ELSEVIER

PII: S0308-8146(96)00344-5

Food Chemistry, Vol. 60, No. 2, pp. 255–262, 1997

0308-8146/97 $17.00+0.00
Analytical, Nutritional and Clinical Methods Section

The food and feed chains – a possible strategy for the production of Certified Reference Materials (CRMs) in the area of mycotoxins?

Achim Boenke

European Commission, Standards, Measurement and Testing Programme (SM & T), 200 Rue de la Loi, B-1049 Brussels, Belgium

(Received 24 July 1996; revised version received 22 October 1996; accepted 22 October 1996)

Mycotoxin measurements at critical steps of the food and feed chains need to be accurate and precise to avoid high contents of mycotoxins in foods and feeds. This article aims to discuss the production of new Certified Reference Materials (CRMs) for measurements performed at such critical steps of the food and feed chains. It concludes that this production is limited in itself due to the following reasons: (a) only relative changes are of interest during initial and final product manufacturing; (b) development of a calibration tree based on a flexible calibration scheme (i.e. from field calibration via matrix CRMs, pure reference compounds to the mass) which ensures the traceability of analyses; (c) influences due to the degree of fungal infestation and invasion, mycotoxin contamination; (d) basic chemical, physical and toxicological (i.e. general routes of absorption, distribution and excretion of toxins in the body as well as organs involved in biotransformation of toxins) behaviour of mycotoxins; (e) raw material and seed production conditions, food and feed processing, storage; (f) consumer habits; (g) health requirements. These reasons form vital parts of the food and feed chains. It is therefore concluded that the food and feed chains provide a possible strategy for establishing the production of new CRMs in the area of mycotoxins. The production of the available CRMs already followed this strategy which is understood to form an integral part of the approach followed by the BCR (predessessor of the SM/T-Programme) for the production of environmental type matrix RMs certified for various contents of different elements. © 1997 Elsevier Science Ltd

INTRODUCTION

An industrial and service oriented society, which aims to continually enlarge the economy, requires the on-going interference of man in various cycles of the eco-system. In order to avoid undesirable side effects such as high contents of toxic compounds in food and feed, measurements at critical steps of the food and feed chains are necessary. Such measurements need to be accurate and precise. It is well recognised that Certified Reference Materials (CRMs) contribute to these needs in a very helpful manner. However, the production of new CRMs for measurements performed at such critical steps is limited in itself because at some of those steps only relative changes are of interest during initial and final product manufacturing (e.g. moisture, pH, fat, protein, measurements of contaminants, etc.) (Heiss, 1988). These relative changes need to be determined by a rapid procedure and/or sensors with a sufficient repeatability and reproducibility. Nevertheless, such rapid procedures and/or sensors require proper calibration and demand reference compounds as RMs certified for their purity to ensure correct calibration and results. The development of a calibration tree based on a flexible calibration scheme (i.e. from field calibration via matrix CRMs, pure reference compounds to the mass) (Marschal, 1994) could provide a useful link and a starting point for the preparation of these reference compound RMs certified for their purity.

In addition, the above limitation on the production of new CRMs is directly connected with the factors that influence the degree of fungal infestation and invasion (Charmley *et al.*, 1995) and mycotoxin contamination. Also, the basic chemical, physical and toxicological behaviour of mycotoxins such as reduction and oxidation reactions, chemisorption to solids, organic solvent/

water partition coefficients, and general routes of absorption, distribution and excretion of toxins in the body and organs involved in biotransformation of toxicants (Klaasen *et al.*, 1986; Smith *et al.*, 1994) are basically influencing the food and feed chains as well as the production of new CRMs. The selection of matrices for CRMs containing, for example, mycotoxins was and is, therefore, based on the raw material and seed production conditions [e.g. changes in pest control, Good Agricultural Practise (GAP), etc.], food and feed processing and storage, chemical, physical and toxicological behaviour as well as on consumers' habits and health requirements.

This article would like to show, by using mycotoxins as examples that the food and feed chains, including influences such as the degree of fungal infestation and invasion, that the basic chemical, physical and toxicological behaviour of mycotoxins, and a suggested calibration tree, form vital parts of a successful strategy for establishing the production of different types of CRMs by not neglecting the economical aspects, consumer habits, and demands of the analytical chemists.

