WHO Technical Report Series
906

EVALUATION OF CERTAIN MYCOTOXINS IN FOOD

Fifty-sixth report of the
Joint FAO/WHO Expert Committee on
Food Additives

World Health Organization
Geneva

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Geneva, 6–15 February 2001

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1. Introduction

The Joint FAO/WHO Expert Committee on Food Additives met in Geneva from 6 to 15 February 2001. The meeting was opened by Mrs A. Kern, Executive Director, Sustainable Development and Healthy Environments, WHO, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and the World Health Organization. Mrs Kern noted that this was the first meeting of the Committee that had been convened to consider only contaminants, which reflected the increasing attention being given to food contaminants by the Codex Committee on Food Additives and Contaminants and the increasing concern among consumers worldwide about the potential risks associated with their intake.

2. General considerations

As a result of the recommendations of the first Joint FAO/WHO Conference on Food Additives, held in September 1955 (*1*), there have been fifty-five previous meetings of the Expert Committee (Annex 1). The present meeting was convened on the basis of the recommendation made at the fifty-fifth meeting (Annex 1, reference *149*), of priorities established by the Codex Committee on Food Additives and Contaminants, and of the recommendation of the Third Joint FAO/WHO/UNEP International Conference on Mycotoxins that FAO and WHO should consider convening a meeting of the Expert Committee devoted specifically to risk assessment of mycotoxins as soon as the requisite databases can be compiled (*2*).

The tasks before the Committee were:

— to elaborate further principles for evaluating contaminants (section 2); and
— to evaluate certain mycotoxins that may be present as contaminants in food (section 3).

2.1 Analytical methods

Use of validated analytical methods is essential to ensure that the results of surveys provide a reliable assessment of intake. Official methods will have usually been validated for analytical performance in collaborative studies, in which characteristics such as accuracy, precision, specificity and practicality have been tested. A number of international organizations are involved in the validation of analytical methods, including AOAC International, the International Organization for Standardization and its European equivalent, the European

Committee for Standardization, and the International Union of Pure and Applied Chemistry. Methods of analysis are accepted by these organizations only after they have been validated within their harmonized protocol for the conduct of collaborative studies.

Use of official validated methods is, however, no guarantee of accurate results. Furthermore, it may not always be possible to use an official method, either because it is not suitable for a particular toxin–matrix combination, because some reagents and instruments are not available, or because it is not cost-effective or practical.

Whenever possible, laboratories that supply analytical data that are used by the Committee should have been accredited by a recognized body to ensure that they are applying a system of analytical quality assurance. Such a system should include, when possible, systematic use of reference materials or certified reference materials and regular participation in inter-laboratory comparison studies. Certified reference materials, which contain certified amounts of the substance of interest, exist for a number of the mycotoxins evaluated by the Committee at its present meeting, namely aflatoxin M_1, ochratoxin A and deoxynivalenol.[1] Certified reference materials are relatively expensive and supplies are limited. It is therefore advisable for laboratories to develop their own reference materials for routine use, the toxin content of which should be established on the basis of the certified reference materials.

Participation in inter-laboratory comparisons, such as proficiency testing schemes, is becoming increasingly important as part of the analytical quality assurance measures that a laboratory must undertake to demonstrate acceptable performance. Various national and international organizations conduct such studies, in which samples are distributed to participants and the analytical results are assessed by the organizers. A number of proficiency testing schemes for mycotoxins exist at the international level, including those organized in the European Union by the Community Reference Laboratory for Milk and Milk Products, in the United Kingdom by the Central Science Laboratory (the Food Analysis Performance Assessment Scheme), and in the USA by the American Oil Chemists' Society.

Regardless of whether accredited methods are used to produce data, laboratories should undertake internal analytical quality assurance measures such as:

[1] Such reference materials may be obtained, for example, from the European Commission Joint Research Centre, Institute for Reference Materials and Measurements, Geel, Belgium.

— intra-laboratory validation of standard operating protocols;
— use of tests for recovery;
— checking the identity and concentrations of standard solutions for calibration; and
— use of tests to confirm the identity of mycotoxins detected in samples.

In the studies evaluated by the Committee at its present meeting, it was usually clear which analytical method had been used; however, much less information was available about analytical quality assurance. For future evaluations, surveillance data should be accompanied by information on the method of analysis used and its validation. In addition, individual, rather than pooled, surveillance data should be reported.

Specifically, the Committee recommended that:

(a) Surveillance data be accompanied by a clear description of the analytical method used and an indication of whether it has been formally validated.
(b) Limits of detection and quantification should be provided, with the definitions used to derive them.
(c) Recoveries determined using "spiked" samples or reference materials should be given with analytical results; the levels of spiking should be defined, and it should be specified whether the analytical data reported were corrected for recovery.
(d) An estimate of the uncertainty of measurement should be given, derived from measurements of repeatability or by calculation.[1]
(e) The source of the standard solution(s) for calibration should be provided, the procedure used to verify its (their) identity and concentration should be described, and the method of preparation of the solution(s) should be given.
(f) There should be an indication whether the laboratory that reported the results was accredited and, if so, for which analyte–matrix combinations.
(g) There should be an indication whether the laboratory that reported the results took part in inter-laboratory comparisons and, if so, for which analyte–matrix combinations.

2.2 Sampling

In order for the results of surveys to be meaningful, representative samples must be collected from carefully selected sources of food (e.g.

[1] Calculations can be done according to the guidelines published by Eurachem: *Quantifying uncertainty in analytical measurement*, 2nd ed.(*3*).

batches or lots, marketplaces and farm shops), which, in turn, should be representative of clearly defined locations (e.g. country or region within a country). These requirements apply throughout the survey. If, for example, the levels of mycotoxin contamination are likely to vary at different times and in different agroclimatic regions, it is essential that representative sources of food be carefully selected from each region. Once a source has been selected, it is equally important that samples be collected using a clearly defined sampling plan designed to give a reasonably representative sample. Although sampling variability is unavoidable, the precision of the sampling plan must be clearly defined and considered acceptable by those responsible for interpreting the results of the survey. If the samples are too small, a wide range of estimated levels of contamination with mycotoxins will be obtained for a given source, and there will be a strong probability that the concentrations will be significantly lower than the true value. It is equally important that a sufficient number of samples be collected from each source to ensure that occasional highly contaminated samples are included.

Most studies of sampling have focused on the development of sampling plans for regulatory purposes, and little work has been done to address the need for specific sampling plans for surveys. Similarly, little or no information was available on the efficacy of sampling plans for the determination of the mycotoxins evaluated by the Committee at its present meeting. Consequently, authoritative recommendations could not be made about the sampling procedures to be used in surveying the concentrations of these toxins in foods. Most of the data that were used for risk characterization at the present meeting were based on sampling protocols of unknown efficacy in a variety of unprocessed, processed, imported and locally produced foods.

Further studies on sampling variability are urgently required so that practicable, economically feasible sampling plans can be developed for convenient, accurate determination of mycotoxins in foods, thus improving the quality of future risk characterizations.

2.3 Data on food consumption

In its assessments of the risks associated with exposure to specific contaminants in food, the Committee determines the total dietary intake of the contaminants at the international level. For this purpose, it uses the regional diets of the WHO Global Environment Monitoring System–Food Contamination Monitoring and Assessment Programme (GEMS/Food) (*4*), which approximate the average consumption of commodities in five defined regions, and are based on

FAO food balance sheets. While data from the food balance sheets tend to result in overestimates of consumption by about 15% (*5*), use of the GEMS/Food regional diets may sometimes result in underestimates of the mean consumption of specific commodities because regional consumption is calculated by averaging data from selected countries in each region. The Committee noted that GEMS/Food is developing an additional 13 regional diets (*6*), which it considered would be more representative of the consumption patterns in different countries.

Most of the values for consumption of food commodities included in the GEMS/Food regional diets are for raw agricultural commodities. The effects of processing, such as milling of cereals and baking of bread, should be taken into account in assessing intake, as processing of raw agricultural commodities may alter the levels of contamination in the final products.

A consumer of a single food item at the 95th percentile of the distribution of consumption might have approximately three times the estimated mean intake calculated for that food, and the intake by a consumer of all foods at that level might be about twice the mean (*7*).

The Committee suggested that data from national food balance sheets or, preferably, from national food consumption surveys, should be used in order to obtain more accurate assessments of intake at the national level. In addition, individual data on levels of contaminants in foods as consumed, such as those obtained from total diet studies, provide the best estimates of intake of contaminants by national populations and by subgroups at risk.

2.4 Availability of data and other issues related to dietary intake

The Committee is occasionally asked by the Codex Committee on Food Additives and Contaminants to estimate the relative health risks associated with specific proposed maximum limits for a particular contaminant. In the past, the Committee has usually had access to pooled data, which are useful for estimating mean intakes. The most commonly used method for estimating intake is to combine data on mean food consumption with weighted mean levels of contamination. While this method provides an estimate of the mean intake of a contaminant at the international level, a probabilistic (stochastic) model is necessary to address relative risks.

Where the data submitted to the Committee were insufficient to calculate the weighted mean intake of foods contaminated with mycotoxins in each GEMS/Food regional diet, the weighted mean intake

based on all the available data was estimated. When there was clear evidence that particular toxinogenic fungi and their associated toxins did not occur in domestic or imported commodities in a particular region, intake for that region was not estimated. This approach allowed the identification of potential risks in relevant regions, with the aim of encouraging surveys of all relevant commodities in those regions.

In view of the complex nature of assessments of individual dietary intake and to permit analysis of particular situations, probabilistic approaches are starting to be applied at the national level. In these approaches, various values can be introduced to ensure the representativeness of all possible outcomes (*5*). Algorithms are used to sample the probability distributions of the input variables randomly. One commonly used sampling technique, the Monte Carlo technique, involves taking values at random from the range of the probability distribution(s). An alternative sampling technique, the Latin hypercube technique, results in an accurate representation of the input distribution and requires fewer iterations. Use of such techniques makes it possible to take into account various permutations of food consumption and contaminant concentrations and to calculate the probability distributions of both the likelihood and the magnitude of dietary intake.

Food consumption and concentrations of chemicals in food can be represented by probability distributions and sampled accordingly. A value for dietary intake can be calculated from the sample values. When this process is repeated many times, a distribution of the probability of dietary intake of chemicals in food can be obtained. The method can also be used to examine the concentrations of a chemical in various foods or to evaluate exposure from various sources. By its nature, probabilistic modelling takes account of the possibility that not all of the foods chosen will contain the chemical in question.

For a probabilistic assessment, the input variables must be described by frequency distributions. Such distributions have an important influence on the outcome of a simulation, and an assessment might be erroneous if an inappropriate distribution is used for an input variable. Use of histograms of the frequency of input distributions based on actual data, rather than distribution functions fitted to the data, can reduce such error, provided the data are sufficient. This technique ensures that none of the individual values used in a simulation is outside the range of the original data. Sensitivity analysis, assessment of the effects of correlations of food consumption pat-

terns, and other techniques can improve the results of probabilistic modelling (*8*).

Data on the concentrations of individual contaminants in food commodities are needed in order to construct distribution curves that allow detailed assessment of intake and its impact on health risks. However, because the distribution curves for contaminants are highly skewed, the potential effect on health of any proposed maximum level that lies at the extreme end of the distribution curve would be limited. The data must be of suitable quality (*9*). To facilitate global cooperation in risk analysis, such data should be submitted according to the protocol developed by GEMS/Food (*10*). The protocol for submission of pooled and individual data on contaminants is being updated to include a description of the sampling method and the performance characteristics of the analytical method used, as described in sections 2.1 and 2.2 of this report. However, data may be submitted in other formats.

