Current situation on regulations for mycotoxins

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Summary

An international inquiry was held in 2002/2003 on worldwide limits and regulations for mycotoxins, under contract with the Food and Agriculture Organization. The survey has shown that, by the end of 2003, more than 100 countries (covering > 90 % of the world’s inhabitants) had specific regulations or detailed guidelines for mycotoxins. Mycotoxins for which currently (proposed) limits and regulations exist include the naturally occurring aflatoxins, aflatoxin M₁, agaric acid, deoxynivalenol, diacetoxy-scirpenol, the fumonisins B₁, B₂ and B₃, HT-2 toxin, ochratoxin A, patulin, phomopsins, sterigmatocystin, T-2 toxin and zearalenone.

Insight will be provided in the mycotoxin regulatory situations in Africa, Asia/Oceania, Europe, Latin America and North America. Overviews will be given about limits that exist for the most regulated mycotoxins. Comparing the situations in 1995 (year of the last worldwide inquiry) and 2003 it appears that, at present, more mycotoxins are regulated in more commodities and products, whereas tolerance limits generally remain the same or tend to decrease. Regulations have become more diverse with newer requirements regarding official procedures for sampling and analytical methodology. At the same time, several regulations have been harmonised between countries belonging to economic communities, or they are in some stage of harmonisation. Nevertheless the regulatory requirements remain substantially different across many countries.

Key words: mycotoxin, limit, regulation, guideline, tolerance

Introduction

In today’s changing world, safety and security have remained basic human needs, and securing safe food has been a major focus of (inter-)national government action over the last years. Both microbiological and chemical hazards are of concern. Of the
chemical hazards, mycotoxins currently form a major food safety issue. The knowledge that mycotoxins can have serious effects on man and animals, have led many countries to establish regulations on mycotoxins in food and feed in the last decades to safeguard the health of humans and the food chain. The setting of mycotoxin regulations is a complex activity, which involves many factors and parties. The first limits for mycotoxins were set in the late 1960s for the aflatoxins. To date, there are more than 100 countries in the world which have specific limits for mycotoxins in foodstuffs and feedstuffs, and the number is still growing.

Papers about limits and regulations for mycotoxins and their rationales have been published several times. The most recent comprehensive review was based on an international inquiry carried out in 1994/1995 for the Food and Agriculture Organization of the United Nations (FAO). It was published as FAO Food and Nutrition Paper 64. Since this publication appeared many new limits and regulations for mycotoxins have come into force or are in development. This had made it necessary to produce an update of this document. A relevant international inquiry was carried out in 2002/2003. A detailed and extensive review of the current worldwide situation on mycotoxin regulations in food and feed will be published as an FAO Food and Nutrition Paper in 2004. In the article below, the developments in the mycotoxin regulatory area will be briefly discussed. Some observations for the various world regions will be presented, and information will be provided on the legal limits established worldwide for the most significant mycotoxins in food and feed. Finally, some conclusions and recommendations will be made.

Factors influencing the constitution of mycotoxin regulations

Several factors, both of a scientific and socio-economic nature, may influence the establishment of mycotoxin limits and regulations. These include:

- the availability of toxicological data of mycotoxins
- the availability of data on the occurrence of mycotoxins in various commodities
- the knowledge of the distribution of mycotoxin concentrations within a lot, in relation to sampling
- the availability of analytical methods
- the legislation in other countries with which trade contacts exist (import and export)
- the need for sufficient food supply

The first two factors provide the necessary information for hazard assessment and exposure assessment respectively, the main ingredients for risk assessment. Risk assessment is the primary scientific basis for the establishment of regulations.