Mycotoxins were chosen as examples because of their ubiquitous nature, toxicological significance, the importance of regulations for their control in foods and feeds (Commission of the European Communities, 1983; Van Egmond & Dekker, 1995), and the worldwide concern since countries in colder climatic areas import foods from areas where at least some mycotoxin levels are high (WHO, 1993). Further, experimental and epidemiological evidence suggest that certain mycotoxins are associated with different forms of human cancer (WHO, 1993; Fink-Gremmels, 1996).

The wheat, maize, peanut butter, full-cream milk powder and animal feed RMs certified for various mycotoxins contents [e.g. aflatoxins, ochratoxin A and deoxynivalenol (Boenke, 1995)] within the EC, BCR and the M&T Programme, which were the predessessors of the EC, Standards, Measurements and Testing Programme (SM/T), already followed this strategy.

In addition, it is understood that this strategy forms an integral part of the approach which was followed by the BCR for the production of environmental-type matrix RMs certified for various contents of different elements (Griepink *et al.*, 1991). This approach grouped these CRMs on the basis of environmental compartments such as water systems, biota, sediment, soil, sludge, waste, plants, animals, food, and man.

DISCUSSION

Influences of the food and feed chain on the production of new pure reference compounds and matrix CRMs

The food and feed chains is part of the world's ecological system and is known as a sequence of autotrophs, such as plants, which support themselves and ultimately all the heterotrophs, such as the herbivore (e.g. hare) the 'primary consumer' (e.g. fox), the 'secondary consumer' (e.g. lynx) and the 'top consumer' (i.e. human being). It determines how living things — plants, animals, fungi, human being — interact with each other and also with their environment in a circular manner. Now, as soon as one step in such a circular system is being influenced or, even stronger, modified due to whatever reasons, it gets out of control and specific actions must be undertaken to bring it back to its circular characteristic. Those specific actions are then highly successful and economically efficient when they are based on accurate and precise conclusions which themselves result from accurate and precise measurements.

Figure 1 provides a small insight into the food and feed chains by focussing on crop growing, harvesting and processing of raw materials, manufacturing of raw and final materials, and their introduction to consumers (i.e. animals and human beings). Links between planting of crops and pesticide treatment due to GAP, between material processing, manufacturing/refining and economical aspects such as import/export of goods are additionally included in this figure. Routes of adsorption, distribution and excretion of toxicants such as mycotoxins in the body are not taken into account in Fig. 1 but are discussed later in more detail.

In order to judge where matrix CRMs or reference compounds certified for their purity are required, one needs to look in Fig. 1 where (a) processing, manufacturing and/or refining of a material is the basic objective and where (b) the final quality of the material, the animal or human health is the basic objective. In the case of (a), measurements to determine relative changings are of importance. Consequently, in-process measurements and the availability of reference compound RMs certified for their purity rather than matrix CRMs to act as accurate calibrants are sufficient and provide a correct and cost-effective solution for a detailed monitoring and control of a processing step in the food and feed chains. However, in the case of (b), measurements to determine the absolute content and/or concentration of a certain product in a material are necessary. It is therefore clear that the availability of matrix CRMs together with reference compound RMs certified for their purity are demanded because such measurements form the basis for legal and economical decisions on the import/export of materials, on the habits and health of animals and human beings, i.e. the consumers. In other words, decisions are taken on which specific actions are required to either bring the circular system back to its circular characteristic or avoid the circular system running out of control.

It is indicated in Fig. 1 where reference compound RMs certified for their purity (see abbreviation R in Fig. 1) and matrix CRMs (see abbreviation C in Fig. 1) are required, based on the above discussion.

Influences of the calibration tree on the production of new pure reference compounds and matrix CRMs

Mycotoxin measurements need to be calibrated in order to ensure traceability to material standards. The heart of any calibration procedure is the need to identify the traceability of each measurement to the relevant basic units, i.e. the SI-units. However, it is unfortunately reality in chemical analyses that an optimal and general calibration procedure allowing direct traceability to the SI-units together with direct and general applicability does not exist at the moment.

In addition, traceability is directly linked to the uncertainty of measurements. This uncertainty can be divided into (a) the uncertainty of the calibrant, (b) the uncertainty with respect to matrix effects and (c) the uncertainty with respect to the comparison of analytical procedures: (a) includes the uncertainty of the certified value of the CRM (where a CRM is used for calibration purposes), the purity of the reference compound and the uncertainty of mass as well as volume of a calibrant solution.

As a consequence of the above, a calibration tree based on a flexible calibration scheme was proposed by Marschal (Marschal, 1994) in order to provide a solution to direct and general traceability in chemical analysis. This calibration tree is based on the mass and on reference compounds whose purities were carefully assessed and continues via primary calibration mixtures and matrix CRMs, in-house test- and/or control-materials up to final field calibration of chemical analyses (Marschal, 1994).

