements and practical considerations. Test portions then undergo the further steps as outlined in Figure 4.

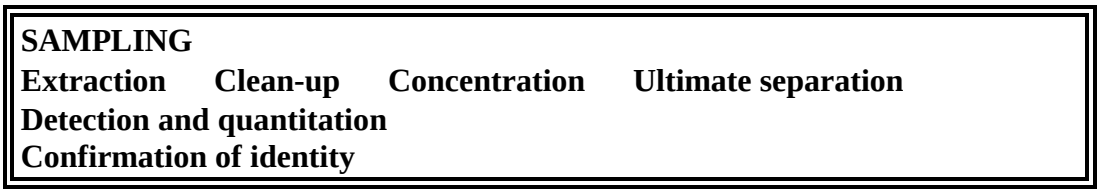

Fig.4. Analytical procedure for mycotoxin determination.

Extraction is usually done with (combinations of) organic solvents and water. Purification of the extract to remove lipids and other substances is usually done by leading the extracts through chromatography columns or prepacked cartridges. The latter are commercially available with many types of adsorbents and in many formats, which may suit the needs of the analyst. The most recent advance in clean-up of extracts containing mycotoxins is the use of immuno-affinity cartridges. These columns are composed of monoclonal antibodies, specific for the toxin of interest , which are immobilized on Sepharose and packed into small plastic cartridges. The immuno-affinity cartridges can be incorporated in fully automated sample preparation systems that take the sample from the extraction stage through to completion of high-performance liquid chromatography (HPLC) in an unattended mode of action (Egmond 1999).

In addition to HPLC, other chromatographic techniques exist to perform the ultimate separation and quantitation: thin-layer chromatography (TLC) and gas-liquid chromatography (GLC). Though still very valuable and widely applied, TLC was largely superseded during 1980s by HPLC. For the determination of residues of mycotoxins in animal products HPLC is nowadays the most commonly applied technique. GLC has limited applications in mycotoxin analysis because it requires volatile components, wheras most mycotoxins are non-volatile. Some trichothecenes are derivatized to volatile components that can be handled then with GLC. Besides the chromatographic techniques, the immuno techniques are worth mentioning. In particular enzym-linked immunosorbent assay (ELISA) has become an important analytical technique in mycotoxin methodology. The simplicity of ELISAs and the large number of samples that can be handled in one day have made these tests important, especially for screening and semi-quantitative group determination. Their lack of specificity at the level of the individual mycotoxins belonging to a group of structurally related compounds might prevent their use as quantitative tools, and therefore they are less suitable for regulatory analysis. It is therefore good laboratory

practice to confirm positive findings obtained with immunoassays by using methods of analysis based on other principles, preferably molecular spectroscopy.

5.2 Method validation and method performance criteria

Methods of analysis used to determine mycotoxins should be reliable and practical. A good and generally accepted approach to validate analytical methods of analysis for mycotoxins is the interlaboratory collaborative study. A leading organisation, devoted to promoting validation and quality measurements in the analytical sciences is AOAC International (see also chapter 1). AOAC International gives ample attention to validation of mycotoxin methods, and AOAC mycotoxin methods are used and quoted in official regulations worldwide. Other major international organisations involved in analytical methods validation are ISO and IUPAC (see also chapter 1). Since 1987 these three organisations follow a harmonised protocol for the conduct of collaborative studies (Horwitz 1989). The purpose of a collaborative study is to establish the characteristics of the methods with respect to accuracy, precision (repeatability and reproducibility), sensitivity, range, specificity, ruggedness, limit of measurement, practicality, and similar attributes under typical laboratory application. Such validated method in combination with an adequate Laboratory Quality Assurance System can then be used with confidence by regulatory agencies, regulated industry, product testing laboratories, and academic institutions to determine compliance with government regulations, to maintain quality control and process requirements, to set and evaluate compliance with terms of procurement contracts, to conduct national and international trade, and to support research (AOAC 1995).

Within the EU, an official (collaboratively studied) method for the determination of aflatoxin B_1 in animal feedstuffs is described at Community level (CEC 1992). For the determination of the aflatoxins in food no specific methods are described at Community level. Laboratories may select any method provided the selected method meets certain specified performance criteria (partly derived by CEN, see 5.2.1) and partly in Commission Decisions 93/256/EEC (CEC 1993 a) and 93/257/EEC (CEC 1993 b) and their updated draft revision (EC 1999). This newer approach is more often followed in the EU now. It has the advantage that, at least theoretically, the analyst can choose a method, that best fits with his needs. In practice the choice is still very limited, because to proof that the method fulfills to the criteria, requires validation through a (time consuming) collaborative study. A different approach: to circumvent the bottleneck of collaboratively studied methods EC criteria are now drafted for suitable and acceptable “in house validation” schemes. Such CRL approved schemes, if performed under the appropiate Quality Assurance system (96/23/EC) will increase flexibility and reduce costs of reliable mycotoxins trace analysis in food of animal origin. It is expected that a similar approach will be adopted for the other mycotoxins, that will be regulated at Community level. Since the Community regulations for various mycotoxins were drafted a few years ago, several collaborative studies according to the harmonised protocol have been carried out in the EU (including a method for aflatoxin M_1 in milk) whereas some are still underway.