Hazard assessment Regulations are primarily made on the basis of known toxic effects. For the mycotoxins currently considered most significant (aflatoxins, ochratoxin
A, patulin, fumonisins, zearalenone and some trichothecenes including deoxynivalenol), the Joint Expert Committee on Food Additives (JECFA), a scientific advisory body of the World Health Organization (WHO) and the FAO, has evaluated their hazard in several sessions. In February 2001, a special JECFA session was completely devoted to mycotoxins$^{11,12}$. The mycotoxins evaluated or re-evaluated at this 56th JECFA meeting included fumonisins B₁, B₂ and B₃, ochratoxin A, deoxynivalenol, T-2 and HT-2 toxins, and aflatoxin M₁. The report addressed several concerns about each mycotoxin: explanation of the mycotoxin, metabolism, toxicological studies, and final evaluation. Along with the mycotoxin evaluations, the committee put forth general considerations on sampling, analytical methods, associated intake issues and control.

**Exposure assessment** In addition to information about toxicity, exposure assessment is another main ingredient of the risk assessment. Reliable data on the occurrence of mycotoxins in various commodities and data on food intake are needed to prepare exposure assessment. The quantitative evaluation of the likely intake of mycotoxins is quite difficult. In its 56th meeting, JECFA stressed the importance of the use of validated analytical methods and the application of analytical quality assurance (see also the section on Methods of Analysis) to ensure that the results of surveys provide a reliable assessment of intake$^{12}$. In most of the JECFA reviews of mycotoxins, the analytical data on the levels of contamination were often inadequate from developed countries and non-existent for developing countries. Because most mycotoxin contamination is heterogeneously distributed, sampling is another important consideration in the development of information on the levels of contamination$^{13}$.  

**Sampling procedures** The distribution of the concentration of mycotoxins in products is an important factor to be considered in establishing regulatory sampling criteria. The distribution can be very heterogeneous, as is the case with aflatoxins in peanuts. The number of contaminated peanut kernels in a lot is usually very low, but the contamination level within a kernel can be very high. If insufficient care is taken for representative sampling, the mycotoxin concentration in an inspected lot may therefore easily be wrongly estimated. Also, consumption of peanuts could lead to an accidental high single dose of aflatoxins, rather than a chronic intake at a relatively low level. A similar situation could occur with pistachio nuts and figs. The risk to both consumer and producer must be considered when establishing sampling criteria for products in which mycotoxins are heterogeneously distributed. The design of sampling procedures has been an international concern for a long time, for instance FAO$^{14}$ and Codex Alimentarius$^{15}$ have been active in this area. Discussions in working groups of these international organisations are continuously carried out to find a harmonized international approach.

**Methods of analysis** Legislation calls for methods of control. Reliable analytical methods will have to be available to make enforcement of the regulations possible. In addition to reliability, simplicity is desired, as it will influence the amount of data that
will be generated and the practicality of the ultimate measures taken. The reliability of analysis data can be improved through use of validated methods of analysis (e.g. methods of AOAC International\textsuperscript{(16)}, certified reference materials (e.g. supplied by the European Commission’s Joint Research Centre/Institute for Reference Materials and Measurements (JRC/IRMM, see http://www.irmm.jrc.be)), and participation in proficiency tests (e.g. FAPAS\textsuperscript{(17)}). Good analytical methodology and analytical quality assurance are prerequisites for adequate food law enforcement. Also important, especially in free trade areas, is how enforcement bodies handle an issue as measurement uncertainty. Within the European Union and the European Free Trade Area the FLEP (Food Law Enforcement Practitioners) Working Party "Mycotoxins" is currently dealing with this issue\textsuperscript{18}.

**Trade contacts** Preferably, regulations should be brought into harmony with those in force in other countries with which trade contacts exist. In fact, this approach has been applied both in the areas of the EU (European Union), MERCOSUR (Mercado Comun del Sur) and Australia/New Zealand, where now harmonized regulations for some mycotoxins exist. Strict regulative actions may lead importing countries to ban or limit the import of commodities, such as certain food grains, which can cause difficulties for exporting countries in finding or maintaining markets for their products. For example, the stringent regulations for aflatoxin B\textsubscript{1} in animal feedstuffs in the EU\textsuperscript{19}, led European animal feed manufacturers to switch from groundnut meal to other protein sources to include in feeds; this had an impact on the export of groundnut meal of some developing countries\textsuperscript{20}. The distortion of the market caused by regulations in importing countries may lead to export of the less contaminated foods and feeds leaving those inferior foods and feeds for the local market. Some countries apply different limits for aflatoxins in certain products depending on the destination.