Now, looking again at Fig. 1, and taking the proposed calibration tree into account, it is, necessary that wherever a matrix CRM is demanded, a reference compound with certified purity is also to be introduced in order to ensure a proper evaluation of the measurement uncertainty and traceability. Only this approach will allow that repeatable, reproducible and accurate measurements of the abolute contents and/or concentrations of any compounds are being applied. This provides the basis for correct and cost-effective decisions in order to avoid trade disputes, damage of the health of animals and the human being, and to determine that the circular system is either running out of control or is being brought back to normal functioning.

Influence of fungal infestation, invasion and the chemical and physical behaviour of mycotoxins on the production of new pure reference compounds and matrix CRMs

The occurrence of mycotoxins within the food and feed chains can be due to different routes of contamination which are of either a direct or indirect nature (Frisvad &

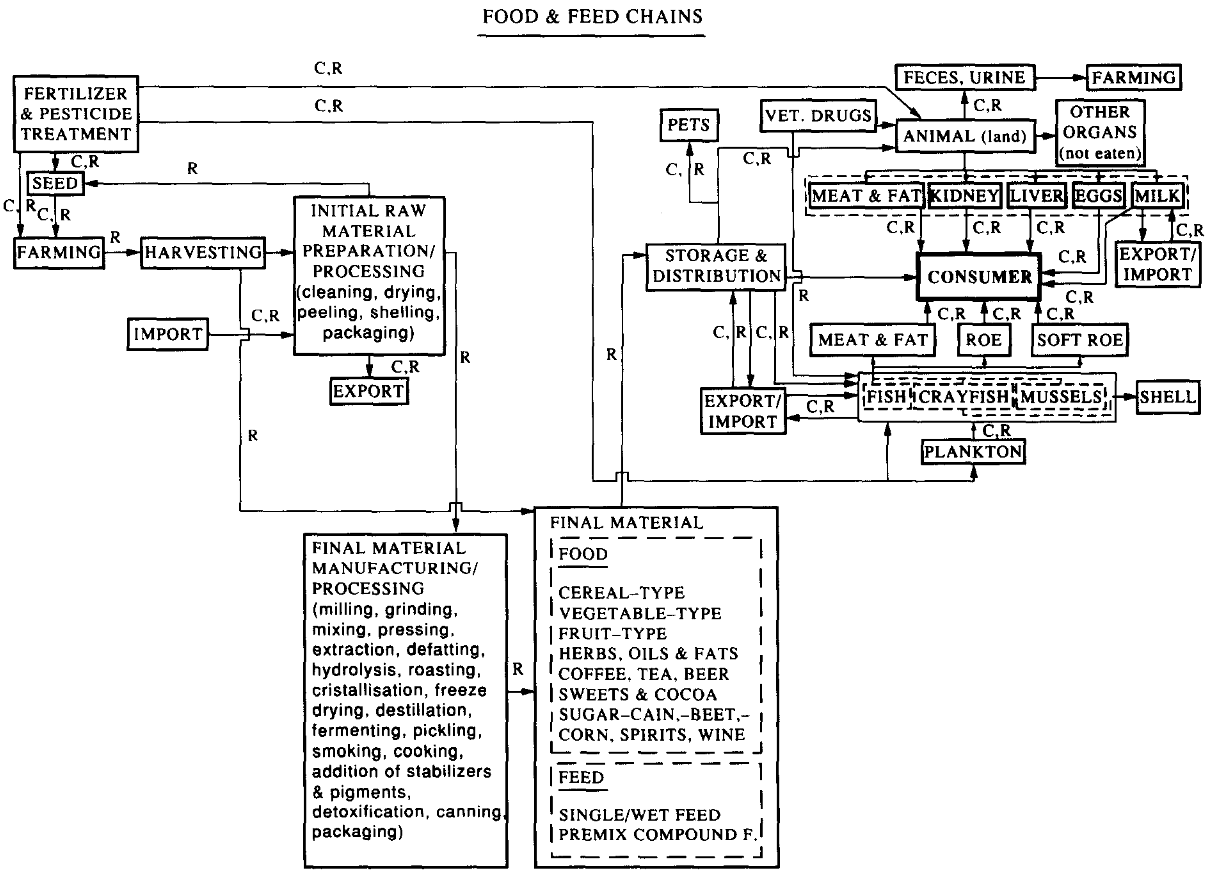

Fig. 1. A small insight in the food and feed chains and indications where reference compound RMs certified for their purity as well as matrix CRMs are needed (C, matrix CRMs; R, reference compound RMs certified for their purity).