2.5 Prevention and control

The prevention and control of mycotoxin formation depend to a large extent on the commodity and fungus of concern, but some general principles apply. Approaches can be used before harvest, immediately after harvest, or during storage. A draft code of practice for preharvest and postharvest control of mycotoxin formation, including suggestions for management systems based on the principles for the application of the Hazard Analysis and Critical Control Points (HACCP) system, has been proposed by the Codex Committee on Food Additives and Contaminants (*11*). The main approaches for preharvest prevention of mycotoxin formation include appropriate agricultural practices, most aspects of which are covered in the Codex draft code of practice and in the report of the Third Joint FAO/WHO/UNEP International Conference on Mycotoxins (*2*). Another approach is to breed plants for resistance to the fungus of concern. Several studies have been conducted on breeding cereal crops for resistance to infection by *Fusarium* spp., with limited practical results. Success has been achieved with crops genetically modified to resist penetration by insects, resulting in a reduction in contamination of maize with fumonisins. Biological control has been of some use against infection by certain *Fusarium* spp., but not particularly those that produce mycotoxins. Competitive exclusion, by the introduction of non-toxinogenic strains in the field, has been used with some success against *Aspergillus flavus* for reduction of aflatoxin B_1 formation in groundnuts and cottonseed. This approach may be useful for other applications.

The main postharvest strategy involves drying commodities, keeping them dry (below a water activity (a_w)[1] of 0.70) and, in addition, cleaning grains and removing the dockage. This and other aspects are covered in the Codex draft code of practice (*11*).

A variety of approaches to control are possible during storage, including use of antifungal chemicals. Various physical means, such as aeration, cooling, hermetic storage and modified atmospheres, have been used effectively to reduce insect and fungal growth in stored grains in some countries, thereby controlling mycotoxin formation. Irradiation with gamma-rays, which is used for insect control, is unsuitable for fungal control since the doses required are greater than those permitted for use in grains. Addition of natural products extracted from medicinal plants has been used successfully on a laboratory scale against a variety of fungi. Addition of biological control agents such as bacteria and yeasts has shown some promise. Use of an integrated approach, combining low levels of more than one control agent, may contribute to fungal control and to reducing contamination by mycotoxins.

The physical and chemical strategies for reducing mycotoxin concentrations in affected commodities include:

— ammoniation, for reduction of the aflatoxin concentrations in feeds;
— processing (see below);
— adsorption onto inert materials; and
— colour sorting, with rejection of discoloured grains and nuts containing mycotoxins.

3. Specific mycotoxins

The Committee evaluated six mycotoxins for the first time (fumonisins B_1, B_2 and B_3, deoxynivalenol and T-2 and HT-2 toxins) and re-evaluated two mycotoxins (aflatoxin M_1 and ochratoxin A).

3.1 Aflatoxin M_1

Aflatoxins may be produced by three species of *Aspergillus* — *A. flavus*, *A. parasiticus* and the rare *A. nomius* — which contaminate plants and

[1] Defined as $a_w = P/P_o$
where:
P = partial pressure of water above the sample
P_o = vapour pressure of pure water at the same temperature.
Water activity is a measure of the "availability" of the water in the sample and not the water content.

plant products. *A. flavus* produces aflatoxins B_1 and B_2, while *A. parasiticus* and *A. nomius* also produce aflatoxins G_1 and G_2. Aflatoxins M_1 and M_2 are the hydroxylated metabolites of aflatoxins B_1 and B_2 and may be found in milk or milk products obtained from livestock that have ingested contaminated feed. The main sources of aflatoxins in animal feeds are groundnut meal, maize and cottonseed meal.

The aflatoxins were evaluated by the Committee at its thirty-first, forty-sixth and forty-ninth meetings (Annex 1, references *77*, *122* and *131*). At its forty-ninth meeting, the Committee considered estimates of the carcinogenic potency of aflatoxins and the potential risks associated with their intake. At that meeting, the Committee reviewed a wide range of studies, conducted in both animals and humans, that provided qualitative and quantitative information on the hepatocarcinogenicity of aflatoxins. The Committee evaluated the potency of these contaminants, linked those potencies to intake estimates and discussed the potential impact of hypothetical standards on the overall risk for certain populations. The Committee noted that aflatoxin B_1 is the most potent carcinogen of the aflatoxins and that most of the available toxicological data relate to aflatoxin B_1. The carcinogenic potency of aflatoxin M_1 is approximately one order of magnitude less than that of aflatoxin B_1. The Committee also noted that the carcinogenic potency of aflatoxin B_1 is substantially higher in carriers of hepatitis B virus (about 0.3 cases per year/100 000 people per ng of aflatoxin B_1/kg of body weight per day), as determined by the presence in serum of the hepatitis B surface antigen ($HBsAg^+$ individuals), than in $HBsAg^-$ individuals (about 0.01 cases per year/100 000 people per ng of aflatoxin B_1/kg of body weight per day). Thus, reduction of the intake of aflatoxins in populations with a high prevalence of $HBsAg^+$ individuals would result in a greater reduction in liver cancer rates than reduction of the intake of aflatoxins in populations with a low prevalence of $HBsAg^+$ individuals. The Committee further noted that vaccination against hepatitis B virus would reduce the number of carriers of the virus, which might reduce the carcinogenic potency of the aflatoxins in vaccinated populations and consequently their risk for liver cancer.

At its forty-ninth meeting, the Committee analysed the effects of applying hypothetical standards for contamination of food with aflatoxin B_1 (10 and 20 µg/kg) and concluded that reducing the standard from 20 µg/kg to 10 µg/kg would not result in any observable difference in the rates of liver cancer.

The present evaluation was conducted in response to a request by the Codex Committee on Food Additives and Contaminants at its Thirty-second Session (*12*) for the Committee to "examine exposure

to aflatoxin M_1 and to conduct a quantitative risk assessment" to compare the application of two standards for contamination of milk (0.05 and 0.5 µg/kg). At its present meeting, the Committee reviewed studies published since its forty-ninth meeting, as well as other information, to elucidate further the carcinogenic potencies of aflatoxin M_1 and aflatoxin B_1 and the differences between animal species in their sensitivity to aflatoxins.

Metabolism

In all species and tissues tested to date, the mutagenicity, carcinogenicity and DNA-binding activity of aflatoxin B_1 appear to result from its activation by cytochrome P450 enzymes to produce aflatoxin B_1-8,9-epoxide. The metabolism of aflatoxin B_1 to the epoxide and to aflatoxin M_1 can be blocked in vitro (in human hepatocytes) and in vivo (in rats) by treatment with oltipraz, an antischistosomal drug, which blocks the formation of the epoxide and induces the major aflatoxin detoxification enzyme, glutathione *S*-transferase. Oltipraz is being tested in phase I and II clinical trials in China for its effectiveness in the prevention of liver cancer; the results of these studies will be useful for clarifying the metabolism and mode of action of aflatoxins in humans.

Studies in human hepatocytes showed wide variation among individuals in the metabolism and activation of aflatoxins. Human hepatocytes appeared to form less of the epoxides of both aflatoxin B_1 and M_1 than did rat hepatocytes. Conjugation of both epoxides with glutathione *S*-transferase appeared to occur more rapidly in mouse than in human hepatocytes. The details of the relationships between aflatoxin metabolism, activation and detoxification in humans remains, however, unclear.

Toxicological studies

Aflatoxin M_1 is cytotoxic, as demonstrated by the results of in vitro studies in human hepatocytes, and its acute toxicity in several species is similar to that of aflatoxin B_1. In ducklings and rats, the acute and short-term toxicity of aflatoxin M_1 was similar to or slightly less than that of aflatoxin B_1. In studies of carcinogenicity, aflatoxin M_1 was about one-tenth as potent as aflatoxin B_1, even in sensitive species such as the rainbow trout and the Fischer rat. The genotoxicity of aflatoxin M_1 in vitro was similar to that of aflatoxin B_1 in some test systems and between one-half and one-sixth of that of aflatoxin B_1 in others.

Observations in humans

No studies were available on the association between dietary intake of aflatoxin M_1 and the risk for liver cancer. The Committee reviewed

the literature on this topic that had been published since the previous evaluation to determine whether the additional studies provided more accurate estimates of the dose–response relationships than those used in 1997. Studies in which recently developed biomarkers of exposure to aflatoxins (e.g. aflatoxin–albumin adducts in serum, aflatoxin–*N*7-guanine adducts in urine, aflatoxin M_1 metabolites in urine or patterns of *p53* gene mutations) were used did not provide additional evidence that would allow more accurate risk assessments. Studies with more sensitive markers of exposure to hepatitis B and/or hepatitis C viruses in patients with liver cancer strongly suggested that the estimated fraction of cases of human liver cancer attributable to these viral infections was increasing. As a consequence, the estimates of the potency of aflatoxin B_1 used by the Committee at its forty-ninth meeting in 1997 are likely to be overestimates. At its present meeting, the Committee made a conservative estimate of the potency of aflatoxin M_1, based on the estimates generated for aflatoxin B_1.

Analytical methods

Screening tests for aflatoxin M_1 in milk and milk products include radioimmunoassays and enzyme-linked immunosorbent assays (ELISAs). Radioimmunoassays have found little application in routine investigations of aflatoxin M_1 in milk, whereas ELISAs are often used. For regulatory purposes, positive results in ELISAs must be confirmed by an accepted reference method.

The quantitative analytical methods for aflatoxin M_1 include thin-layer and liquid chromatography. Many of these methods were developed for the analysis of milk and milk powder but can be used for other dairy products. Five such methods have been studied in formal collaborative studies, and their performance characteristics have been published. With the development of liquid chromatography in the 1980s, most laboratories abandoned use of thin-layer chromatography for the analysis of aflatoxin M_1. Use of immunoaffinity cartridges for clean-up of milk extracts was introduced subsequently, and the combination of immunoaffinity and liquid chromatography now offers the best means for efficient clean-up and precise determination of low concentrations of aflatoxin M_1. A method involving a combination of immunoaffinity and thin-layer chromatography and a low-cost, computer-based densitometer is being validated for the detection of low concentrations of aflatoxins in a formal collaborative study.

Sampling protocols

Protocols for sampling of granular feed products for aflatoxins have been evaluated, but little work has been done to evaluate protocols

for sampling of milk for aflatoxin M_1. Nevertheless, the European Union, the Southern Common Market (MERCOSUR) and the USA have designed several protocols for sampling of milk for aflatoxin M_1. A European Commission Directive specifies that a sample of at least 0.5 kg (or 0.5 l) composed of no fewer than five increments should be collected from a batch of milk mixed by manual or mechanical means. The batch is considered to be acceptable for human consumption if the concentration of aflatoxin M_1 does not exceed the permitted limit. In the USA, the Food and Drug Administration stipulates that samples should consist of at least 10 pounds (4.5 kg) of milk, composed of no fewer than 10 randomly selected portions.

As the distribution of aflatoxin M_1 in liquid milk can be expected to be reasonably homogeneous, sampling of liquid milk for aflatoxin M_1 will involve less uncertainty than sampling of granular feed products for aflatoxins. Most of the uncertainty in estimating aflatoxin M_1 in milk is probably associated with the analytical procedure.