5.2.1 CEN criteria

Performance criteria of analytical methods for mycotoxins in foodstuffs have recently been published in a CEN report (CEN 1999). The CEN working group "Biotoxins" is responsible for the selection and elaboration of European Standards of methods of analysis for mycotoxins in the European Union. The criteria were drawn up as performance characteristics for guidance to select methods of analysis.

The CEN performance characteristics are based on published data of European interlaboratory studies, supplemented by experiences from European mycotoxin experts. They were arrived upon by consensus within the CEN working group "Biotoxins". Desired performance characteristics of analytical methods for mycotoxins, currently of interest to the working group and partly adopted already in European Legislation are given for aflatoxins and ochratoxin A in table VIII.

Table VIII Performance characteristics of methods of analysis

Mycotoxin	Level [µg/kg]	RSD_r %	RSD_R %	Recovery %
Aflatoxin B_1	< 1	≤ 40	≤ 60	50 to 120
Aflatoxin B_1	1 - 10	≤ 20	≤ 30	70 to 110
Aflatoxin B_1	> 10	≤ 15	≤ 20	80 to 110
Aflatoxin B_1, B_2, G_1, G_2	< 1	-	-	50 to 120
Aflatoxin B_1, B_2, G_1, G_2	1 - 10	≤ 40	≤ 60	70 to 110
Aflatoxin B_1, B_2, G_1, G_2	> 10	≤ 30	≤ 50	80 to 110
Aflatoxin M_1	10 - 50	≤ 30	≤ 50	60 to 120
Aflatoxin M_1	> 50	≤ 20	≤ 30	70 to 110
Ochratoxin A	< 1	≤ 40	≤ 60	50 to 120
Ochratoxin A	1-10	≤ 20	≤ 30	70 to 110

Meaning of symbols:
RSD_r = relative intra-laboratory standard deviation (as derived from collaborative studies)
RSD_R = relative between-laboratory standard deviation (as derived from collaborative studies)

Performance characteristics have also been published for the mycotoxins patulin, fumonisin B_1 en B_2, deoxynivalenol, nivalenol, T-2 toxin and zearalenone.

5.2.2 EC criteria for aflatoxin methods used in official checking

In Directive 98/53/EC (EC 1998 b) a section is included with specific requirements of the methods of analysis used in official checking of the levels of aflatoxins in certain foodstuffs. These requirements indicate that no specific methods for the determination of aflatoxin levels in foodstuffs are prescribed at Community level and that laboratories may select any method provided the selected method meets the following criteria given in table IX.

Table IX Performance criteria for methods of analysis for aflatoxins to be used for food control purposes in the European Union

Criterion	Concentration range	Recommended value	Maximum permitted value
Blanks	all	Negligible	
Recovery - Aflatoxin M_1	0.01-0.5 µg/l	60 to 120 %	
	>0.05 µg/l	70 to 110 %	
Recovery - Aflatoxins B_1, B_2, G_1, G_2	<1.0 µg/l	50 to 120 %	
	1-10 µg/l	70 to 110 %	
	>10 µg/l	80 to 110 %	
Precision RSD_R	all	As derived from Horwitz equation	2x value derived from Horwitz equation
Precision RSD_r may be calculated as 0.66 times precision RSD_R at the concentration of interest			

Notes:
- Values apply to both B_1 and sum of B_1+ B_2 + G_1 + G_2,
- if sum of individual aflatoxins B_1+ B_2 + G_1 + G_2 are to be reported, then response of each to the analytical system must be either known or equivalent,
- the detection limits of the methods used are not stated as the precision values are given at the concentrations of interest,
- the precision values are calculated from the Horwitz equation, i.e.:

  $$RSD_R = 2^{(1-0,5 \log C)}$$

  where:
  - RSD_R is the relative standard deviation calculated from results generated under reproducibility conditions [(S_R/x) x 100].
  - C is the concentration ratio (i.e. 1 = 100g/100 g. 0.001 = 1000mg/kg)

This is a generalised precision equation which has been found to be independent of analyte and matrix but solely dependent on concentration for most “routine methods” of analysis.

5.3 Methods for Aflatoxins

5.3.1 Aflatoxin B_1 in animal products.

Thanks to EU regulations on aflatoxin B_1 in animal feedstuffs and the very low carry over rates of this toxin to animal products, there is hardly a need for analytical methodology. Nevertheless AOAC validated analytical methods for aflatoxins in liver (methods 982.24 and 982.25) and eggs (method 978.15) are available for those who wish to carry out analyses of animal products for aflatoxin B_1 (AOAC International 1996). These methods are based on thin layer chromatography (TLC) and are applicable at levels of approx. > 0.1 µg/kg. If application of more advanced techniques are desired in laboratories, they can try to modify the AOAC method 999.07. This method (immunoaffinity cleanup / HPLC / postcolumn derivatization / fluorescence detection) was recently successfully studied in an EU/AOAC International collaborative study (Stroka, in press)

5.3.2 Reference method for Aflatoxin M_1 in milk(powder)

Recently an interlaboratory study was conducted with a method that combines immunoaffinity and HPLC for the determination of aflatoxin M_1 in raw liquid milk (Dragacci 1999). The collaborative study yielded excellent results. The precision characteristics are summarized in table X. The mean recovery was 69 % at a spiking level of 0.05 µg/l.