Samson, 1992). Consequently, either the food and feed is directly infected by the fungus or an already contaminated food or feed ingredient is contaminating other food and feeding stuffs. The degree of mycotoxin formation on crops is dependent on the weather conditions, the susceptibility of the crop to fungal invasion, temperature, moisture content, mechanical injury, damage caused by animals, harvesting, milling and storage conditions (e.g. moisture content and oxygen concentration, etc.) (Charmley *et al.*, 1995; Smith *et al.*, 1994). These influencing conditions causing the occurrence of mycotoxins due to the existence and effect of the following basic chemical and physical processes grouped by different phases are well known from environmental contaminants such as polycyclic aromatic hydrocarbons (PAHs) or polychlorinated biphenyls (PCBs) (Hites & Eisenreich, 1987):

1. Chemical and physical processes in gaseous phases:
   - adsorption/desorption;
   - coagulation/wash out;
   - photolysis/oxidation;
   - gas exchange;
   - vaporisation (Henry's law);
   - formation of aerosols.
2. Chemical and physical processes in aqueous phases:
   - hydrolysis/photolysis;
   - conjugation/biotransformation;
   - adsorption/desorption;
   - acid/base reactions;
   - ligand exchange.
3. Chemical and physical processes in solid phases:
   - adsorption/desorption;
   - coagulation/precipitation;
   - ligand exchange;
   - red-ox-reactions;
   - conjugation/biotransformation.

These processes are either used in combination with each other or separately for the detoxification of commodities contaminated with mycotoxins (Charmley *et al.*, 1995; Phillips *et al.*, 1995).

Knowledge of the chemical and physical behaviour of different mycotoxins based on the above processes and the availability of, for example, chemisorption indices and other physicochemical data for different mycotoxins will consequently limit later on the number of different matrix RMs to be produced.

In addition, a number of different mycotoxins are produced by a limited quantity of fungi species, while others are produced by a relatively large range of fungi species from several genera. Most of the *ca* 300 mycotoxins which have been identified up to now, have been demonstrated under laboratory conditions, whereas only a relative small number of ~20 have been shown so far to occur naturally in food and feed at substantial levels and frequency to be of concern to food safety (Smith *et al.*, 1994).

This above knowledge on physicochemical data and the natural occurrence of mycotoxins in food and feed at substantial levels and frequency to be of concern also reduce the number of different mycotoxins for which matrix RMs need to be certified due to possible similarities.

Consequently, mould-damaged foodstuffs (e.g. agricultural products such as cereals, oilseeds (groundnuts), fruits and vegetables, consumer foods and compounded animal feeds), mycotoxin residues in animal tissues and animal products (e.g. milk, dairy products, meat), mould-ripened foods (e.g. cheeses, fermented meat products, other fermentations), and fermented products are potential target matrices for the occurrence of mycotoxins (Smith *et al.*, 1994) and substantial candidates for matrix CRMs.

Additionally, the indications of required matrix CRMs and reference compound RMs with certified purity in Fig. 1 do not need to be increased. Based on possible similarities of the chemical and physical behaviour of different mycotoxins, many more matrix RMs certified for the contents of various mycotoxins may therefore not be necessary.

Influence of the toxicological behaviour of mycotoxins on the production of new pure reference compounds and matrix CRMs

The toxicity of chemicals, in general, is dependent on their content present in the target organ (Klaasen *et al.*, 1986). This content depends on the disposition of the toxicant which is its absorption, distribution, biotransformation, and excretion. These factors are shown in Fig. 2.

Little or no toxicity will result if the toxicant content is low in the target organ, whereas if high contents are present, toxicity will result. If the toxicant is more lipophilic, it can be absorbed through the skin, across the lungs, or through the gastrointestinal tract. They can later be accumulated unless the body manages to transform them into more water-soluble transformation products which can be excreted via urine, bile, feces, expired air, and perspiration. Toxicants which are more water-soluble by their very nature can be excreted directly via the explained routes. The following organs, tissues and fluids are therefore of basic toxicological importance and are relevant matrices for depression of toxicants in general: Liver, lung, kidney, brain, soft tissue, fat, blood and lymph, urine, and feces. Additionally, blood is the relevant body fluid for transport of toxicants and other compounds in general (Klaasen *et al.*, 1986).