Effects of processing

Numerous studies have been conducted on the effects of processing on the concentration of aflatoxin M_1 in milk, the results of which are variable. Most studies show that the concentration is not appreciably reduced by heat treatment, nor by processing yoghurt, cheese, cream, milk powder or butter, although aflatoxin M_1 is redistributed differentially in these products.

Aflatoxin M_1 can be partially eliminated from milk by physical or chemical procedures, which include use of adsorbents, hydrogen peroxide and ultraviolet radiation. These treatments are not readily applicable by the dairy industry, however, and their safety has not been tested; moreover, the costs may be prohibitive for large-scale application.

Food consumption and dietary intake assessment

Data on the concentrations of aflatoxin M_1 in milk were submitted by Argentina, Brazil, Canada, Indonesia, Norway, the United Arab Emirates, the USA and several Member States of the European Union; some data were also obtained from the literature. In certain cases, the data reflected biased or limited sampling designs and may not have been representative of the country or area in which the samples were obtained. Important information (on the number of samples, the mean and individual sample values and analytical quality assurance) was not available for some data sets; this does not imply that the data were not of good quality, but that they should be interpreted with caution. All the data on aflatoxin M_1 that were submitted were used in the evaluation.

The dietary intake of aflatoxin M_1 was estimated from data on concentrations in milk and from data on milk consumption in the GEMS/Food regional diets (*4*). The weighted mean concentration of aflatoxin M_1 in milk was highest (0.36 µg/kg) in the Far Eastern diet, followed by the European-type diet (0.023 µg/kg), the Latin American diet (0.022 µg/kg), the Middle Eastern diet (0.005 µg/kg) and the African diet (0.002 µg/kg). These mean concentrations were based on 1191, 10 778, 893, 231 and 15 milk samples, respectively. The intake of aflatoxin M_1 from milk was calculated to be 12 ng/person per day in the Far Eastern diet, 6.8 ng/person per day in the European-type diet, 3.5 ng/person per day in the Latin American diet, 0.7 ng/person per day in the Middle Eastern diet and 0.1 ng/person per day in the African diet. The intake calculated from the European regional diet was used for the assessment of cancer risk because this diet included the highest milk consumption. If all milk consumed were contaminated with aflatoxin M_1 at the proposed maximum levels of 0.05 µg/kg or 0.5 µg/kg, the intake of aflatoxin M_1 from milk in the European-type diet would be 15 ng/person per day or 150 ng/person per day, respectively.

One approach for determining the potential effects on dietary intake at the two proposed maximum levels for aflatoxin M_1 in milk involves estimating intake on the basis of the mean concentration in all samples and in all samples containing aflatoxin M_1 at less than 0.5 µg/kg or less than 0.05 µg/kg for a given population. The three calculated concentrations were multiplied by the milk consumption of the population of interest to determine the intake of aflatoxin M_1. As the most recent data on aflatoxin M_1 in milk in some of the Member States of the European Union (7573 samples reported in 1999) indicated that the concentration in all samples was less than 0.05 µg/kg, the choice of either maximum level would not affect intake. Similarly, data from Canada (81 samples reported in 1997–98) showed that the aflatoxin M_1 concentration in all milk samples analysed was less than 0.015 µg/kg (the limit of detection), so intake would not be affected at either level. The USA submitted individual data for 3620 samples collected between 1995 and 2000. On the basis of these data, the intakes of aflatoxin M_1 from milk were estimated to be 0.030 ng/kg of body weight per day for all samples and 0.023 and 0.0035 ng/kg of body weight per day, respectively, when the proposed maximum levels of 0.5 µg/kg and 0.05 µg/kg were used. As none of the other submissions or data from the literature included individual values for milk samples containing aflatoxin M_1 at a concentration higher than either of the proposed maximum levels, similar calculations could not be performed.

Another approach to determining the effect of the proposed maximum levels on dietary intake is to generate distribution curves for the concentrations of aflatoxin M_1 in milk in the GEMS/Food regional diets. The distribution was constructed assuming log normality and using the mean aflatoxin M_1 concentrations in foods in the regional diets and the maximum values reported. The distribution curve for the European-type diet showed that if 0.05 µg/kg and 0.5 µg/kg were set as the maximum levels for aflatoxin M_1 contamination, they would be at the extreme upper end of the distribution and consequently would have no effect on intake. The distribution curve for the Middle Eastern diet, for which there were relatively few data, was similar to that for the European-type diet. For Latin American diets, selection of a maximum level of 0.5 µg/kg would also have no effect; however, use of a maximum level of 0.05 µg/kg would probably reduce intake. In the Far Eastern diet, in which milk is more heavily contaminated, intake of aflatoxin M_1 would be decreased at both proposed levels; however, it should be noted that milk consumption is low in that diet.

Prevention and control

About 0.3–6.2% of aflatoxin B_1 in animal feed is transformed to aflatoxin M_1 in milk. A linear relationship has been found between intake of aflatoxin B_1 in contaminated feed at concentrations of 5–80 µg/kg and the aflatoxin M_1 content of milk, as follows:

$$\underset{\text{(ng/kg of milk)}}{\text{Concentration of aflatoxin } M_1} = \underset{\text{(µg/cow per day)}}{[1.19 \times \text{aflatoxin } B_1 \text{ intake}]} + 1.9$$

Thus, production of milk containing aflatoxin M_1 at 0.05 µg/kg (the proposed Codex limit) would require that the average intake of aflatoxin B_1 by dairy cows be limited to approximately 40 µg per day. On the basis of a daily feed consumption of 12 kg of compound feeds per cow, application of a limit of 40 µg of aflatoxin B_1 would mean that the content of aflatoxin B_1 in the feed would have to be no more than 3.4 µg/kg in order to meet the limit of 0.05 µg/kg for aflatoxin M_1.

The most effective means for controlling aflatoxin M_1 in the food supply is to reduce the amount of aflatoxin B_1 in the feed of dairy cows. Specific regulations exist in many countries to control aflatoxin B_1 in the animal feed supply, but it might be difficult to design an effective control programme in countries where cottonseed and maize are incorporated into animal feed, because of the heterogeneous distribution of aflatoxin in these commodities, which results in a high degree of sampling variability.

The concentration of aflatoxin B_1 in feed can be reduced by good manufacturing practice and good storage practices. If preventive measures fail, however, the aflatoxin B_1 concentration in feed can be reduced by blending or by physical or chemical treatment. The physical treatments include application of heat, irradiation with microwaves, gamma-rays, X-rays or ultraviolet light, and adsorption on to hydrated sodium calcium aluminosilicate and other inert materials. The most successful chemical procedure for degrading aflatoxins in animal feed is treatment with ammonia. This procedure is used with agricultural commodities in various countries and leads to decomposition of 95–98% of the aflatoxin B_1 present.

Evaluation

Since aflatoxin M_1 is a metabolite of aflatoxin B_1 and is presumed to induce liver cancer in rodents by a similar mechanism, estimates of the carcinogenic potency of aflatoxin B_1 can be used to determine the risk associated with intake of aflatoxin M_1. No adequate epidemiological studies exist on the dose–response relationships between the intake of aflatoxin M_1, exposure to hepatitis B or C virus, and liver cancer. The Committee therefore assumed that aflatoxin M_1 acts similarly to aflatoxin B_1 with hepatitis B (and possibly hepatitis C) virus. On the basis of the carcinogenicity studies in the Fischer rat, the Committee estimated the carcinogenic potency of aflatoxin M_1 to be 0.001 cases per year/100 000 people per ng of aflatoxin M_1/kg of body weight per day in $HBsAg^-$ individuals and 0.03 cases per year/100 000 people per ng of aflatoxin M_1/kg of body weight per day in $HBsAg^+$ individuals.

The estimates of the carcinogenic potency of aflatoxin M_1 were combined with estimates of intake from the GEMS/Food European-type diet. The carcinogenic potency of aflatoxin M_1 in a population in which the prevalence of hepatitis B virus infection is P was projected to be the weighted combination of the potency estimates for $HBsAg^-$ individuals and $HBsAg^+$ individuals, as follows:

$$\text{Carcinogenic potency} = (0.001 \times [1-P]) + (0.03 \times P)$$

Three rates of prevalence of $HBsAg^+$ individuals (1%, 5% and 25%) were considered to span the range of rates of infection with hepatitis B virus observed in various populations in western and south-east Asian countries. The risks for liver cancer were projected to be the product of the average potency values and the intake estimates for aflatoxin M_1 from a European-type diet, corresponding to a relatively high intake of milk and milk products. The projected risks associated with the intake of aflatoxin M_1 were calculated for the two proposed maximum levels, 0.5 µg/kg and 0.05 µg/kg. It was assumed that all

products were contaminated at the two proposed maximum levels, giving worst-case projections of the population risk. Projected risks were also calculated for the weighted mean of 0.023 µg/kg of milk for the GEMS/Food European-type diet (see Table 1).

The calculations showed that, with worst-case assumptions, the projected risks for liver cancer attributable to use of the proposed maximum levels of aflatoxin M_1 of 0.05 µg/kg and 0.5 µg/kg are very small. For example, in a population with a prevalence of hepatitis B virus infection of 1%, which is typical for western Europe and the USA, the additional numbers of liver cancer cases associated with contamination of all milk with aflatoxin M_1 at 0.5 µg/kg versus 0.05 µg/kg would be 29 (i.e. 32 – 3.2) cancers per 1000 million persons per year. The potency of aflatoxin M_1 appears to be so low in $HBsAg^-$ individuals that a carcinogenic effect of aflatoxin M_1 intake in those who consume large quantities of milk and milk products in comparison with non-consumers of these products would be impossible to demonstrate.

Hepatitis B virus carriers might benefit from a reduction in the aflatoxin concentration in their diet, and the reduction might also offer some protection to hepatitis C virus carriers. Reduction of the current concentrations of aflatoxins in the diet in most developed countries is unlikely to produce an observable reduction in the rates of liver cancer.

On the basis of the above, the Committee concluded that the liver cancer burden could best be reduced by giving priority to vaccination campaigns against hepatitis B and to prevention of infection with hepatitis C; the latter would require greater control of blood and blood products and the use of sterile medical equipment.

3.2 Fumonisins B_1, B_2 and B_3

Fumonisins are mycotoxins produced by fungi of the genus *Fusarium*. *F. verticillioides* (Sacc.) Nirenberg (= *F. moniliforme* (Sheldon)) and the related *F. proliferatum* (Matsushima) Nirenberg are the only species that produce significant quantities of fumonisins, but at least 10 other *Fusarium* species also produce these toxins. *F. verticillioides* and *F. proliferatum* are among the most common fungi associated with maize, the most frequently contaminated food, and can be recovered from both damaged and undamaged maize kernels. These species cause *Fusarium* kernel rot of maize, an important disease in hot climates. A strong relationship also exists between insect damage and *Fusarium* kernel rot due to other *Fusarium* species such as *F. graminearum.* Temperature stress may also play a role, especially in cultivars grown outside their area of adaptation. As *F. verticillioides*

Table 1
Projected risk of liver cancer attributable to intake of aflatoxin M_1 in milk and comparison of the impact of the application of the proposed maximum levels

Concentration of aflatoxin M_1 in milk (µg/kg)	Prevalence of $HBsAg^+$ individuals in population (%)	Average carcinogenic potency (cases per year/ 100000 population per ng/kg of body weight per day)	Intake of aflatoxin M_1[a]		Prevalence of liver cancer attributable to aflatoxin M_1 intake (cases per year/1000 million population)
			(ng/person per day)	(ng/kg of body weight per day)[b]	
0.023 (weighted mean)	1	0.0013	6.8	0.11	1.5
	5	0.0025	6.8	0.11	2.8
	25	0.0083	6.8	0.11	9.4
0.05 (proposed maximum level)	1	0.0013	15	0.25	3.2
	5	0.0025	15	0.25	6
	25	0.0083	15	0.25	20
0.5 (proposed maximum level)	1	0.0013	150	2.5	32
	5	0.0025	150	2.5	60
	25	0.0083	150	2.5	200

$HBsAg^+$: Hepatitis B surface antigen detected in serum.
[a] Based on a European-type diet; assumes that all products were contaminated at the proposed maximum level.
[b] Based on a body weight of 60 kg.

and *F. proliferatum* grow over a wide range of temperatures but only at relatively high water activities (above about 0.9), fumonisins are formed in maize only before harvest or during the early stage of drying. Except under extreme conditions, fumonisin concentrations will not increase during grain storage. Formation of fumonisins in the field is positively correlated with the occurrence of *F. verticillioides* and *F. proliferatum*, which predominate during late maturity. Fumonisins are widely distributed geographically, and their natural occurrence in maize has been reported in many areas of the world. Of particular concern are the high levels found in maize produced and consumed by particular subpopulations such as subsistence farmers. Considerable annual variations in contamination have been noted. Fumonisins occur infrequently in other foods, such as sorghum, asparagus, rice, beer and mung beans.