Table X. Method performance for determination of aflatoxin M1 in liquid milk (Dragacci 1999)

Sam-ples	N	M (µg/l)	SD_r (µg/l)	r (µg/l)	RSD_r (%)	SD_R (µg/l)	R (µg/l)	RSD_R (%)	HORRAT
A	12	0.023	0.0040	0.0113	17	0.0061	0.0173	27	0.33
B	12	0.046	0.0056	0.0158	12	0.0104	0.0293	23	0.31
C	12	0.103	0.0077	0.0217	8	0.0220	0.0622	21	0.33

Meaning of the symbols:
A: batch with presumptive value of 0.027 µg AFM_1/L;
B: batch with presumptive value of 0.055 µg AFM_1/L;
C: batch with presumptive value of 0.121 µg AFM_1/L;
N: number of laboratories;
M: overall mean;
SD_r (SD_R): standard deviation for repeatability (for reproducibility);
r (R): repeatability (reproducibility) value;
RSD_r (RSD_R) relative standard deviation for repeatability (reproducibility);
HORRAT: value calculated as the ratio of the RSD_R resulting from the trial to the predicted RSD_R. A HORRAT value of 1 indicates an RSD_r value corresponding exactly to the Horwitz equation and HORRAT values bracketing a value of 1 or smaller indicate acceptable precision.

The method is based on IDF standard 171 (Tuinstra 1993). The principle of the method is based on extraction of aflatoxin M1 from the sample and clean-up of aflatoxin M_1 through the immunoaffinity column. Aflatoxin M_1 as the antigen is selectively complexed by the specific antibodies bound on the solid support into an antibody-antigen complex. The column is washed with water to remove all other matrix components of the sample. Aflatoxin M_1 is eluted from the column with a

small volume of pure acetonitrile. The eluate is concentrated and applied to High Performance Liquid Chromatography (HPLC) coupled with fluorescence detection. The quantification limit is 0.005 μg/l. The method can also be applied to skimmed milk and low fat milk.

5.4 Possible methods for Ochratoxin A

Methods for ochratoxin A (OA) in animal and human tissues and fluids were recently reviewed by Valenta (Valenta 1998). The review is directed mainly to chromatographic methods for the determination of ochratoxin A. Immunochemical methods are not discussed. The review includes sampling, sample storage, extraction, spiking procedures, clean-up, detection and determination, and confirmation procedures. Recently also a LC/MS/MS method was described for the analysis and confirmation of ochratoxin A in pig kidney (Jørgensen 1999). If laboratories wish to apply one of these procedures, selection should be made in compliance with the CEN criteria (5.2.1). Currently this will be difficult, however, because performance characteristics derived from accepted collaborative studies on ochratoxin A in animal products are not available. Recent SMT/AOAC collaborative studies of immunoaffinity/HPLC methods have been carried out for ochratoxin A in cereals and coffee. However these methods have not been studied for animal products and modifications, at least in the sample extraction step will be required. If laboratories plan to use one of these or of the above-referred methods to carry out formal investigations of animal products for ochratoxin A, in-house validation should be done, preferably according to recently approved EU guidelines for analytical methods of animal products (EC 1999).

5.5 Reference materials and calibrants

Interlaboratory studies often showed in the past quite different analytical results by investigations of samples, drawn from the same homogeneous batch. In compliance with the principles of Quality Assurance the mycotoxin measurements by different laboratories have to be reliable and comparable. A Quality Assurance programme includes, where possible, the use of (certified) reference materials besides many other quality system elements. Certified reference materials (CRMs) are stable, homogeneous products with certified values of the analyte(s) of interest. The European Union's Community Bureau of Reference, formerly BCR and nowadays renamed as Standards, Measurements and Testing Programme, is charged with the development of the CRMs and in case of food of animal origin in cooperation with the responsible CRL. The EC Joint Research Centre, Institute for Reference Materials and Measurements in Geel, Belgium, takes care of the storage, stability control and distribution of the CRMs. The development of new CRMs is closely related to the critical points in the food and feed chains. An overview (Boenke 1997) of the different CRMs or RMs available for mycotoxin analysis is given in Table XI.