**Food supply** The regulatory philosophy should not jeopardize the availability of some basic commodities at reasonable prices. Especially in the developing countries, where food supplies are already limited, drastic legal measures may lead to lack of food and to excessive prices. At the time of writing, for instance, the dramatic food security situation in parts of Africa has led to measures that prioritise food sufficiency above food safety. Mycotoxins are an important problem as evidenced by occasional outbreaks of human mycotoxicoses and the role of aflatoxins in liver cancer in western Africa and fumonisins in oesophageal cancer in South Africa\textsuperscript{21}.

**Synopsis** Weighing the various factors at the interface of science, food security and regulations is not a trivial activity and common sense is a major factor for reaching a decision. Public health officials are confronted with a complex problem: mycotoxins, and particularly the carcinogenic mycotoxins, should be excluded from food as much as possible. Since the substances are present in foods as natural contaminants, however, human exposure cannot be completely prevented, and exposure of the population to
some level of mycotoxins has to be tolerated. Despite the dilemmas, mycotoxin regulations have been established in the past decades in many countries, and newer regulations are still being drafted.

**Mycotoxin regulations in food and feed in 2003 and current developments**

In 2002/2003 an international inquiry was carried out, under contract with FAO, among the Agricultural Services of the Dutch Embassies around the world, with the request to gather up-to-date information from the local authorities on the situation regarding mycotoxin regulations, in as many countries of the world as possible. Where this procedure did not lead to the desired information, personal contacts were used. By the end of 2003, data were received from approx. 90 countries. Together with information gathered in previous inquiries, detailed information became available about the existence or absence of specific mycotoxin limits and regulations in food and feed, in approximately 120 countries. All data were interpreted to the best of our knowledge and tabulated. At the time of writing, the processing and correction stages were not finalized yet and a few more months were envisaged, before the whole exercise could be completed. Publication of the survey is expected in 2004 as an FAO Food and Nutrition Paper. Some provisional conclusions with respect to current limits and regulations for the most significant mycotoxins can be made from first impressions of the updated information. They are given hereafter.

![Fig. 1](image_url)

**Fig. 1. Countries known to have regulations (gray), unknown whether or not they have regulations (white) and known to have no specific regulations (black) for mycotoxins in foodstuffs and feedstuffs.**

**General observations** On a worldwide basis, at least 100 countries had mycotoxin regulations for food and/or feed in 2003 (see Fig. 1), an increase of approx. 30% as compared to 1995. In fact all countries with mycotoxin regulations at least have regulatory limits for aflatoxin B1 or the sum of aflatoxins B1, B2, G1 and G2 in foods and/or feeds, a situation that was also observed in 1995. For several other mycotoxins
specific regulations exist as well (i.e. aflatoxin M₁; the trichothecenes deoxynivalenol, diacetoxyscirpenol, T-2 toxin and HT-2 toxin; the fumonisins B₁, B₂, and B₃; agaric acid; the ergot alkaloids; ochratoxin A; patulin; phomopsins; sterigmatocystin; zearalenone). The number of countries regulating mycotoxins have significantly increased over the years. Comparing the situations in 1995 and 2003, it appears that in 2003 more mycotoxins are regulated in more commodities and products, whereas tolerance limits generally remain the same or tend to decrease. Regulations have become more diverse with newer requirements regarding official procedures for sampling and analytical methodology. At the same time, several regulations have been harmonised between countries belonging to economic communities (EU, MERCOSUR, Australia/New Zealand).

Specific observations per region On a regional basis the following provisional observations can be made:

In Africa, 14 countries are known to have specific mycotoxin regulations in 2003. Most of the existing mycotoxin regulations in Africa concern the aflatoxins. For the majority of the African countries specific mycotoxin regulations (probably) do not exist. The mycotoxin issue in Africa needs to be viewed, however, in the overall context of local food safety, health and agricultural issues. The establishment of mycotoxin regulations will have limited effects in terms of health protection as many farmers grow agricultural produce for their own consumption.