In the special case of mycotoxins such as aflatoxins and ochratoxins, in particular, metabolic processing in liver cells and non-hepatic tissues (Liu & Massey, 1992) is essential for their mutagenicity and geno-

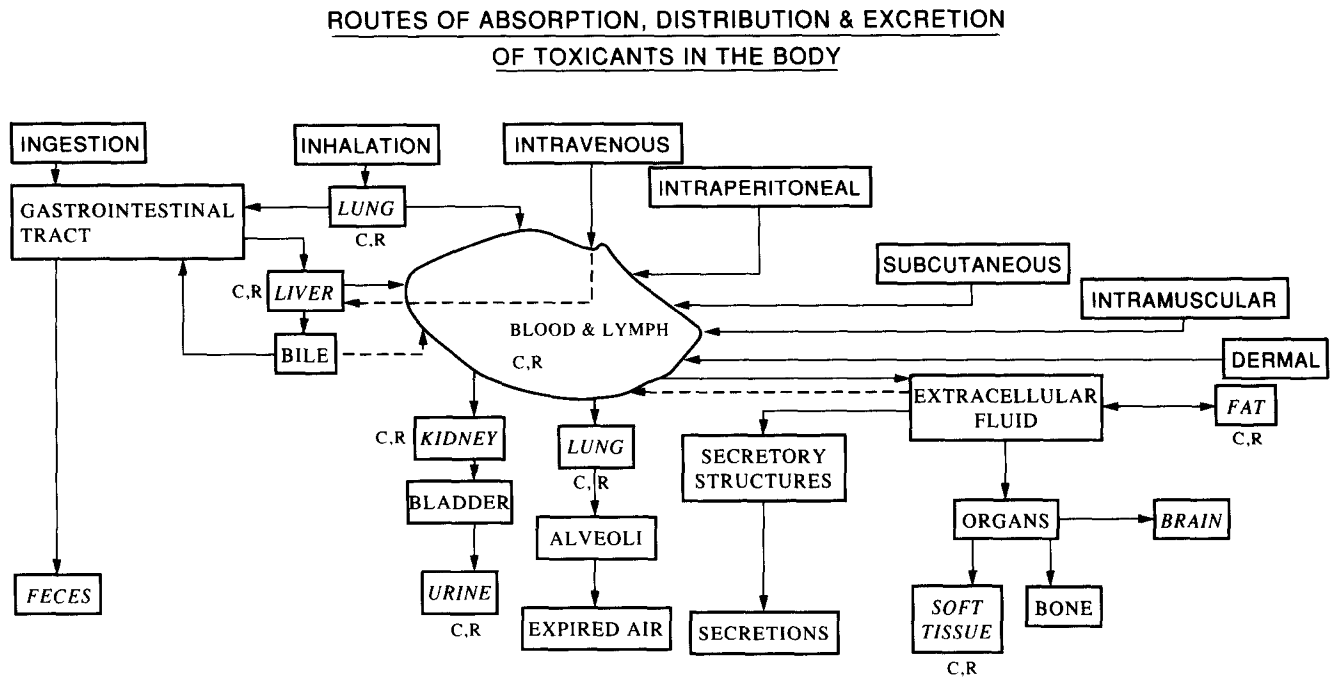

Fig. 2. Routes of absorption, distribution, and excretion of toxicants in the body as well as indications where reference compound RMs certified for their purity as well as matrix CRMs are needed. (C: matrix CRMs, R: reference compound RMs certified for their purity).

toxicity. Biotransformation processes as generally outlined above play a key role in mycotoxin carcinogenicity (Klaasen *et al.*, 1986; Fink-Gremmels, 1996).

Based on the above information, it can be proposed that: (a) blood is a candidate fluid for continuous monitoring purposes which therefore requires measurements of relative and absolute changes of mycotoxin concentrations, and (b) liver, kidney, soft tissue, and fat are candidate organs and/or tissues for the measurement of absolute mycotoxin contents. These different characteristics of organs and tissues with respect to basic measurement requirements strongly suggest concentrating the matrix CRM production on the matrices specified under (b). This basic proposal has to be finally reviewed in the light of the following general aspects relevant for a hypothetical mycotoxicosis: genetic aspects (e.g. species, breed and strain), physiological aspects (e.g. age, hormones, nutrition, intestinal miroflora, infection and parasitism) and environmental aspects (e.g. climatic conditions, chemicals, husbandry and management) (Smith *et al.*, 1994). It is understood that these different aspects will definitely lead to different target organs for animals than for human beings. This will additionally allow a further selection combined with a reduction of the production of new matrix CRMs.