Fumonisin B_1 is the diester of propane-1,2,3-tricarboxylic acid and 2*S*-amino-12*S*,16*R*-dimethyl-3*S*,5*R*,10*R*,14*S*,15*R*-pentahydroxyeicosane in which the C-14 and C-15 hydroxy groups are esterified with the terminal carboxy group of propane-1,2,3-tricarboxylic acid. Fumonisin B_2 is the C-10 deoxy analogue of fumonisin B_1 in which the corresponding stereogenic units on the eicosane backbone possess the same configuration. The full stereochemical structures of fumonisin B_3 and B_4 are unknown, although the amino-terminal end of fumonisin B_3 has the same absolute configuration as that of fumonisin B_1.

The present evaluation was conducted in response to a request by the Codex Committee on Food Additives and Contaminants at its Thirty-second Session (*12*) for the Committee to evaluate fumonisins B_1, B_2 and B_3. These mycotoxins had not been evaluated previously by the Committee. In 2000, the International Programme on Chemical Safety (IPCS) prepared a monograph on fumonisin B_1 (*13*), which provided much of the background information for the evaluation.

As most biological data were available on fumonisin B_1, and as maize is the major source of intake, the Committee focused its evaluation on toxicological studies of fumonisin B_1 and on studies of the analysis and intake of contaminated maize and maize products. In many studies on fumonisins, culture materials and naturally contaminated maize were used, which can contain several other fumonisins, primarily fumonisins B_2 and B_3. The toxicological profiles of fumonisins B_2 and B_3 are very similar to that of fumonisin B_1. Various chemical derivatives of fumonisins have been tested in a number of biological test systems to gain insight into their structure–activity relationships. Briefly, fumonisins B_1, B_2 and B_3 are more toxic in vivo than their hydrolysed or *N*-acetylated counterparts. The free amino group appears to play a specific role in the biological activity of these compounds.

Absorption, distribution, metabolism and excretion

In all animal species studied, fumonisins are poorly absorbed from the digestive tract and are rapidly distributed and eliminated. Liver and kidney retain most of the absorbed material, and fumonisin B_1 persists longer in rat liver and kidney than in plasma. In pregnant rats and rabbits, very low concentrations of fumonisin B_1 were recovered in the uterus and placenta. No fumonisin B_1 was detected in fetuses, indicating the absence of placental transfer. There was little evidence of significant transfer during lactation, and fumonisins do not appear to be metabolized in vitro or in vivo. Although fumonisins are not metabolized by cytochrome P450 enzymes, fumonisin B_1 can alter the activity of these enzymes through mechanisms that alter sphingolipid biosynthesis. Fumonisins are structurally related to sphingoid bases. Removal of the tricarballylic acid (propane-1,2,3-tricarboxylate) side-chains, presumably by the microbial flora of the gut, converts fumonisin B_1 into a substrate for ceramide synthase. The product of the enzyme reaction, like fumonisin B_1, inhibits the enzyme in vitro.

Toxicological studies

In all animal species studied, the liver was a target for fumonisin B_1; the kidney was also a target in many species. In the kidney, the early effects are often increases in free sphingoid bases, apoptosis of renal tubule cells and cell regeneration. In the liver, apoptotic and oncotic necrosis, proliferation of oval cells, and hyperplasia and regeneration of the bile duct are early signs of toxicity. In rats and trout fed known cancer initiators and studied using various tumour initiation/promotion protocols, purified fumonisin B_1 enhanced the development of liver cancer. Brief administration of high doses or longer administration of low doses that caused significant hepatotoxicity resulted in the appearance of foci positive for glutathione *S*-transferase (placental form), hepatocellular nodules and other precursors of liver tumour development. In rodents, the toxicity of fumonisin B_1 was strain- and sex-dependent. For example, male rats of the BDIX strain appeared to be more sensitive to the hepatotoxic effects of fumonisin B_1, whereas those of the Fischer 344N, Sprague-Dawley and RIVM:WU strains appeared to be more sensitive to its nephrotoxic effects. In mice, the liver was more sensitive than the kidney to the toxicity of fumonisin B_1, and females were more sensitive than males. In long-term feeding studies, purified fumonisin B_1 caused both liver and kidney tumours in rodents. The kidney carcinomas induced in male Fischer 344N rats by fumonisin B_1 were a highly malignant variant of renal tubule tumour, but the significance of their aggressive nature was unclear. The no-observed-effect levels (NOELs) for renal cancer and renal toxicity in Fischer 344N rats were 0.67 and 0.2 mg/kg of

Table 2
Dose–response relationship for renal toxicity and renal tumours in male Fischer 344N rats fed diets containing purified fumonisin B_1 for 2 years

Dose of fumonisin B_1 (mg/kg of body weight per day)	Number of animals showing signs of renal toxicity or renal tumours		
	Cytotoxic or regenerative lesions	Atypical tubule hyperplasia	Renal tumours
Untreated controls	0/42	0/48	0/48
0.22	0/40	0/40	0/40
0.67	23/33	0/48	0/48
2.2	42/42	4/48	10/48
6.6	43/43	9/48	16/48

Table 3
Dose–response relationship for renal toxicity in male Fischer 344N rats fed diets containing purified fumonisin B_1 for 90 days

Dose of fumonisin B_1 (mg/kg of body weight per day)	Number of animals showing signs of renal toxicity
Untreated controls	0/10
0.1	0/10
0.2	0/10
0.6	9/10
1.9	10/10
5.7	10/10

body weight per day, respectively (Tables 2 and 3). The NOELs for liver cancer in male BDIX rats and in female $B6C3F_1$ mice in which feed intake was restricted were 0.8 and 1.9 mg/kg of body weight per day, respectively.

Studies in rodents, non-human primates and other animal species fed diets containing culture material from an *F. verticillioides* isolate that produces predominantly fumonisin B_1 (MRC 826) or maize naturally contaminated with fumonisins showed toxic effects in the liver and kidney that were similar to those observed in studies in animals given purified fumonisin B_1. Both MRC 826 and naturally contaminated maize caused liver tumours in rats at doses similar to those that caused liver tumours in rodents fed purified fumonisin B_1. The NOEL for the toxicity of total fumonisins in vervet monkeys fed a diet containing *F. verticillioides* culture material was 0.11 mg/kg of body weight per day for both the kidney and the liver.

Purified fumonisin B_1, *F. verticillioides* culture material and naturally contaminated maize all induced not only hepatic toxicity but also

leukoencephalomalacia in equids and pulmonary oedema and hydrothorax in pigs. Both pulmonary oedema and hydrothorax appeared to occur secondarily to cardiovascular dysfunction in pigs. Cardiovascular effects have also been seen in other species. Outbreaks of equine leukoencephalomalacia and porcine pulmonary oedema associated with consumption of fumonisin-contaminated maize have been reported in several countries, including the USA. The NOEL for fumonisin B_1 in equine leukoencephalomalacia was equivalent to 0.3 mg/kg of body weight per day in animals fed diets containing *F. verticillioides* culture material. In pigs fed *F. verticillioides* culture material, evidence of pulmonary oedema was detected at a concentration of fumonisin B_1 equivalent to 0.4 mg/kg of body weight per day. In pigs fed naturally contaminated maize, the concentration of fumonisin B_1 required to induce pulmonary oedema was much higher, although the NOEL for liver toxicity was similar (equivalent to 0.2 mg/kg of body weight per day).

Several biochemical modes of action have been postulated to explain the toxicological effects reported in animals given fumonisin-contaminated feed. Two hypotheses involve disruption of lipid metabolism as the initial step. The first proposed mechanism involves disruption of sphingolipid metabolism through inhibition of ceramide synthase. The demonstrated consequences of inhibition of this enzyme in liver and kidney are changes in all the major pools of sphingolipids, including increased concentrations of free sphingoid bases and their metabolites and decreased biosynthesis of ceramide and other sphingolipids containing ceramide. Glycerophospholipid metabolism is also affected. Clear evidence of fumonisin-induced disruption of sphingolipid metabolism has been obtained in all target tissues except brain and in all species tested. The second proposed mechanism involves disruption of the metabolism of fatty acids and glycerophospholipids. Fumonisin-induced changes in fatty acid profiles and prostaglandins have been demonstrated in vivo in rat liver. These two proposed lipid-based mechanisms of action are similar in many respects with regard to their ultimate effects on cell physiology and are consistent with the results of in vitro and in vivo studies (short-term toxicity studies and long-term carcinogenicity studies in rodents). Fumonisins also affect sites of cellular regulation that are apparently independent of the disruption of lipid metabolism, but cancer and the other toxic effects observed in animals appear to depend on disruption of various aspects of lipid metabolism, membrane structure and signal transduction pathways mediated by lipid secondary messengers. The demonstrated cellular effects include alterations in cell proliferation, rates of apoptosis, intercellular communication and cell adhesion,

induction of oxidative stress and modulation of gene expression. Since the proposed biochemical mechanisms of action involve alterations in de novo biosynthetic pathways, nutritional factors could play an important role in determining the potency of fumonisin B_1 and the observed toxicological effects in rodents.

The observations reported in the available in vivo studies are consistent with a proposed mode of action for fumonisin B_1 that is dependent on perturbed lipid metabolism. The resulting increase in cell death coupled with regenerative cell proliferation, possibly by generation of oxidative damage, could in turn lead to an increase in the incidence of tumours in target tissues. The primary evidence for sustained cell loss and regeneration is the observation of such effects in rat kidneys.

In a small number of studies in vitro and a single study in vivo, neither fumonisin B_1 nor any other fumonisin was shown unequivocally to be genotoxic. Similarly, no adducts of fumonisin with DNA have been found.

While there was evidence that fumonisins are embryotoxic in vitro, no published data exist to support the conclusion that fumonisins cause developmental or reproductive toxicity in farm animals. Except in one study in hamsters, embryotoxicity occurred in laboratory animals (rats, mice and rabbits) secondarily to maternal toxicity.

Observations in humans

Consumption of mouldy sorghum or maize containing fumonisin B_1 at concentrations of up to 64 mg/kg was associated with an outbreak of human disease in India involving gastrointestinal symptoms. The grain was also reported to be contaminated with other toxinogenic fungi.