Asia and Oceania cover a very large part of the globe, with most countries laying in the tropics and subtropics, so it is expected that most mycotoxin problems be caused by fungi, which grow at higher temperatures. An exception is New Zealand, which has a temperate to cool climate and separate mycotoxin issues from Asia and northern Australia. In the whole region, 26 countries are known to have specific mycotoxin regulation. Regulations for total aflatoxins dominate in food, whereas in feed regulations for aflatoxin B₃ dominate. Australia and New Zealand have harmonised their mycotoxin regulations, which include limits for the "exotic" mycotoxins agaric acid and phomopsins.

In Europe, 39 countries are known to have specific mycotoxin regulations in 2003. Europe has extensive and detailed regulations for mycotoxins in food. In the EU, now consisting of 15 countries but soon expanded with another 10 countries, harmonised regulations or guidelines exist for several of the mycotoxins. They currently concern aflatoxins in various foodstuffs, aflatoxin M₁ in milk, ochratoxin A in cereals and dried vine fruits, patulin in apple products, deoxynivalenol in cereals and cereal products and aflatoxin B₁ in various feedstuffs. It is of interest to note that many of the EU candidate countries have mycotoxin regulations, that are often more detailed than those currently in force in the EU. In the coming time (2004 and following years) a significant further expansion of EU-harmonised mycotoxin regulations is expected both for foods and feeds.
The major Latin American agricultural crops (maize, wheat, coffee, cotton, soybeans, barley, sunflower, groundnuts and tree nuts, cocoa and dairy products) are highly susceptible to fungal contamination and mycotoxin production, and 19 Latin-American countries are known to have in force specific mycotoxin regulations for food and feed. The aflatoxin regulations in food are often set for the sum of the aflatoxins B₁, B₂, G₁ and G₂. Harmonised regulations for aflatoxins exist in MERCOSUR member states (a trading block consisting of Argentina, Brazil, Paraguay and Uruguay). Incidentally other countries indicate they follow MERCOSUR regulations.

North America consists only of two countries, the USA and Canada. Both have mycotoxin regulations since many years, and advanced techniques exist for sampling and analysis. In both countries limits for aflatoxins are set for the sum of the aflatoxins B₁, B₂, G₁ and G₂. In addition to limits for mycotoxins, Canada has also established tolerances for the percentage Fusarium-damaged kernels in wheat and for the percentage ergot in various crops for animal feed. In the USA detailed tolerance levels exist for the sum of the fumonisins B₁, B₂ and B₃ in a wide variety of maize products, which is rather unique.

**Codex Alimentarius maximum limits** The Codex Alimentarius Commission (CAC) is an international organisation, supported by FAO and WHO, aiming at facilitating world trade and protecting the health of the consumer by developing international standards for foods and feeds. Currently 168 countries are member of Codex Alimentarius. Within the CAC, the Codex Committee on Food Additives and Contaminants (CCFAC) derives maximum limits (standards) for additives and contaminants in food, that are decisive in trade conflicts. The CCFAC develops standards in a procedure which follows the principles of risk analysis as far as possible, according to rules and methods as they are laid down in the general Codex Procedural Manual and more specifically in the Codex General Standard for Contaminants and Toxins in Food. In the mycotoxin area CCFAC has established standards for total aflatoxins in unprocessed peanuts, for aflatoxin M₁ in milk and for patulin in apple juice. A draft standard has been developed for ochratoxin A in wheat, barley, rice and derived products and proposed standards for DON in cereals are currently under discussion.