As a consequence of the above proposal, it is indicated in Fig. 2 where reference compound RMs certified for their purity (see abbreviation R) and matrix CRMs (see abbreviation C) would be required.

Influence of economical and legal aspects and consumer habits on the production of new pure reference compounds and matrix CRMs

Economical and legal aspects, consumer habits and measurement aspects are all interconnected and can therefore not be seen separately. In other words, in order to protect the consumer (here: animals and human beings) European legislation needs to take into

Table 1. European Union agricultural trade and self-sufficiency, 1990–1991 (European Commission, Directorate General for Economic and Financial Affairs 1994)

Product	Self-sufficiency %	Net exports(+)/imports(−) (1000 tonnes)	1990 USD Mio.
Cereals	120	24981	4519
Fresh fruit	85	−4417	−9054
Fresh vegetables	106	1861	—
Meat	120	1218.4	236
All agricultural and food products	—	—	−26366[a]

[a]In addition to deficits shown for fruit and vegetables, the major contributions to this overall net import figure comes from coffee, cocoa, tea and spices (-4174 Mio.USD) and animal feed (-4211 Mio.USD) amongst other goods.

account the consumer habits by covering as many different food and feed products as is reasonably possible in order to fix acceptable maximum values (e.g. for aflatoxins) which are measurable but also to avoid trade barriers which hinder the freedom of movement of products and lead to distortion of competition (Verardi & De Froidmont-Görtz, 1995; Verardi & Rosner, 1995).

To allow identification of the food and feedstuffs which are important for consumer habits, economic, legal and measurement aspects, it is suggested here to look at the status of agricultural production in the European Union (EU) and international trade based on the European point of view. This shows that 56% of the EU's total area is used for agricultural activities and that agriculture forms a significant part of the overall economy of the EU. Further evidence for that may be the fact that total expenditure on agriculture occupies about 60% of the total European Community (EC) budget. In addition to that there are much smaller but still significant proportions of national government expenditures. Export of EU farm products reaches a level of 8.5% compared to the world export level of 12%. The Community is the second largest agricultural exporter after the US, with trade based on cereals, particularly wheat, milk powder, cheese and meat amongst other goods (see Table 1). The following five product groups are relevant as they contribute largely to EU agricultural export: cereals, fresh vegetables, milk, beef and veal, and pigmeat, each accounting for over 10% of total EU agricultural output. However, about a third of grown cereals and nearly all grass (roughage) is fed

Table 2. Overview on the different CRMs and RMs for mycotoxin analysis as well as their corresponding certified values or current status

CRM no. or RM no.	Matrix	Mycotoxin	Certified value (mass fraction or mass conc.)	Uncertainty (mass fraction or mass conc.)
CRM 385	Peanut butter	Aflatoxin B_1	7.0 $\mu g\ kg^{-1}$[d]	$\pm 0.8\ \mu g\ kg^{-1}$
		Aflatoxin B_2	1.1 $\mu g\ kg^{-1}$[d]	$\pm 0.2\ \mu g\ kg^{-1}$
		Aflatoxin G_1	1.7 $\mu g\ kg^{-1}$[d]	$\pm 0.3\ \mu g\ kg^{-1}$
		Aflatoxin G_2	0.3 $\mu g\ kg^{-1}$[d]	$\pm 0.2\ \mu g\ kg^{-1}$
		Total aflatoxins	10.1 $\mu g\ kg^{-1}$[b]	$\pm 1.5\ \mu g\ kg^{-1}$[c]
CRM 401	Peanut butter	Aflatoxin B_1	$<0.2\ \mu g\ kg^{-1}$[d]	—
		Aflatoxin B_2	$<0.2\ \mu g\ kg^{-1}$[d]	—
		Aflatoxin G_1	$<0.3\ \mu g\ kg^{-1}$[d]	—
		Aflatoxin G_2	$<0.2\ \mu g\ kg^{-1}$[d]	—
		Total aflatoxins	$<0.9\ \mu g\ kg^{-1}$[d]	—
CRM 282		Aflatoxin M_1	$<0.05\ \mu g\ kg^{-1}$[a]	—
CRM 283	Full-cream		0.09 $\mu g\ kg^{-1}$[d]	$+0.04\ \mu g\ kg^{-1}$ $-0.02\ \mu g\ kg^{-1}$
	Milk powder			
CRM 285			0.76 $\mu g\ kg^{-1}$[d]	$\pm 0.05\ \mu g\ kg^{-1}$
CRM 377	Maize flour	Deoxynivalenol (DON)	<0.05 mg kg^{-1}[d]	—
CRM 378			0.43 mg kg^{-1}[d]	± 0.04 mg kg^{-1}
CRM 379	Wheat flour		0.67 mg kg^{-1}[d]	± 0.02 mg kg^{-1}
CRM 396			<0.05 mg kg^{-1}[d]	—
CRM 262	Defatted	Aflatoxin B_1	$<3\ \mu g\ kg^{-1}$[d]	—
CRM 263	Peanut meal		43.3 $\mu g\ kg^{-1}$[d]	$\pm 2.8\ \mu g\ kg^{-1}$
CRM 264			206 $\mu g\ kg^{-1}$[d]	$\pm 13\ \mu g\ kg^{-1}$
CRM 375	Compound feed		$<1\ \mu g\ kg^{-1}$[d]	—
CRM 376			9.3 $\mu g\ kg^{-1}$[d]	$\pm 0.5\ \mu g\ kg^{-1}$
CRM 471	Wheat flour	Ochratoxin A	$<0.6\ \mu g\ kg^{-1}$[e]	—
CRM 472			8.2 $\mu g\ kg^{-1}$[d]	$\pm 1.0\ \mu g\ kg^{-1}$[f]
Not yet	Pig kidney		First intercomparison completed Second intercomparion planned	
RM 423	Chloroform	Aflatoxin M_1	Study planned	
Not yet	Maize and/or maize products	Fumonisins	Two intercomparisons completed	
Not yet	Cereal type materials	Trichothecenes (nivalenol, HT-2, T-2)	Intercomparisons and feasibility studies started	