The available evidence for an association between intake of fumonisins and human cancer was limited to a few correlation studies. Typically, these involved a few regions in which the populations were broadly classified with regard to their risk for oesophageal or liver cancer. The regions were then compared with respect to the proportion of fumonisin-contaminated food samples and the level of contamination. Sometimes, the measures of intake of fumonisins were indirect, and the incidence of disease was related to consumption of certain foods, notably maize. Taken together, the results of these studies could be interpreted as indicating an association between fungal contamination of foods and oesophageal cancer or liver cancer. However, bias, chance or confounding factors could not be excluded, and hence there was only limited evidence of an independent carcinogenic effect of fumonisins.

A specific role for fumonisins in the development of neural tube defects has been proposed. The hypothesis includes a critical role of fumonisins in disruption of folate membrane transport, but no specific studies have been designed or performed to confirm this mechanism in vivo.

Analytical methods

Two validated analytical methods based on liquid chromatography have been developed for fumonisins. The first method, which uses solvent extraction with strong anion exchange for clean-up of samples, has been validated for fumonisins B_1, B_2 and B_3 in maize. Although this method has been used to determine fumonisin concentrations in maize-based foods, recovery from certain food matrices can be problematic. A second method with higher extraction efficiency and using immunoaffinity columns for sample clean-up has been validated for fumonisins B_1 and B_2 in maize and cornflakes. Although methods for unequivocal detection based on liquid chromatography with detection by mass spectrometry are also available, their high cost prohibits their routine use. Screening tests based on thin-layer chromatography and, for the fumonisins of the B series, ELISAs have also been developed. No methods specific for fumonisin B_4 have been described, and little is known about its occurrence, although the limited evidence suggests that it occurs at lower concentrations than the fumonisins evaluated at the present meeting. The absence of a method to determine the concentration of fumonisin standard solutions for calibration remains a problem. In the laboratories that provided data on the natural occurrence of fumonisins in maize and maize-based foods for the current assessment, liquid chromatography was used predominantly, together with solvent extraction, solid-phase extraction (for clean-up of samples) and quantification by pre-column formation of fluorescent *o*-phthaldialdehyde derivatives. The limits of detection were generally $\leqslant 50\,\mu g/kg$ and the analytical recovery greater than 70%.

Sampling protocols

Variance in the sampling of shelled maize for fumonisins was studied after collection of a large bulk sample and riffle-division into 1.1-kg test samples. For batches contaminated with fumonisins at a concentration of 2 mg/kg, the coefficients of variation associated with sampling (1.1-kg sample), sample preparation (milling and 25-g analytical portion) and analysis were 17%, 9.1% and 9.7%, respectively; these coefficients were independent of the fumonisin(s) tested (fumonisin B_1, B_2 or B_3, or all fumonisins). The coefficient of variation associated with the whole test procedure (sampling, sample preparation and

analysis) was 21%, which was of the same order of magnitude as that for the measurement of aflatoxins in shelled maize by a similar test procedure.

Effects of processing

The effects of various food-processing procedures on the levels of fumonisin contamination have been studied. For example, maize screenings contain higher concentrations of fumonisins than whole grain. Separation and removal of screenings is a useful method for reducing the amount of fumonisins entering storage. Steeping maize in aqueous solutions during wet milling results in extraction of fumonisins and is thus effective in reducing the concentration in maize products. Fumonisins are fairly heat-stable, and the toxin content is significantly reduced only during processes in which the temperature exceeds 150 °C. Dry milling of maize results in the distribution of fumonisins into the different maize constituents. In wet milling, some fumonisins are extracted into the steeping water. There is little degradation of fumonisins during fermentation. Alkaline cooking and heating (nixtamalization) of maize result in hydrolysis of fumonisins, but do not completely detoxify contaminated maize. During processing, many parameters affect the fate of these mycotoxins. In addition, they may be converted to products of unknown toxicity.

Food consumption and dietary intake assessment

The distributions of the estimated intake of fumonisin B_1 at the international level were based on the GEMS/Food regional diets (*4*) and a published distribution of the concentrations of fumonisin B_1 in maize. Data that supported use of the published distribution were submitted by Argentina, Brazil, Canada, China, Denmark, Sweden, the United Kingdom, Uruguay and the USA. The intake estimates were made on the assumption that all the maize consumed contained fumonisin B_1 at the concentration found in the unprocessed maize samples that were used to construct the distribution curve. While the mean concentration of fumonisin B_1 used was 1.4 mg/kg of unprocessed maize (median, 0.42 mg/kg), surveys conducted over several years have shown that the median or mean concentration in maize varies greatly. The mean concentration of fumonisin B_1 in sound maize in international trade in any given year could be expected to be between 0.2 and 2.5 mg/kg. Use of these concentrations of fumonisin B_1 with the intakes of maize in the GEMS/Food regional diets would alter the mean of the expected distribution of intake of fumonisin B_1 from one-seventh to twice that reported here.

Table 4
Estimated intake of fumonisin B_1, based on GEMS/Food regional diets

Distribution	Estimated intake of fumonisin B_1 (μg/kg of body weight per day)				
	African diet	European-type diet	Far Eastern diet	Latin American diet	Middle Eastern diet
Mean	2.4	0.2	0.7	1.0	1.1
90th percentile	7.3	0.6	2.1	2.9	3.3

Table 5
National estimates of intake of fumonisin B_1

Country or area	Intake (μg/kg of body weight per day)	
	Mean or median	90th percentile
Argentina	0.2	NR
Canada	0.02	0.08
Netherlands	0.06[a]	NR
	1.0[b]	NR
Switzerland	0.03	NR
United Kingdom	0.03	0.1
USA	0.08	NR

NR: Not reported or not calculated.
[a] Whole population.
[b] Regular eaters of maize.

The mean estimated intake of fumonisin B_1 ranged from 0.2 μg/kg of body weight per day in the European-type diet to 2.4 μg/kg of body weight per day in the African diet (Table 4).

The Committee also considered published national estimates of intake of fumonisin B_1 from Argentina, Canada, the Netherlands, Switzerland and the USA. The intake of fumonisin B_1 was also estimated on the basis of data on the consumption of food containing maize and the associated concentrations of fumonisin B_1 submitted by the United Kingdom. The national estimates of intake were lower than the international estimates presented here because they took into account the effects of processing and because they were prepared from more specific data, i.e. the intake of food as consumed rather than the intake of raw agricultural commodities. The mean national estimates of intake of fumonisin B_1 ranged from 0.02 to 1.0 μg/kg of body weight per day (Table 5). These estimates included a number of assumptions that ensure conservatism, such as the assumption that all

persons consumed food containing fumonisin B_1 at the default concentration. Finally, the Committee noted that subsistence farmers, who grow and eat their own maize, might consume larger amounts of fumonisin B_1 than those reported here.

Where they had been quantified in the same sample, the ratio of fumonisin B_1:B_2:B_3 was approximately 10:3:1. To approximate the intake of all three fumonisins, therefore, the intake values obtained for fumonisin B_1 should be increased by 40%.

Prevention and control

The strategies for preharvest reduction of contamination by fumonisins include agricultural practices, plant breeding and genetic engineering. However, any plant breeding programme should take into account the growth conditions in specific regions to ensure full adaptation of the variety(ies) developed.

The main means to prevent fumonisin contamination after harvest is immediate drying of the grain. Treatment with chemical preservatives before storage or use of physical means such as temperature reduction or modified atmospheres during storage can also prevent fungal growth and subsequent mycotoxin formation.

Evaluation

Nephrotoxicity, which was observed in several strains of rat, was the most sensitive toxic effect of pure fumonisin B_1. Since the available studies clearly indicated that long-term renal toxicity is a prerequisite for carcinogenesis induced by this mycotoxin, the potential for renal carcinogenesis is subsumed by the dose–response relationship for renal toxicity. Therefore, the pivotal studies that could serve as the basis for establishing a tolerable intake of fumonisin B_1 were the short-term and long-term toxicity studies in rodents (see Tables 2 and 3). On the basis of these studies, the overall NOEL for renal toxicity was 0.2 mg/kg of body weight per day.

The Committee allocated a group provisional maximum tolerable daily intake (PMTDI) of 2 μg/kg of body weight to fumonisins B_1, B_2 and B_3, alone or in combination, on the basis of the NOEL of 0.2 mg/kg of body weight per day and a safety factor of 100. All of the intake estimates for fumonisin B_1 based on the available data on national consumption were well below the group PMTDI. This remained true even when these estimates were increased by 40% to account for the presence of fumonisins B_2 and B_3.

The Committee was aware of an unpublished risk assessment in which the data on renal tumours had been used, and noted that the

estimated risk was negligible at intakes below the group PMTDI established at the present meeting.

Recommendations

The Committee acknowledged the need for research in the areas recommended in WHO Environmental Health Criteria, No. 219 (*13*). The Committee made the following additional recommendations:

- Since renal toxicity is a prerequisite for fumonisin-induced carcinogenicity in male rats, the incidence of renal tumours in male rats should be modelled by biologically based procedures.
- Studies should be conducted on the biochemical and physiological mechanism(s) underlying the aggressive nature of fumonisin-induced renal tubule carcinomas in Fischer 344N rats, including the effects of fumonisin B_1 on expression of cell adhesion molecules.
- The biochemical and physiological mechanisms for the apparently different sensitivities of Fischer 344N and BDIX rats to fumonisin-induced hepatic toxicity should be studied.
- Studies should be conducted to determine whether dietary factors such as folate, vitamin E and choline modify renal or hepatic toxicity induced by fumonisin B_1 in laboratory animals.
- The ability of fumonisin B_1 to modify folate transport at the cellular level and through the placenta to the fetus should be investigated.
- Studies should be conducted to determine the role of inhibition of ceramide biosynthesis by fumonisins in protecting cells from ceramide-mediated apoptosis induced by mitochondrial dysfunction.
- The relationship between fumonisin intake and human disease in areas where nixtamalized maize products account for a large part of the human diet should be investigated. Particular emphasis should be placed on hepatic and renal diseases and other diseases suspected to be associated with fumonisin B_1 intake, such as nasopharyngeal and oesophageal cancers and neural tube defects.
- The ability of fumonisins to modify expression of receptors for microbial pathogens and toxins that are associated with renal and hepatic disease in humans should be investigated.

3.3 Ochratoxin A

Ochratoxin A is produced by *Penicillium verrucosum*, by *Aspergillus ochraceus* and several related *Aspergillus* species, and by *A. carbonarius* together with a low percentage of isolates of the closely related *A. niger*. These three groups of species differ in their ecological niches, in the commodities affected, and in the frequency of their occurrence in different geographical regions. *P. verrucosum* grows

only at temperatures below 30 °C and at a water activity above 0.80. It is therefore found only in cool temperate regions and is the source of ochratoxin A in cereals and cereal products in Canada and Europe. As cereals are widely used in animal feeds in Europe and ochratoxin A is relatively stable, this mycotoxin is also found in some animal products in that region, especially pig kidney and liver. As *P. verrucosum* does not occur in the tropics and subtropics, cereals from those regions are unlikely to contain ochratoxin A from this source. *A. ochraceus* grows at moderate temperatures and at a water activity above 0.8. It is found sporadically in a wide range of stored food commodities, including cereals, but is seldom the source of substantial concentrations of ochratoxin A. It may also infect coffee beans during sun-drying and is a source of ochratoxin A in green coffee beans. *A. carbonarius* grows at high temperatures and is associated with maturing fruits, especially grapes. Because of its black spores, it is highly resistant to sunlight and survives sun-drying. It is the source of ochratoxin A in fresh grapes, dried vine fruits and wine; it is also a source of ochratoxin A in coffee.