**Specific observations with respect to limits for aflatoxins in food, dairy products and feed**

The number of countries regulating aflatoxins has significantly increased over the years. The regulations for aflatoxins are often detailed and specific for various foodstuffs, for dairy products and for feedstuffs. Compared to the situation in 1995, the maximum tolerated levels for aflatoxin B₁ in food have not changed dramatically, although the range of limits has narrowed. The most frequently occurring limit is now 2 μg/kg (see Fig. 2). Most of the countries applying this limit belong to the EU, the European Free Trade Association (EFTA) and candidate EU countries. Another major
limit is visible at 5 $\mu g/kg$, in force in a large group of countries, spread over Africa, Asia/Oceania, Latin America and Europe. The USA and Canada do not have a single limit for aflatoxin B$_1$.

![Number of countries](image)

**Fig. 2.** Specific regulatory limits for aflatoxin B$_1$ in food, in 56 countries.

As in 1995, also in 2003 many countries regulated the aflatoxins with limits for the sum of the aflatoxins B$_1$, B$_2$, G$_1$, and G$_2$, sometimes in combination with a specific limit for aflatoxin B$_1$. The range of limits has narrowed a little and the most frequently occurring limit is 4 $\mu g/kg$ (see Fig. 3), again a limit found back in the harmonised regulations in the EU, EFTA and candidate EU countries, where dual limits for both aflatoxin B$_1$ and for total aflatoxins are enforced. Another major peak occurs at 20 $\mu g/kg$, applied by many countries in Latin America (where it is also a MERCOSUR harmonised limit), and several in Africa. Also the USA, one of the first countries that established an aflatoxin action limit, follows the 20 $\mu g/kg$ limit.

Regulations for aflatoxin M$_1$ are now seen in approx. 60 countries, a more than 3-fold increase as compared to 1995! The EU, EFTA and candidate EU countries that contribute in major part to the largest peak (seen in Fig. 4) at 0.05 $\mu g/kg$, but also some countries in Africa, Asia and Latin America apply this limit. The other peaking limit is at 0.5 $\mu g/kg$. This higher regulatory level is applied in the USA, several Asian and European countries, and it occurs most frequently in Latin America, where it is established as a harmonised MERCOSUR limit.

For feedstuffs many aflatoxin regulations exist. Whereas many more countries regulate aflatoxin B$_1$ in feedstuffs for dairy cattle in 2003 (see Fig. 5) as compared to 1995 (45 in 2003 versus 25 in 1995), this is not the case for the countries that regulate the sum of the naturally occurring aflatoxins (17 in 1995 and 17 in 2003, see Fig. 6). Figure 5 shows that a limit of 5 $\mu g/kg$ dominates the distribution pattern of aflatoxin B$_1$ regulations. This limit is applied in the EU and EFTA countries, it is also followed in
many of the candidate EU countries, and it is only sporadically seen outside Europe. Strict application will normally be effective to prevent that aflatoxin M₁ levels in milk exceeds 0.05 µg/kg (where these countries have set their corresponding limit for aflatoxin M₁ in milk). The other main limit for aflatoxin B₁ in feedstuffs occurs at 20 µg/kg, and it is applied in some other European countries, and sporadically in Africa, Asia and Latin America.
Fig. 5. Specific regulatory limits for aflatoxin B₁ in animal feed, in 45 countries.

Fig. 6. Specific regulatory limits for the sum of aflatoxins B₁, B₂, G₁ and G₂ in animal feed, in 17 countries.

Fig. 6 depicts the distribution of the limits for total aflatoxins in animal feeds that are also given to dairy cattle. A rather flat distribution is apparent, and a further analysis reveals that regulatory levels for the sum of the aflatoxins B₁, B₂, G₁ and G₂ occur in the feed regulations of all the world's continents, but in particular in the America's.