[a]probable content is in the range 0.01–0.02 $\mu g\ kg^{-1}$; -, not given due to the certification of a less than value.
[b]Calculated by linear addition of the mean of means obtained for the four aflatoxins.
[c]Calculated by quadratic addition of the variances obtained for the four individual aflatoxins and additional application of a safety factor.
[d]Includes allowance for incomplete recovery during certification measurements.
[e]This is a finite value not corrected for recovery.
[f]This uncertainty is taken as the half-width of the 95% confidence interval of the mean together with applied recovery correction and allowance is included for the uncertainty of the purity of the ochratoxin A calibrant.

directly to animals, and does not appear as output [European Commission (1994), Directorate General for Economic and Financial Affairs, 1994].

Based on the above information on the status of European agricultural production, on European trade of agricultural products, on the reduced pesticide application based on GAP, on the increased utilisation of biological farming, and on the consumers' preference for naturally or biologically growing foodstuffs, it is clear that cereals, vegetables, milk and milk products, meat and meat products are relevant candidate matrices for matrix CRMs. Taking into account the demand of analytical chemists to make matrix CRMs available for each analytical problem and comparing it to the economic and legal aspects and the consumer habits based on the above knowledge, it seems possible, on the one hand, that the demand of the analytical chemists for matrix CRMs for all analytical problems could therefore be reconciled and, on the other hand, it confirms the indication of the reduced production of new matrix CRMs in Figs 1 and 2.

CONCLUSIONS

The question: 'Are the food and feed chains a possible strategy for the production of certified reference materials (CRMs) in the area of mycotoxins?' can be answered with 'Yes' because all the discussed influences form vital parts of the food and feed chains. In fact, all those influences provide a further insight into the food and feed chains as they explain the occurrence of mycotoxins at various stages of it and additionally underline that it is a circular system.

Due to the positive answer to this strategy, the second question to be answered is: 'Do those RMs already certified for their mycotoxin contents fit into this strategy?' In this case also the answer is positive because the matrices which were chosen are fully in line with the proposed priority matrices and include full cream milk powder, cereals such as wheat and maize, peanut butter as a type of food product which belongs to a class where mycotoxin contamination is quite common, and two different major feedstuffs (see Table 2). The chosen mycotoxins, e.g. aflatoxins (B_1, B_2, G_1, G_2, M_1 and total), deoxynivalenol (DON), and ochratoxin A (Boenke, 1995), represent those which are economically and legally important as well as characteristic for this class of mycotoxins because of their chemical, physical and toxicological behaviour.

In addition, this strategy is understood to form an integral part of the approach which was followed by the BCR for the production of environmental-type matrix RMs certified for various contents of different elements (Griepink *et al.*, 1991). This approach grouped the produced CRMs on the basis of environmental compartments such as water systems, biota, sediment, soil, sludge, waste, plants, animals, food and man.