Ochratoxin A was first reviewed by the Committee at its thirty-seventh meeting (Annex 1, reference *94*), when it established a provisional tolerable weekly intake (PTWI) of 112 ng/kg of body weight based on the deterioration of renal function observed in pigs, for which the lowest-observed-effect level (LOEL) was 0.008 mg/kg of body weight per day, and a safety factor of 500. At that time, the Committee recommended that further studies be conducted to elucidate the role of ochratoxin A (and other mycotoxins) in nephropathy in pigs and humans, the mechanisms of induction of tumours and the role of phenylalanine in antagonizing the nephrotoxic effects of ochratoxin A. The Committee re-evaluated ochratoxin A at its forty-fourth meeting (Annex 1, reference *116*), when it considered toxicological data that had become available since its previous evaluation, including studies on epidemiology, genotoxicity and nephrotoxicity. At that meeting, the Committee reconfirmed the PTWI established at its thirty-seventh meeting, rounded it to 100 ng/kg of body weight, and reiterated its request for further studies on ochratoxin A.

The present evaluation was conducted in response to a request by the Codex Committee on Food Additives and Contaminants at its Thirty-first Session (*14*) for the Committee to perform a risk assessment of the consequences of establishing a maximum level of 5 µg/kg or 20 µg/kg for ochratoxin A in cereals and cereal products.

The Committee considered several new studies that had become available since the previous evaluation of ochratoxin A. These

included further studies of its absorption, distribution (including secretion into the milk of experimental animals), metabolism and excretion, as well as studies of its biochemistry, genotoxicity, immunotoxicity, neurotoxicity, embryotoxicity and hepatotoxicity, and studies on the mechanisms of cytotoxicity and nephrotoxicity. Epidemiological data and data derived from surveys of food commodities for ochratoxin A and of food consumption were also considered, and used to estimate intakes in various countries and regions of the world.

Absorption, distribution, metabolism and excretion

Ochratoxin A is slowly absorbed from the gastrointestinal tract. It is distributed via the blood, mainly to the kidneys, lower concentrations being found in liver, muscle and fat. Transfer to milk has been demonstrated in rats, rabbits and humans, but is minimal in ruminants, owing to metabolism of ochratoxin A by the rumen microflora. The major metabolite of ochratoxin A in all species examined is ochratoxin α. Ochratoxin α and the other metabolites that have been identified are all reported to be less toxic than ochratoxin A. Ochratoxin A is excreted in the urine and faeces; the relative contribution of each of these excretory routes in different species is influenced by the extent of enterohepatic recirculation of ochratoxin A and its binding to serum macromolecules. These factors are also important in determining the serum half-life of ochratoxin A, which varies widely among species, e.g. 24–39 h in mice, 55–120 h in rats, 72–120 h in pigs, 510 h in one macaque monkey and 840 h in a human volunteer.

Toxicological studies

Ochratoxin A has been shown to be nephrotoxic in all mammalian species tested. Its main target is the renal proximal tubule, where it exerts cytotoxic and carcinogenic effects. Significant sex- and species-related differences in sensitivity to nephrotoxicity were evident, with pigs being the most sensitive, followed by rats and mice. The doses at which carcinogenicity was observed in rodents were higher than those that caused nephrotoxicity. The Committee reconsidered the report of a carcinogenicity study conducted by the National Toxicology Program in the USA in 1989 and noted the consistent presence and severity of karyomegaly in male and female rats and the aggressive nature of the renal tumours. However, the biological basis and significance of these observations were unclear.

Gene mutations were induced in bacteria and in mammalian cells in a few genotoxicity studies, but not in most. Ochratoxin A did, however, induce DNA damage, DNA repair and chromosomal aberrations in mammalian cells in vitro and DNA damage and chromosomal

aberrations in mice treated in vivo. Putative DNA adducts were found consistently with a ^{32}P-postlabelling method in the kidneys of mice and rats dosed with ochratoxin A, but none of these adducts was demonstrated to contain fragments of ochratoxin A. It was therefore uncertain whether ochratoxin A interacts directly with DNA or whether it acts by generating reactive oxygen species (free radicals). There was no indication that a reactive metabolite of ochratoxin A was generated in vivo. Ochratoxin A is thus genotoxic both in vitro and in vivo, but the mechanism of its genotoxicity is unclear, and there is no evidence that it is mediated by direct interaction with DNA. The doses used in the genotoxicity studies were in the same range as those at which the incidence of renal tumours was increased in mice. In rats, however, the incidences of nephrotoxicity and renal tumours were increased at much lower doses; therefore, the contribution of the genotoxicity of ochratoxin A to neoplasia in rats is unknown.

Ochratoxin A can cross the placenta, and it is embryotoxic and teratogenic in rats and mice. It has been shown to have immunosuppressive effects in a number of species. Prenatal administration of ochratoxin A to rats caused immunosuppression, but perinatal administration stimulated certain aspects of the immune response in rats. Ochratoxin A inhibited the proliferation of B and T lymphocytes and affected the late stages of T-lymphocyte activation in vitro. However, the immunological and teratogenic effects have been observed only at doses much higher than those that caused nephrotoxicity.

Observations in humans

Ochratoxin A has been found in human blood samples, most notably in countries in the cool temperate climatic areas of the northern hemisphere; however, no cases of acute intoxication in humans have been reported. The Committee noted that ochratoxin A was found frequently and at high average concentrations in blood samples obtained from people living in regions where a fatal human kidney disease (known as Balkan endemic nephropathy) occurs, which is associated with an increased incidence of tumours of the upper urinary tract. Nevertheless, similar average concentrations have been reported in several other European countries where this disease is not observed. The Committee concluded that the epidemiological and clinical data available do not provide a basis for calculating the carcinogenic potency of ochratoxin A in humans and that the etiology of Balkan endemic nephropathy may involve other nephrotoxic agents.

Analytical methods

Reliable, validated methods have been developed for the analysis of ochratoxin A in maize, barley, rye, wheat (including bran and whole

meal), roasted coffee, wine and beer, which are based on liquid chromatography with fluorescence detection. The limit of quantification was 0.03 µg/kg for wine and beer and 0.3–0.6 µg/kg for other commodities. These methods have also been used successfully to analyse ochratoxin A in a number of other cereals, cereal products and dried fruit. Two certified reference materials (blank and naturally contaminated wheat) are available for quality assurance purposes. Screening methods based on thin-layer chromatography are available but have been used in only a few laboratories. Data obtained by analytical methods with a limit of quantification greater than 5 µg/kg were not considered in this evaluation, as this was the lower concentration for which the Codex Committee on Food Additives and Contaminants requested a risk assessment. There are no formally validated methods for the analysis of ochratoxin A in human blood. The available methods are based on liquid chromatography with fluorescence detection and have limits of quantification ranging from about 0.1 to 2 ng/ml.

Sampling protocols

The Committee noted that an acceptable sampling procedure had been described in only 10 of the 22 studies on cereals considered at the present meeting, whereas no description was reported for the remaining 12. No sampling protocols for the determination of ochratoxin A in foods have been published, and details of the variability in sampling have not been reported. Adequate sampling procedures should be used in future surveys of ochratoxin A in cereals and cereal products.

Effects of processing

Milling has been reported to reduce substantially the concentration of ochratoxin A in white flour, but it has little effect on the levels in wholemeal flour. Milling is a physical process: the ochratoxin A removed from the grain during the production of white flour remains in the bran and other fractions, some of which may be used in foods. Ochratoxin A is relatively stable to heat: at 100 °C, a 50% reduction in the concentration was achieved after 2.3 h in wet wheat and after 12 h in dry wheat. The processes involved in the manufacture of breakfast cereals and biscuits resulted in substantial reductions in ochratoxin A content, but little or no reduction was found during the manufacture of egg noodles and pasta. Decaffeination of coffee reduced the ochratoxin A concentration by about 90%. The reduction obtained by roasting coffee varied, but may also be as much as 90%.

Levels and patterns of contamination of food commodities

Most (85%) of the studies that were reviewed by the Committee were from Europe; studies were also submitted from Africa (1%), North

America (6%), South America (7%) and Asia (1%). The concentrations of ochratoxin A in the various commodities were highly variable; 1.4% and 0.6% of samples contained more than 5 µg/kg and more than 20 µg/kg, respectively. The levels of contamination were higher for the cereals (1.2% and 0.3% of samples contained more than 5 µg/kg and more than 20 µg/kg, respectively) than for the cereal products (0.3% and 0.05% of samples contained more than 5 µg/kg and more than 20 µg/kg, respectively). The weighted mean concentrations of ochratoxin A that were used to estimate intake were: 0.94 µg/kg for cereals, 0.19 µg/kg for cereal products, 0.32 µg/kg for wine, 0.86 µg/kg for coffee, 2.3 µg/kg for dried vine fruit and 0.44 µg/kg for grape juice. The incidence of contamination varied between the different commodities. Higher rates were reported for the same commodity when analytical methods with low limits of quantification were used.

Food consumption and dietary intake assessment

Intake of ochratoxin A at the international level was assessed on the basis of data on mean consumption combined with the weighted mean level of contamination. As ochratoxin A contamination of food occurs mainly in Europe, data on food consumption in the GEMS/Food European-type diet (*4*) were considered the most relevant for risk assessment. The submitted data on levels of contamination were aggregated according to the recommendations of a FAO/WHO workshop (*9*) to obtain a weighted mean. With this approach, the mean total intake of ochratoxin A was estimated to be 45 ng/kg of body weight per week, assuming a body weight of 60 kg.

Cereals and wine contributed about 25 and 10 ng/kg of body weight per week, respectively, to the mean intake, whereas grape juice and coffee each contributed 2–3 ng/kg of body weight per week. Other food products (dried fruits, beer, tea, milk, cocoa, poultry, pulses) each contributed less than 1 ng/kg of body weight per week. Most of the results submitted for pig meats and pig meat products were based on analysis of liver and kidney, whereas the estimated figure for consumption in the GEMS/Food European-type diet was based on pig meats. The resulting estimate of 1.5 ng/kg of body weight per week was therefore considered by the Committee to be a gross overestimate of intake of ochratoxin A.

A probabilistic approach was used to assess intake of ochratoxin A from cereals and cereal products, in which a simulated distribution of contamination and the distribution of cereal consumption in France were used. This example, which was considered to be realistic for the European-type diet, showed that consumers of cereals at the 95th

percentile would have an intake of ochratoxin A of 92 ng/kg of body weight per week. Application of the proposed maximum limit of 5 µg/kg, as opposed to 20 µg/kg, would have a statistically significant effect on intake of ochratoxin A only for those consumers with intakes above the 95th percentile. However, the difference would be very small (84 ng/kg of body weight per week at the median and 92 ng/kg of body weight per week at the 95th percentile), in view of the distribution of the level of contamination indicated by the available data.