Table XI. Overview*) on the different BCR CRMs and RMs for mycotoxin analysis as well as their corresponding certified values or current status

Number	Matrix	Mycotoxin	Certified value	Uncertainty
CRM 385	peanut butter	aflatoxin B_1	7.0 µg/kg	± 0.8 µg/kg
		aflatoxin B_2	1.1 µg/kg	± 0.2 µg/kg
		aflatoxin G_1	1.7 µg/kg	± 0.3 µg/kg
		aflatoxin G_2	0.3 µg/kg	± 0.2 µg/kg
		total aflatoxins	10.1 µg/kg	± 1.5 µg/kg
CRM 401		aflatoxin B_1	< 0.2 µg/kg	-
		aflatoxin B_2	< 0.2 µg/kg	-
		aflatoxin G_1	< 0.3 µg/kg	-
		aflatoxin G_2	< 0.2 µg/kg	-
		total aflatoxins	< 0.9 µg/kg	-
CRM 282	full-cream	aflatoxin M_1	< 0.05 µg/kg	-
CRM 283	milk powder		0.09 µg/kg	+ 0.04 µg/kg
				- 0.02 µg/kg
CRM 285			0.76 µg/kg	± 0.05 µg/kg
CRM 377	maize flour	deoxynivalenol	< 0.05 µg/kg	-
CRM 378			0.43 µg/kg	± 0.04 µg/kg
CRM 379	wheat flour		0.67 µg/kg	± 0.02 µg/kg
CRM 396			< 0.05 µg/kg	-
CRM 262	defatted	aflatoxin B_1	< 3 µg/kg	-
CRM 263	peanut meal		43.3 µg/kg	± 2.8 µg/kg
CRM 264			206 µg/kg	± 13 µg/kg
CRM 375	compound feed	aflatoxin B_1	< 1 µg/kg	-
CRM 376			9.3 µg/kg	± 0.5 µg/kg
CRM 471	wheat flour	ochratoxin A	< 0.6 µg/kg	-
CRM 472			8.2 µg/kg	± 1.0 µg/kg
RM 423	chloroform	aflatoxin M_1	information value: 9.93 µg/ml	
Not yet	maize	zearalenone	Feasibility studies completed, interlaboratory studies underway	
Not yet	maize and/or maize products	fumonisins	two interlaboratory studies completed	
Not yet	cereal type	trichothecenes (nivalenol, HT-2, T-2)	interlaboratory and feasibility studies underway	

*) In accordance with Boenke (Boenke 1997), CRL updated December 1999

The number of CRMs for mycotoxins in feed and food of vegetable origin is substantially greater than that for food of animal origin. With respect to the latter category, currently only full-cream milkpowders, certified for their aflatoxin M_1 content, and an aflatoxin M_1 calibrant solution in chloroform are available. The current milkpowder CRM supplies are nearly exhausted and the IRMM is planning to produce new batches in 2000. In the 1990s, attempts were made to develop lyophilized kidney materials, certified for their ochratoxin A mass fraction within a project of the BCR. The project was not successful however, and CRMs for ochratoxin A in products of animal origin have never become available.

5.6 Proficiency tests

Proficiency testing is becoming increasingly important as part of the QA measures a laboratory has to undertake to demonstrate acceptable performance. Participating in a proficiency testing program helps laboratories identify and correct the weaknesses in their system. Various national and international organisations offer possibilities for laboratories to take part in proficiency testing schemes and check sample surveys. Homogeneous and stable test materials are distributed to participants, analysis undertaken using the normal method of analysis, and results reported back within a reasonable time. The results are statistically processed by the organiser and reports are issued to the participants, usually giving all results for the round plus individual z-scores for the analyte determined. Proficiency tests at the European level for mycotoxins in animal products are scarce, and thus far limited to aflatoxin M_1 in milk. The CRL for Milk and Milk products (Paris) has conducted such proficiency tests for EU NRLs for Milk and Milk Products in 1996 (Dragacci 1996 b) and in 1998 (Grosso 1999). The organizers concluded that, considering the very low aflatoxin M_1 levels of the distributed samples, the NRLs network displayed a very good analytical competency for the determination of aflatoxin M_1 in milk at the EU regulation level. It is foreseen that proficiency tests for aflatoxin M_1 will be expanded in the near future to include also NRLs for Residues that are charged with mycotoxin tasks. The UK-based Food Analysis Performance Assessment Scheme (FAPAS), with worldwide participation possibilities, started proficiency testing for aflatoxin M_1 in milk early 1999. Aflatoxin M_1 rounds are now ongoing with FAPAS and samples go out every 3-4 months.

6 Recommendations and future activities

The recommendations given hereafter are provisional. They are based on current knowledge on mycotoxins in food of animal origin, as summarized in this CRL document. New information and new developments in mycotoxin methodology, as well as other views that may exist within the NRLs for residues and for milk and milk products may lead to changes in the recommendations in the near future.

- Aflatoxin M_1 in milk and milk products should be regularly monitored, although there is currently no reason for concern. Various methods of analysis can be used. Their performance should be controlled by regularly using (certified) reference materials and by taking part in proficiency tests. For screening purposes ELISAs may be suitable, for reference purposes the HPLC method, as collaboratively studied under auspices of SMT and AOAC International is recommended.
- Monitoring for ochratoxin A (in pig kidney) is of second priority compared to aflatoxin M_1 in milk(products). If there is a reason for concern, immunochemical and HPLC methods could be used, provided that an "fit for the purpose" validation is carried out, so as to guarantee adequate performance.
- Monitoring for aflatoxin B_1 and other aflatoxins in animal products (meat, organs, eggs) is normally not needed. Should the need arise in exceptional circumstances, (modified) AOAC methods could be applied.
- Monitoring for residues of other mycotoxins in animal products is currently not recommended with a possible exception for zearalenone in relation to the inspection for the banned "hormonal" growth promotor zeranol.