Specific observations with respect to limits for other mycotoxins in food Since 1995 many more countries have now regulated patulin, mostly in fruit products as apple juice. The countries that have set regulations or guideline levels for patulin, are remarkably united about the desired limit (50 μg/kg, see Fig. 7). Harmonised EU limits for patulin have recently come into force for various products. This makes patulin one of the most regulated mycotoxins in the world. Validated analytical methodology (AOAC, CEN) is readily available to determine patulin in fruit juice at a level of 50 μg/kg.
The developments in the area of regulations for ochratoxin A show similarities as those for patulin: a significant increase in number of countries that apply limits, and good agreement about the desired limit for cereals and cereal products (see Fig. 8). Cereals are considered the major source of human exposure to ochratoxin A. The current and proposed limits for ochratoxin A may need to be reviewed in the near future, pending the outcome and subsequent evaluation by JECFA of an ongoing EC-supported
Fig. 9. Specific regulatory limits for deoxynivalenol in wheat (flour), in 37 countries.

Fig. 10. Specific regulatory limits for zearalenone in maize and other cereals, in 16 countries.

project on “Mechanisms of ochratoxin A induced carcinogenicity as a basis for an improved risk assessment.” This project is aimed at establishing whether or not the carcinogenicity of ochratoxin A is considered to arise through a threshold or non-threshold approach.

As is the case with patulin and ochratoxin A, a few dozens of countries have set regulatory or guideline limits for deoxynivalenol (DON, see Fig. 9). Whereas in 1995 this trichothecene was only sporadically regulated in food, it has become a toxin of high concern in monitoring programmes and amongst regulatory authorities since the late 1990’s when mg/kg concentrations were reported to occur in cereals and cereal products, particularly in Europe. The peak at 750 μg/kg is dominated by the countries of the EU, that currently apply this (unofficial) guideline limit for DON in flour used as raw materials since several years.

Zearalenone, an oestrogenic mycotoxin is now regulated in 16 countries (see Fig. 10), as compared to 6 in 1995. Limits in maize and other cereals currently vary from 50
Fig. 11. Specific regulatory limits for fumonisins in maize, in 6 countries.

to 1000 µg/kg. Figure 10 betrays a tendency of setting limits at higher rather than at lower limits.

Fumonisins were discovered in the late 1980's. Whereas in 1995 fumonisins were only subject of regulations in one country, this number has now increased to 6, with limits for maize ranging from 1000-3000 µg/kg (see Fig. 11). Although proportionally a very significant increase, the number of fumonisins-regulating countries is too small to draw meaningful conclusions about generally agreed limits.

Concluding remarks

Comparing the situations in 1995 and 2003, apparently in 2003 more countries are known to have regulations for more mycotoxins in more commodities and products. Regulations have become more diverse with newer requirements regarding official procedures for sampling and analytical methodology. This reflects the general concerns that governments have regarding the potential effects of mycotoxins on the health of humans and animals. At the same time harmonisation of tolerance levels is taking place in some free trade zones (EU, EFTA, MERCOSUR, Australia/New Zealand), and harmonisation efforts are being undertaken for goods moving in international commerce (Codex Alimentarius). This process is slow however, because of the different views and interests of those involved.

Whereas harmonised tolerance levels would be beneficial from the point of view of trade, one might argue this would not necessarily be the case from the point of view of (equal) human health protection around the world. Risk characterisation involves hazard assessment and exposure assessment. The hazard of mycotoxins to individuals is probably more or less the same all over the world (although other factors sometimes play a role as well, e.g. hepatitis B virus infection in relation to the hazard of aflatoxins). Exposure is not the same, because of differences in levels of contamination and dietary habits in various parts of the world. National governments or regional communities
should encourage and fund activities that contribute to reliable exposure assessment of mycotoxins in their regions. The availability of inexpensive, validated and easily performed analytical methodology and the application of Analytical Quality Assurance are basic ingredients to come to meaningful data on occurrence, and their development must therefore be stimulated. Efforts to come to improved hazard assessment should preferably be coordinated and funded at the international level. Chronic toxicity studies carried out under Good Laboratory Practice conditions are very time consuming, expensive, and not necessarily bound to certain regions. These studies should be carried out in internationally recognized centres of excellence and their results evaluated by international groups of experts, e.g. JECFA.

The regulations enacted and those under development for mycotoxins in food and feed, should be the result of sound cooperation between interested parties, drawn from science, consumers, industry and policy makers. Only then can realistic protection be achieved.

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