Prevention and control

As the formation of ochratoxin A depends on the fungal source, the type of crop and its geographical location, control of ochratoxin A production by each fungal species was considered separately. Control of *A. ochraceus*, which occurs primarily in stored foods, consists of the standard methods for preventing the growth of any fungus in dried foods. The major commodities in which *A. ochraceus* may produce ochratoxin A are stored grains. The traditional means of avoiding fungal growth in grains is to dry them rapidly and thoroughly and to keep them dry. The moisture content of grains must be reduced to a water activity below 0.8 in order to prevent formation of ochratoxin A by *A. ochraceus*. Further effective approaches to grain storage include fumigation, aeration and cooling, sealed storage and controlled atmospheres, especially in tropical and subtropical regions where insect damage is a major problem. Controlled atmosphere storage is based on continuous application of atmospheres with a low oxygen or a high carbon dioxide concentration. Use of modified atmospheres to control insects may also contribute to controlling fungi. Some fumigants used for insect control may also control fungi.

As ochratoxin A is apparently formed in green coffee beans after harvest, agricultural practice has little or no influence on the concentration of the toxin in dried beans. Control measures for ochratoxin A in coffee are therefore based on good manufacturing practice, i.e. rapid and effective drying, good storage practice and, in some countries, colour sorting to reject mouldy or damaged beans.

The available evidence indicates that *A. carbonarius* and *A. niger* are not pathogens on fruit such as grapes and hence cannot gain entry to undamaged fruit. However, mechanical or chemical damage to fruit or damage caused by insects or microorganisms may permit fungal invasion of fruit tissue. Controlling the growth of *A. carbonarius* and *A. niger* in grapes therefore relies on controlling pathogenic fungi, mechanical damage and splitting due to rain before harvesting.

The occurrence of ochratoxin A from *P. verrucosum* in grains from Canada and Europe was attributed to insufficient drying or

inadequate storage. Analysis and segregation of defective lots could be used to reduce the concentration of ochratoxin A in dried grains used for human food.

Evaluation

The Committee concluded that the new data raised further questions about the mechanisms by which ochratoxin A causes nephrotoxicity and renal carcinogenicity and the interdependence of these effects. The mechanism by which ochratoxin A causes carcinogenicity is unknown, although both genotoxic and non-genotoxic modes of action have been proposed. The Committee noted that studies to resolve these issues are in progress and would wish to review the results when they become available. The Committee retained the previously established PTWI of 100 ng/kg of body weight, pending the results of the studies on the mechanisms of nephrotoxicity and carcinogenicity, and recommended a further review of ochratoxin A in 2004. In reaching this conclusion, the Committee noted the large safety factor that had been applied to the NOEL for nephrotoxicity in deriving the PTWI, which corresponds to a factor of 1500 applied to the NOEL for carcinogenicity in male rats, the most sensitive species and sex for this end-point.

The most sensitive adverse effect in several mammalian species is nephrotoxicity, and this is likely also to be true in humans. Although an association between the intake of ochratoxin A and nephropathy in humans has been postulated, causality has not been established. The Committee noted that the intake of ochratoxin A by consumers of cereals at the 95th percentile may approach the PTWI from this source alone. Given the distribution of ochratoxin A contamination of cereals, application of a limit of 5 µg/kg or of 20 µg/kg would make no significant difference to the average intake. The estimated intake of cereal consumers at the 95th percentile would be about 84 and 92 ng/kg of body weight per week, respectively, in the European-type diet. While intake below the PTWI would not present an appreciable risk, the Committee was unable, on the basis of the available data, to arrive at a quantitative estimate of the risk for nephrotoxicity if the PTWI were to be exceeded. Efforts are needed to ensure that intakes of ochratoxin A do not exceed the PTWI, and this could best be achieved by lowering overall contamination by appropriate agricultural, storage and processing practices.

Recommendations

The Committee made the following recommendations:

- Studies should be conducted to clarify the mechanisms by which ochratoxin A induces nephrotoxicity and carcinogenicity.

- Appropriate sampling procedures should be developed for food commodities likely to be contaminated with ochratoxin A.
- Better surveys are needed, particularly in regions of the world other than Europe, in order to assess intake of ochratoxin A in these regions.
- Epidemiological investigations should be encouraged to explore the role of ochratoxin A in chronic renal disease.
- Studies should be conducted to improve understanding of the occurrence and ecology of the fungi that produce ochratoxin A, especially in fresh produce.

3.4 Trichothecenes

3.4.1 *Deoxynivalenol*

Deoxynivalenol (DON, vomitoxin) is a type B trichothecene mycotoxin, an epoxy-sesquiterpenoid. Surveys have shown that deoxynivalenol occurs predominantly in grains such as wheat, barley, oats, rye and maize and less often in rice, sorghum and triticale. The occurrence of deoxynivalenol is associated primarily with *Fusarium graminearum* (*Gibberella zeae*) and *F. culmorum*, both of which are important plant pathogens, causing *Fusarium* head blight in wheat and *Gibberella* ear rot in maize. A direct relationship between the incidence of *Fusarium* head blight and contamination of wheat with deoxynivalenol has been established. The incidence of *Fusarium* head blight is most affected by moisture at the time of flowering (anthesis). The timing of rainfall, rather than the amount, is the most critical factor. *F. graminearum* grows optimally at a temperature of 25 °C and at a water activity above 0.88. *F. culmorum* grows optimally at 21 °C and at a water activity above 0.87. The geographical distribution of the two species appears to be related to temperature, *F. graminearum* being the more common species and occurring in warmer climates. Deoxynivalenol has been implicated in incidents of acute mycotoxicoses in both humans and farm animals.

Deoxynivalenol has not been evaluated previously by the Committee.

Toxicological studies

Deoxynivalenol is metabolized by, in particular, de-epoxidation and glucuronidation, generally to less toxic substances.

Deoxynivalenol may have adverse health effects after acute, short-term or long-term administration to experimental animals. After administration as a single dose, deoxynivalenol has two characteristic toxicological effects: decreased feed consumption (anorexia) and emesis (vomiting). Both effects have been linked to increased central

serotoninergic activity. Single doses of deoxynivalenol also damage rapidly dividing cells, such as those of the gastrointestinal tract. These characteristic effects have also been observed with other trichothecenes, although differences in potency were seen.

Many early studies were conducted in livestock given feed containing cereals contaminated with deoxynivalenol. In later studies, purified deoxynivalenol was generally administered to experimental animals (laboratory rodents). In the studies in livestock, feed naturally contaminated with deoxynivalenol tended to be more toxic than feed to which purified deoxynivalenol had been added. This result was attributed to the presence of additional fungal metabolites. Low concentrations of zearalenone or the 3- or 15-acetyl precursors of deoxynivalenol were found in some cases.

After short-term or long-term administration of deoxynivalenol to experimental animals, one of the most consistent effects observed in most species was reduced growth, and this was often the most sensitive parameter in routine studies of toxicity. At higher doses, the thymus, spleen, heart and liver were affected. In a 2-year study in mice, a slight reduction in body weight observed at the lowest dose (0.1 mg/kg of body weight per day) was considered to be biologically insignificant. Since no other changes were seen at this dose, the NOEL was 0.1 mg/kg of body weight per day.

A working group convened by the International Agency for Research on Cancer (IARC) in 1993 placed deoxynivalenol in Group 3, not classifiable as to its carcinogenicity to humans. A carcinogenicity study in mice conducted since that time showed fewer tumours of the liver in treated male mice than in controls. The Committee concluded that the lower incidence was due to the reduced body weight of the treated animals. No significant difference in tumour incidence was seen in female mice.

Deoxynivalenol was not mutagenic in bacteria, but chromosomal aberrations were observed both in vitro and in vivo, suggesting that it is genotoxic. However, in the single in vivo study that was available, most of the aberrations consisted of gaps in the chromosomes, and the overall significance of the results was considered to be equivocal.

Deoxynivalenol was teratogenic but not maternally toxic when given to pregnant mice at 5 mg/kg of body weight per day by gavage over a short critical period (days 8–11) of gestation, but not when given at 2.5 mg/kg of body weight per day. When deoxynivalenol was administered in the feed, the NOEL for maternal toxicity and fetotoxicity was 0.38 mg/kg of body weight per day.

The results of two studies in mice suggested that deoxynivalenol can suppress host resistance to *Listeria monocytogenes* and *Salmonella enteritidis*, with a NOEL of 0.25 mg/kg of body weight per day in the first study and a LOEL of 0.12 mg/kg of body weight per day in the second. Deoxynivalenol also affected the immune response. In mice, the NOEL for this effect was 1 mg/kg of body weight per day, while in pigs given naturally contaminated feed, the NOEL was 0.08 mg/kg of body weight per day.

Observations in humans

Many outbreaks of acute disease involving nausea, vomiting, gastrointestinal upset, dizziness, diarrhoea and headache have been reported in Asia, which have been attributed to consumption of grains contaminated with *Fusarium* spp. and, more recently, to the presence of deoxynivalenol at concentrations of 3–93 mg/kg in grains for human consumption. Occasionally, other trichothecenes were present as well, but at much lower incidences and much lower concentrations. When the contaminated food was replaced with uncontaminated food, the signs and symptoms disappeared. In one study, these effects were not observed after consumption of grains containing deoxynivalenol at reported concentrations of 0.4–13 mg/kg, but under-reporting or false-negative results at the higher concentrations may have occurred. In two studies, none of the health effects described above were observed after consumption of grains containing deoxynivalenol at 0.02–3.5 mg/kg. Most of the studies on acute effects in humans were population-based or ecological studies.

Sampling protocols and analytical methods

A number of studies of variability in sampling for deoxynivalenol have been reported. In one study, a 225-kg bulk sample was collected from each of six batches of barley, and riffle-divided into 16 test samples of 0.1 kg, 16 test samples of 0.8 kg and 16 test samples of 7 kg, and analysed. The results indicated that the variation associated with sample preparation and the analytical steps was of greater significance than the variation in sampling for all sizes of test sample; the former was not substantially reduced by increasing the size of the test sample. In a similar study, a 20-kg bulk sample was taken from each of 24 commercial batches of wheat, and riffle-divided into 32 test samples of 0.45 kg each. For a batch concentration of deoxynivalenol of 5.0 mg/kg, the coefficient of variation was 6.3% for sampling, 10% for sample preparation and 6.3% for the analytical steps. The total variation was 13%. The low variation associated with the sampling step (relative to that for other mycotoxins and other food commodities) was partly due to the high kernel count of wheat (about 30

kernels per gram), which is about 10 times higher than that of shelled maize and 30 times higher than that of shelled groundnuts.

Official methods and other validated methods have been developed for the analysis of deoxynivalenol in cereals and other foodstuffs. The introduction of improved columns based on charcoal, alumina and modified diatomaceous earth for clean-up of samples before determination by thin-layer, gas or liquid chromatography has simplified and accelerated the analysis of deoxynivalenol. Use of such columns in combination with gas chromatography and detection by electron capture or mass spectrometry after derivatization of deoxynivalenol is the most common technique for quantifying this mycotoxin. This technique allows simultaneous determination of deoxynivalenol and other trichothecenes at concentrations of a few nanograms per gram, even in complex food matrices. However, the matrix may interfere with analytical methods using gas chromatography. Liquid chromatography with fluorescence detection after post-column derivatization or ultraviolet detection in combination with rigorous clean-up of samples is a suitable alternative. Liquid chromatography with detection by mass spectrometry can be used for direct, simultaneous determination of several trichothecenes, but its high cost prohibits its routine use. Thin-layer chromatography, particularly high-performance, is still a convenient method for quantifying deoxynivalenol. Thin-layer chromatography and ELISA methods are also suitable means for screening for this toxin.

Inter-laboratory comparisons clearly showed that further improvements are needed in analytical methods for deoxynivalenol with respect to recovery and the accuracy and precision of measurements. More widely available reference materials and regular international comparative studies are needed to ensure improved internal and external quality assurance.