Activities of the CRL for Residues in Bilthoven for 2000 are expected to include:

- Provision of NRLs for residues with a reference method for aflatoxin M_1 in milk (Dragacci 1999), after this method has been approved by SMT and AOAC International.
- An enquiry among the NRLs for Residues about their facilities and quality systems relative to mycotoxin methodology in animal products.
- Investigations of the possibilities to extend the existing proficiency studies for aflatoxin M_1 in milk and milk products (in which the NRLs for Milk and Milk Products participate), to include the NRLs for Residues.

7 References

Note on explanation and use of the ARODOC number:
The Laboratory for Residue Analysis, inclusively the Community Reference Laboratory, is using an electronic management information system, in which each document has obtained a specific number, called ARODOC number. The specific ARODOC number is recorded electronically in a database with the bibliographic data of the document. If one of the following documents is requested, it is advisable to include the ARODOC number mentioned.

Anonymus (1997). Aflatoxins – the 1996 survey by the Milchwirtschaftliche Verein Baden-Wuerttemberg eV. Aflatoxin–Jahresrückblick 1996 des Milchwirtschaftlichen Vereins Baden-Württemberg e.V. Molkerei Zeitung. Welt der Milch 51: 91-92 [In German] ARODOC 19257

AOAC (1995). AOAC official methods program. Associate referee's manual on development, study, review, and approval process. AOAC International, Gaithersburg US ARODOC 19265

AOAC International (1996). Official methods of analysis of AOAC International. Sixteenth edition 1995, Volume II, AOAC International, Gaithersburg US, ISBN 0-935584-54-4 ARODOC 15775

Asplin FD, Carnaghan RBA (1961). The toxicity of certain groundnut meals for poultry with special reference to their effect on ducklings and chickens. Vet. Rec. 73: 1215-1219 ARODOC 19249

Boenke A (1997). The food and feed chains - a possible strategy for the production of Certified Reference Materials (CRMs) in the area of mycotoxins? Food Chemistry 60 255-262 ARODOC 19223

Bononi A, Quarantelli A, Mazzali I., Cabassi E, Corradi A, Di Lecce R, Ubaldi A, Fusari A, Chizzolini R (1995). Effects of aflatoxins B_1 and G_1 on production efficiency, meat yield and quality in fattening pigs (experimental contribution). In: Quality control and requirements of food of animal origin [editors Polidori P & Renaud J]. Proceedings of a workshop held in Verona, Italy from 9 to 12 March, 1994, FAO Rome. REU Technical Series 40: 141-142 ARODOC 16641

Bukovjan K, Hallmannová A, Karpenko A (1992). Concentration of aflatoxin B1 in the organs of game living in the wild. Konzentration von Aflatoxin B_1 in Organen frei lebenden Wildes. Fleischwirtschaft 72: 794-796 [In German] ARODOC 18663

Castegnaro M, McGregor D (1998). Carcinogenic risk assessment of mycotoxins. Revue Méd. Vét. 149: 671-678 ARODOC 18913

CEC (Commission of the European Communities) (1991). Commission Directive of 13 February 1991 amending the Annexes to Council Directive 74/63 EEC on undesirable substances and products in animal nutrition (91/126/EEC). Official J. European Comm. L 60: 16-17 ARODOC 19699

CEC (Commission of the European Communities) (1992). Commission Directive 92/95 EEC of November 9, 1992 amending the Annex of the Seventh Directive (76/372/EEC) establishing Community methods of analysis for the official control of feedstuffs. Annex: Determination of aflatoxin B_1 with high performance liquid chromatography. Official J. European Comm. L 327: 54-62 ARODOC 19251

CEC (Commission of the European Communities) (1993 a). Commission Decision of 14 April 1993 laying down the methods to be used for detecting residues of substances having a hormonal or a thyrostatic action (93/256/EEC). Official J. European Commun. L 118: 64-72 ARODOC 9980

CEC (Commission of the European Communities) (1993 b). Commission Decision of 15 April 1993 laying down the reference methods and the list of national reference laboratories for detecting residues (93/257/EEC). Official J. European Commun. L 118: 75-79 ARODOC 9981

CEC (Commission of the European Communities) (1996). Reports of the Scientific Committee for Food (Thirty-fifth series); Opinions of the Scientific Committee for Food on aflatoxins, ochratoxin A and patulin. Directorate-General Industry, Brussels. 35th series 45-50 ARODOC 19261.