However, when applying this strategy, it is concluded that the availability of mycotoxins as reference compounds certified for their organic and inorganic purity is an area where more activities and effort are required as they are an essential milestone to ensure traceability. Additional reasons for the production of reference compound RMs certified for their purity are the introduction of the proposed calibration tree, the limited availability of mycotoxin calibrants combined with partly insufficient purity certificates, and of course their requirements for the calibration of in-process measurement systems such as chemical and/or biological sensors for detoxification processes, etc.

When following this strategy further, the area of mycotoxin degradation and biotransformation products is also not yet covered. This area will probably become even more important in the future as final material manufacturing and processing such as fermentation and detoxification require in-process measurements in order to demonstrate the absence of such toxins. The production of reference compound RMs with certified purity of relevant mycotoxins such as aflatoxin and ochratoxin A degradation and biotransformation products seems to be the logical consequence which would close the last gap in this strategy for these specific mycotoxins.

REFERENCES

Boenke, A. (1995). BCR-and M & T-activities in the area of mycotoxin analysis in food and feedstuffs. *Natural Toxins*, **3**, 243–247.

Charmley, L. L., Trenholm, H. L., Prelusky, D. B. & Rosenberg, A. (1995). Economic losses and decontamination. *Natural Toxins*, **3**, 199–203.

Commission of the European Communities (1983). Third Commission directive of 28 July 1983 amending the annex to Council directive 74/63/EEC on the fixing of maximum permitted levels for undesirable substances and products in feeding stuffs. *Official Journal of the European Communities*, **L222**, 31–32.

European Commission (1994). Directorate General for Economic and Financial Affairs. European economy — reports and studies, EC Agricultural Policy for the 21st Century, Vol. No. 4, ISSN 0379-0991, printed in Belgium, pp. 23–26 and 42–75.

Fink-Gremmels, J. (1996). Mycotoxins: mechnism of action at the molecular level, oral presentation at the IX. IUPAC Symposium on mycotoxins and phycotoxins, 27–31 May, Rome, Italy.

Frisvad, J. C. & Samson, R. A. (1992). Filamentous fungi in foods and feeds: ecology, spoilage and mycotoxin production. In *Foods and Feeds*, Vol. 3, eds D. K. Arora, K. G. Mukerji & E. H. Marth, *Handbook of Applied Mycology*. Marcel Dekker Inc., New York, pp. 31–68.

Griepink, B., Maier, E. A., Quevauviller, P. & Muntau, H. (1991). Certified reference materials for the quality control of analysis in the environment. *Fresenius J. Anal. Chem.*, **339**, 599–603.

Heiss, R., ed. (1988). *Lebensmitteltechnologie*, 2nd Edition, Springer Verlag, Heidelberg.

Hites, R. A. & Eisenreich, S. J. (eds) (1987). Sources and fates of aquatic pollutants, advances in chemistry, series 216, American Chemical Society: Washington DC.

Klaasen, C. D., Amdur, M. O. & Doull, J. (eds) (1986). Toxicology — The basic science of poisons, 3rd edition, Macmillan Publishing Company, New York.

Liu, L. & Massey, T. E. (1992). Bioactivation of aflatoxin B_1 by lipoxigenases, prostaglandin-H synthase and cytochrome P450 monooxygenase in guinea-pig tissues. *Carcinogenesis*, **13**, 533–539.

Marschal, A. (1994). Traceability and calibration in analytical chemistry and materials testing In *Proceedings of the 2nd EUROLAB Symposium*, 25–27 April, Florence, Italy.

Phillips, T. D., Sarr, A. B. & Grant, P. G. (1995). Selective chemisorption and detoxification of aflatoxins by phyllosilicate Clay. *Natural Toxins*, **3**, 204–213.

Smith, J. E., Solomons, G. L., Lewis, C. W. & Anderson J. G. (1994). Mycotoxins in human nutrition and health. EUR-Report No. EUR16048EN, Brussels, Belgium.

Van Egmond, H. P. & Dekker, W. H. (1995). Worldwide regulation for mycotoxins in 1994. *Natural Toxins*, **3**, 332–336.

Verardi, G. & De Froidmont-Görtz, I. (1995). Overview on Community legislation in the field of official control of mycotoxins in feedstuffs. *Natural Toxins*, **3**, 248–256.

Verardi, G. & Rosner, H. (1995). Some reflections on establishing a Community legislation on mycotoxins. *Natural Toxins*, **3**, 337–340.

WHO (1993). World Health Organization — International Agency for Research on Cancer. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 56, Lyon.