CEC (Commission of the European Communities) (1998 a). Commission Decision of 3 September 1998 establishing the list of national reference laboratories for the detection of residues (notified under document number C(1998) 2487). Official J. European Commun. L 251: 39-42 ARODOC 20011

CEC (Commission of the European Communities) (1998 b). Commission Decision of 9 October 1998 on financial aid from the Community for the operation of certain Community reference laboratories in the veterinary field (notified under document number C(1998) 2998; Decision 98/587/EC). Official J. European Commun. L 282: 73-75 ARODOC 18867

CEG (Commissie van de Europese Gemeenschappen) (1996). Beschikking van de Commissie betreffende financiële steun van de Gemeenschap voor de werkingskosten van het Rijksinstituut voor Volksgezondheid en Milieuhygiëne (RIVM) in Bilthoven (Nederland), dat is aangewezen als communautair referentielaboratorium voor het opsporen van bepaalde residuen (Slechts de tekst in de Nederlandse taal is authentiek) (96/519/EG). [In Dutch; only the Dutch text is authentic]. Publikatieblad van de Europese Gemeenschappen L 217: 10-11 ARODOC 16612

CEN (Comité Européen de Normalisation) (1999). Food analysis - Biotoxins - Criteria of analytical methods of mycotoxins. CEN report CR 13505: 8 pp ARODOC 19258

Charmley E, Trenholm HL, Thompson BK, Vudathala D, Nicholson JWG, Prelusky DB, Charmley LL (1993). Influence of level of deoxynivalenol in the diet of dairy cows on feed intake, milk production, and its composition. J. of Dairy Science 76: 3580-3587 ARODOC 14211

Codex Alimentarius Commission Position paper on ochratoxin A (1998). CX/FAC 99/14 ARODOC 19250

Council of the EU (1996 a). Council Directive 96/22/EC of 29 April 1996 concerning the prohibition on the use of stockfarming of certain substances having hormonal or thyrostatic action and of beta-agonists, and repealing Directives 81/602/EEC, 88/146/EEC and 88/299/EEC. Official J. European Communities L 125: 3-9 ARODOC 16793

Council of the EU (1996 b). Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC, and 86/469/EEC, and Decisions 89/187/EEC and 91/664/EEC. Official J. European Communities L 125: 10-32 ARODOC 16794

Dailey RE, Reese RE, Brouwer EA (1980). Metabolism of [14C]-zearalenone in laying hens. J. Agric. Food Chem. 28: 286-291 ARODOC 11748

Dragacci S, Fremy JM (1996 a). Application of immunoaffinity column cleanup to aflatoxin M1 determination and survey in cheese. J. Food Protection 59: 1011-1013 ARODOC 19256

Dragacci S, Marchond N, Venant A (1996 b). First proficiency testing for aflatoxin M_1 determination in dried milk in the European Union. CRL document reference AFM_1 06/96 CNEVA, Paris, ARODOC 16460

Dragacci S, Grosso F (1999). Validation of analytical methods to determine the content of aflatoxin, ochratoxin and patulin in foodstuffs of vegetable origin. Report EUR 18856 EN, CNEVA Paris FR, 31 pp. ISBN 92-828-5926-6 ARODOC 19266

EC (European Commission) (1998 a). Commission Regulation (EC) No 1525/98 of 16 July 1998 amending Regulation (EC) No 194/97 of 31 January 1997 setting maximum levels for certain contaminants in foodstuffs. Official J. European Comm. L 201: 43-46 ARODOC 18847

EC (European Commission) (1998 b). Commission Directive 98/53/EC of 16 July 1998 laying down the sampling methods and the methods of analysis for the official control of the levels for certain contaminants in foodstuffs. Official J. European Comm. L 201: 93-101 ARODOC 18848

EC (European Commission) (1999). Commission Decision laying down analytical methods to be used for detecting certain substances and residues thereof in live animals and animal products according to Council Directive 96/23/EC (Revision of Commission Decision 93/256/EC), Final version: 52 pp. ARODOC 18877

Eaton DL and Groopman JD (Eds.) (1994). The Toxicology of Aflatoxins. Human Health, Veterinary, and Agricultural Significance. Academic Press Inc, San Diego, California: 544 pp. ISBN 0-12-228255-8 ARODOC 11486

Egmond HP van, Vreman K, Deyll WE, Paulsch W (1978). The content of sterigmatocystin(s) in whole milk as result from feeding the cow with sterigmatocystin contaminated concentrate. Het gehalte aan sterigmatocystine(n) in volle melk ten gevolge van het voeren van met sterigmatocystine gecontamineerd krachtvoer. LCLO rapport 45/78, maart 1978 [In Dutch] ARODOC 8045

Egmond HP van (1989). Mycotoxins in dairy products. Elsevier Applied Science, London, UK and New York, US. 272 pp. ISBN 1-85166-369-x, ARODOC 7026

Egmond HP van (1996-1999). Draft Associate Referee Reports of AOAC International 1996 ARODOC 16456, 1997 ARODOC 19704, 1998 ARODOC 18382, 1999 ARODOC 18388

Egmond HP van, Speyers GJA (1999). Natural toxins I. Mycotoxins. In: International FOOD SAFETY Handbook, (Eds. Heyden CA van der, Younes M, Fishbein L, Miller, S) Marcel Dekker Inc., New York, US 811 pp. ISBN 0-8247-9354-4 ARODOC 19263

Erasmuson AF, Scahill BG, West DM (1994). Natural zeranol (alpha-zearalanol) in the urine of pasture-fed animals. J Agric Food Chem 42: 2721-2725 ARODOC 16953

FAO [Egmond HP van, Dekker WH] (1997). Worldwide Regulations for Mycotoxins 1995. A Compendium. FAO Food & Nutrition Paper 64: 45 pp. ISBN 92-5-103960-7 ARODOC 10171

Fink-Gremmels J, Georgiou NA (1996). Risk assessment of mycotoxins for the consumer. In: Residues of veterinary drugs, Wageningen Pers, 159-174 ISBN 90-74134-27-0 ARODOC 16366

Forgacs J & Carll WT (1962). Mycotoxicoses. Advances in Veterinary Science 7: 273-382 ARODOC 19248

Gareis M (1996). Fate of ochratoxin A on processing of meat products. Food Addit. Contam. 13: 35-37 ARODOC 17039

Grosso F, Dragacci S, Lombard B (1999). Second Community proficiency testing for the determination of aflatoxin M_1 in milk (November 1998). Report AFSSA/TOMI/FG9901 May 1999, 13 pp. ARODOC 19695

Hammer P, Bluethgen A, Walte HG (1996). Carry over of fumonisin B_1 into milk of lactating cows. Milchwissenschaft 51: 691-695 ARODOC 19227

Helferich WG, Garrett WN, Hsieh DPH, Baldwin RL (1986). Feedlot performance and tissue residues of cattle consuming diets containing aflatoxins. J. Anim. Sci. 62: 691- 696 ARODOC 19697

Hewitt SA, Currie JW, Elliott CT, Cannavan A, Blanchflower WJ, McEvoy JDG, Kennedy DG (1996). Fusarium spp. toxins, a natural source of zeranol in bovine bile. Proceedings Euroresidue III part I : 496-500 ARODOC 16646

Horwitz W (1989). Guidelines for collaborative study of procedure to validate characteristics of a method of analysis. J. Assoc. Official Anal. Chem. 72: 694-704 ARODOC 19264

IARC (1999). Lists of IARC Evaluations last updated January 20, 1999: http://193.51.164.11/monoeval/grlist.html, ARODOC 19219

ILSI Europe (1996). Occurrence and significance of ochratoxin A in food. (Eds. Battaglia R, Hatzold T, Kroes R) Food Additives and Contaminants 13: supplement 57 pp ARODOC 16465

Jørgensen K, Vahl M (1999). Analysis of ochratoxin A in pig kidney and rye flour using liquid chromatography tandem mass spectrometry (LC/MS/MS). Food Addit. Contaminants 16: 451-456 ARODOC 19686

Kennedy DG, Hewitt SA, McEvoy JDG, Currie JW, Cannavan A, Blanchflower WJ, Elliott CT (1998). Zeranol is formed from Fusarium spp. Toxins in cattle in vivo. Food Add Contaminants 15: 393-400 ARODOC 18495

Krogh P (1992). Role of ochratoxin in disease causation. Food Chemistry and Toxicology 30: 213-224 ARODOC 18521

Kuiper-Goodman T, Scott PM (1989). Risk assessment of the mycotoxin ochratoxin A. Biomedical and Environmental Sci. 2: 179-248 ARODOC 18840

Maryamma KI, Rajan A, Nair MG, Ismail PK, Manomohan CB, Gangadharan B (1989). Aflatoxin residues in animal tissues and animal products. Kerala J. Vet. Sci. 20: 116-124 ARODOC 19696

Miles CO, Erasmuson AF, Wilkins AL, Towers NL, Smith BL, Garthwaite I, Scahill BG, Hansen RP (1996). Ovine metabolism of zearalenone to α-zearalanol (zeranol). J. Agric. Food Chem. 44: 3244-3250 ARODOC 15334

Mirocha CJ, Robison TS, Pawlosky RJ, Allen NK (1982). Distribution and residue determination of [3H]zearalenone in broilers. Toxicol. Applied Pharmacology 66: 77-87 ARODOC 13979

Norred WP, Cole RJ, Dorner JW, Lansden JA (1987). Liquid Chromatographic Determination of Cyclopiazonic Acid in Poultry Meat. J. Assoc. Off. Anal. Chem. 70: 121-123 ARODOC 18917

Pietri A, Bertuzzi T, Bertuzzi P, Piva G (1997). Aflatoxin M1 occurrence in samples of Grana Padano cheese. Food Addit. Contam. 14: 341-344 ARODOC 18304

Prelusky DB, Scott PM, Trenholm HL, Lawrence GA (1990). Minimal transmission of zearalenone to milk of dairy cows. J. of Environm. Science and Health 25: 87-103 ARODOC 19217

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

Ramazza V, Brina N, Zucchi M (1998). The presence of natural zeranol (α-zearalanol) in the urine of fattening meat cattle. Results of a survey in northern Italy. Contribution to the Proceedings of the third symposium on residue analysis and veterinary drugs, Bruges, June 2-5, 9 pp. ARODOC 15016

Rensburg SJ van (1986). Role of mycotoxins in endemic liver and oesophageal cancer. In: Mycotoxins and Phycotoxins (Eds. Steyn PS and Vleggaar R). Elsevier Science Publishers, Amsterdam: 483-494 ARODOC 19262

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 (Matsushima) Nirenberg culture material. Mycopathologia 133: 123-126 ARODOC 17495

Scott PM (1978). Mycotoxins in feeds and ingredients and their origin. J. Food Protection 41: 385-398 ARODOC 16615

Sizoo EA, Egmond HP van (1997). The occurrence of aflatoxin M_1 in infant foods, prepared on the basis of milk(products); Dutch samples, taken in 1989 and 1994. Het voorkomen van aflatoxine M_1 in babyvoedingsmiddelen bereid op basis van melk(producten). Nederlandse monsters getrokken in 1989 en 1994. Rapport 388802 012. National Institute of Public Health and the Environment, Bilthoven, NL [In Dutch] ARODOC 16361

Stoloff L, Trucksess MW (1978). Survey for aflatoxin B1 in chicken eggs. J. Assoc. Off. Anal. Chem. 61: 995-996 ARODOC 19698.

Stoloff L, Trucksess MW (1979). Distribution of Aflatoxins B1 and M1 in contaminated calf and pig livers. J. Assoc. Off. Anal. Chem. 62: 1361-1363 ARODOC 18666

Stroka J, Anklam E, Jörissen U, Gilbert J (in press). Immunoaffinity column clean-up with liquid chromatography using post-column bromination for the determination of aflatoxins in peanut butter, pistachio paste, fig paste and paprika powder: Collaborative study. J. AOAC International. ARODOC 19703

Top H van den, Paulsch W, Egmond HP van (1991). Ochratoxin A in meat products, porcine blood plasma and porcine dried plasma. Ochratoxine A in vleeswaren, varkensbloedplasma en varkensplasmapoeder. RIVM rapport 388608 001, februari 1991, 14 pp. [In Dutch] ARODOC 7713

Tuinstra LGMTh, Roos AH, Trijp JMP van (1993). Liquid chromatographic determination of aflatoxin M_1 in milk powder using immunoaffinity columns for cleanup: interlaboratory study. J. AOAC Int. 76: 1248-1254 ARODOC 19267

Valenta H, Goll M (1998). Determination of ochratoxin A in regional samples of cow's milk from Germany. J. Chromatogr. A 815: 75 - 92 ARODOC 18636

Yousef AE, Marth EH (1989). Stability and degradation of aflatoxin M_1. In: Egmond HP van. Mycotoxins in dairy products. Elsevier Applied Science, London, UK and New York, US: 127-161 ISBN 1-85166-369-x ARODOC 19247

8.0 Glossary of abbreviations

Institutes or Organisations

AOACI	Association of Official Analytical Chemists International
BCR	Bureau Communautaire de Référence [*in French*]
CCFAC	Codex Committee on Food Additives and Contaminants
CEC	Commission of the European Communities
CEN	Comité Européen de Normalisation [*in French*]
COST	Cooperation in the Field of Scientific and Technical Research
CRL	Community Reference Laboratory
EC	European Commission
EEC	European Economic Community
EU	European Union
FAO	Food and Agriculture Organisation
FAIR	Food Agro-Industrial Research
FAPAS	Food Analysis Performance Assessment Scheme
IAEA	International Atomic Energy Agency
IARC	International Agency for Research on Cancer
IDF	International Diary Federation
ILSI	International Life Sciences Institute
IRMM	Institute of Reference Materials and Measurements
ISO	International Standardisation Organisation
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint Expert Committee on Food Additives and Contaminants
MERCOSUR	Mercado Común del Sur [*in Spanish*]
NRL	National Reference Laboratory
SCF	Scientific Committee on Food
SCOOP	Scientific Co-operation on Questions relating to Food
SMT	Standards, Measurements and Testing
UK	United Kingdom
UNEP	United Nations Environmental Program
WHO	World Health Organisation

Analytical Methods

ELISA	Enzyme-linked Immunosorbent Assay
GLC	Gas Liquid Chromatography
HPLC	High Performance Liquid Chromatography
TLC	Thin Layer Chromatography

Chemical compounds

AFB_1	Aflatoxin B_1
AFM_1	Aflatoxin M_1
CRMs	Certified Reference Materials
F2-toxin	Zearalenone
PR-toxin	*Penicillium roqueforti* toxin
RAL	Resorcylic Acid Lactone
RMs	Reference Materials
ZEL	Combination of zearalenone, α-zearalenol and β-zearalenol

Otherwise

CAS	Chemical Abstracts Service

Codes and entities

Alpha-2 code	*Entity (short name)*
AT	Austria
BE	Belgium
CH	Switzerland
CZ	Czech Republic
DE	Germany
DK	Denmark
FI	Finland
GB	United Kingdom
HU	Hungary
IT	Italy
NL	Netherlands
NO	Norway
PL	Poland
US	United States
YU	Yugoslavia

Alpha-3 code	*Entity (short name)*
USA	United States of America