Levels and patterns of contamination of food commodities

Data on the concentrations of deoxynivalenol in food commodities were received from Argentina, Brazil, Canada, China, Finland, Germany, Italy, the Netherlands, Norway, Sweden, the United Kingdom, Uruguay and the USA, and were also obtained from the literature. Gas chromatography with detection by electron capture or mass spectrometry was the most common technique used for the quantification of deoxynivalenol, followed by thin-layer chromatography, liquid chromatography and ELISA, respectively. Data were excluded from the evaluation when no information was provided on the analytical method or sampling protocol used. The remaining data were used

only if the samples had been collected at random and if the analytical methods used were considered to be adequate.

Deoxynivalenol was found to be a frequent contaminant of cereal grains such as oats (834 samples, 68% positive), barley (1662 samples, 59% positive), wheat (11444 samples, 57% positive), rye (295 samples, 49% positive), maize (5349 samples, 41% positive) and rice (154 samples, 27% positive). It was also detected in buckwheat, popcorn, sorghum, triticale and in some processed food products such as wheat flour, bread, breakfast cereals, noodles, infant foods and cooked pancakes. In addition, it has been reported in barley products, malt and beer. The mean concentrations in data sets in which samples containing deoxynivalenol were found were 4–760 µg/kg for oats, 4–9000 µg/kg for barley, 1–5700 µg/kg for wheat, 13–240 µg/kg for rye, 3–3700 µg/kg for maize and 6–5100 µg/kg for rice.

The submitted data showed wide annual variations in the deoxynivalenol concentrations in most of the cereals tested. The results emphasize the need for regular screening for this mycotoxin in cereal crops.

The Committee considered that the probability of deoxynivalenol being carried over to food products of animal origin was small, because animals refuse feed in which the mycotoxin is present at high concentrations, and because it undergoes rapid metabolism and elimination in livestock species.

Food consumption and dietary intake assessment

Intake of deoxynivalenol at the international level was assessed on the basis of the mean level of contamination combined with data on the mean estimated food consumption from the GEMS/Food regional diets (*4*).

Most of the data on mean concentrations of deoxynivalenol that were available for this evaluation were pooled; i.e. each data point represented the mean concentration in a number of individual samples. Data on processed food products were excluded from the estimates of dietary intake. A total of 375 data points (representing about 23000 individual samples) were included in the assessment, of which 243 were reported from countries represented by the GEMS/Food European-type diet. The remaining 132 data points represented the nine commodities (barley, maize, oats, rice, rye, wheat, popcorn, sorghum, triticale) in the other four GEMS/Food regional diets. As few data were available on the concentrations of deoxynivalenol in all commodities in regions other than Europe, a single mean concentration weighted by sample size was calculated for each commodity from

the available data. The weighted mean deoxynivalenol concentration in each commodity was multiplied by the respective value for consumption in each of the five GEMS/Food regional diets.

The total intake of deoxynivalenol was estimated to range from 0.77 µg/kg of body weight per day in the African diet to 2.4 µg/kg of body weight per day in the Middle Eastern diet. The major source of intake in three of the five regional diets (European-type, Latin American and Middle Eastern) was wheat (64–88% of total intake), whereas the sources in the other two regional diets were more varied (wheat, rice and maize in the African diet and wheat and rice in the Far Eastern diet). These estimates of average intake were based on the assumption that consumers choose foods randomly with respect to the distribution of contaminant concentrations and will, therefore, over time, have an intake that approximates to the mean of that distribution. Although it was not possible to estimate intakes at high levels from the available data, the 95th percentile may be approximated by multiplying the average intake by a factor of 2 for a single food commodity or 3 for the total diet. Possible reductions in contamination by deoxynivalenol as a result of processing were not taken into consideration in this assessment.

In general, more data on the occurrence of deoxynivalenol in food products are required to make better estimates of intake. The Committee noted that the distribution of concentrations of contaminants for processed products might differ from that for raw cereals, given that contamination tends to be more homogeneous after processing. Despite the uncertainty associated with the data on both concentration and food consumption, they provide useful preliminary estimates of contamination and intake of deoxynivalenol at the international level.

Prevention and control

Preharvest measures to control infection with *Fusarium* spp. can also reduce the formation of deoxynivalenol. Reducing the inoculum of *Fusarium* spp. in host debris and other reservoirs in the field seems to be one important control measure. Consequently, reduced tillage appears to increase the concentration of deoxynivalenol in subsequent crops. Crop rotation is also important in reducing the inoculum, and rotation of wheat and maize with non-host crops has been recommended. Use of appropriate fungicides and relevant timing of application of fungicides are other important measures for controlling *Fusarium* head blight. Good agricultural practice, such as immediate drying after harvest and proper storage, prevents further contamination with deoxynivalenol.

Physical, chemical and biological methods have been used for decontaminating grains containing trichothecenes. Some of the treatments reduced the concentration of toxin, while others were ineffective. Cleaning methods, such as gravity separation and washing procedures, can reduce deoxynivalenol concentrations in wheat and maize. The effectiveness of milling practices in reducing the concentration of deoxynivalenol in flour depends to a large extent on the degree of fungal penetration of the endosperm. Thermal processing is usually ineffective. Chemical and biological decontamination processes cannot yet be applied on a commercial scale.

Evaluation

The results of a 2-year study in mice treated in the diet did not suggest that deoxynivalenol presents a carcinogenic hazard. The Committee considered that this study was appropriate for evaluation of other long-term effects. Although the mean body weight of animals given the lowest dose was lower than that of controls, the difference was considered not to be biologically significant, and no toxicological changes were observed at this dose. The Committee established a PMTDI of 1 µg/kg of body weight on the basis of the NOEL of 100 µg/kg of body weight per day in this study and a safety factor of 100. The Committee concluded that intake of deoxynivalenol at this level would not result in adverse effects on the immune system, growth or reproduction.

The Committee recognized that deoxynivalenol can cause outbreaks of acute illness in humans; however, the available data did not permit the establishment of a level below which no acute effects would be expected to occur.

Estimation of the dietary intake of deoxynivalenol on the basis of single weighted mean concentrations and the GEMS/Food regional diets resulted in values that exceeded the PMTDI for four of the five regional diets. The Committee noted that there was considerable uncertainty in the intake estimates because of uncertainties in the values for concentration and consumption used in the assessment. Furthermore, food processing would be expected to reduce the concentrations of deoxynivalenol to varying extents, which would result in lower estimates of dietary intake.

Recommendations

The Committee made the following recommendations:

- Comparative studies on the toxicity and toxicokinetics of deoxynivalenol would help to clarify species differences in sensitivity.

- Studies are needed on the combined effects of deoxynivalenol and other trichothecenes that may be present in human food. As the trichothecenes have similar toxic properties, albeit with different potencies, the Committee recommended that toxic equivalence factors be developed for the trichothecenes, if sufficient data become available. Since deoxynivalenol is the most extensively studied trichothecene, the Committee further recommended that toxic equivalence factors be established relative to deoxynivalenol.
- In view of the widespread human exposure to deoxynivalenol, further studies on the genotoxicity of this mycotoxin should be conducted, as well as a study of its carcinogenicity in a second species (rat).
- More detailed, analytical, epidemiological studies of human disease should be conducted in those areas of the world where the presence of *Fusarium* head blight in wheat or *Gibberella* ear rot in maize is a cyclic, endemic event. Such data would help to establish a dose–response relationship between the intake of deoxynivalenol (and other trichothecenes) and acute illness, and would allow the identification of a NOEL based on human data.
- The accuracy and comparability of analytical measures of deoxynivalenol in processed foods for use in surveys should be improved.
- Additional data on the distribution of contamination of grains with deoxynivalenol and on national food consumption patterns, particularly in countries where this mycotoxin is prevalent, are needed.
- Information on the effects of processing and its impact on levels of contamination with deoxynivalenol are needed for better estimates of dietary intake.
- Better tools should be developed for the prevention of diseases of cereal crops caused by *Fusarium* spp. that result in production of deoxynivalenol.

3.4.2 *T-2 and HT-2 toxins*

T-2 and HT-2 toxins are type A trichothecene mycotoxins, which are closely related epoxy-sesquiterpenoids. Surveys have revealed the presence of T-2 and HT-2 toxins in grains such as wheat, maize, oats, barley, rice, beans and soya beans as well as in some cereal-based products. T-2 and HT-2 toxins have been reported to be produced by *Fusarium sporotrichioides*, *F. poae*, *F. equiseti* and *F. acuminatum.* The most important of these species is *F. sporotrichioides*, a saprophyte (i.e. not pathogenic to plants) which grows between –2 °C and 35 °C and only at high water activities (above 0.88). In consequence, T-2 and HT-2 toxins are not normally found in grains at

harvest but result from water damage to grains, such as may occur when they remain for extended periods in the field at or after harvest, especially in cold weather, or become wet during storage.

T-2 and HT-2 toxins have not been evaluated previously by the Committee.

Absorption, distribution, metabolism and excretion

T-2 toxin is readily metabolized by the gut microflora of mammals to several metabolites. HT-2 toxin is a primary metabolite in the gut and is absorbed into the blood after ingestion of T-2 toxin. Metabolism continues in the liver (with biliary excretion), resulting in a substantial, combined first-pass effect in the gut and liver. The metabolites of T-2 toxin include HT-2, 3′-hydroxy-HT-2, 3′-hydroxy-T-2, T-2 tetraol, de-epoxy 3′-hydroxy-T-2 triol, de-epoxy 3′-hydroxy-HT-2 and 3′-hydroxy-T-2 triol. Glucuronide conjugates are also formed extensively in most species (with the exception of cats). T-2 toxin and its metabolites are eliminated rapidly. In rats, more than 95% of a radioactively labelled oral dose of 0.15 mg/kg of body weight per day was excreted within 72 h. In the same study, a dose of 0.6 mg/kg of body weight per day was eliminated more slowly, suggesting potentially saturable metabolism or elimination pathways at doses that are relevant to those used in the studies of toxicity considered in this evaluation.

Toxicological studies

T-2 toxin is a potent inhibitor of protein synthesis both in vivo and in vitro. The effective concentration for protein inhibition in vitro is lower than those for all other effects that have been demonstrated.

The metabolites have not been studied in detail, but several primary metabolites appeared to be less toxic than the parent compound in vitro. Furthermore, T-2 toxin was 10 times more toxic when inhaled than after oral intake, suggesting that the first-pass effect reduces the toxicity, at least after acute exposure. The Committee noted that, although T-2 toxin is generally assumed to be considerably (e.g. 10-times) more toxic than deoxynivalenol, a comparison of the LOELs for similar species and end-points (see section 3.4.1) suggests that the toxicity of these trichothecenes is roughly similar when they are ingested with food.

Strain and sex differences in susceptibility to the toxicity of T-2 toxin have been observed in mice given single oral doses by gavage in studies designed to evaluate such variation. A sex difference was also observed after administration by inhalation. The cause of the differences has not been identified. Differences in susceptibility to T-2

toxin among species are also suggested from a comparison of the results of short-term studies. In a study in which cats received T-2 toxin orally in gelatin capsules at a dose of 0.06 mg/kg of body weight per day, severe toxic effects were observed, including haemorrhage in the intestinal tract, lymph nodes and heart, that led to death within 1–7 weeks. In contrast, relatively mild effects were observed in 7-week-old pigs given T-2 toxin in the diet at doses of up to 0.13 mg/kg of body weight per day for 3 weeks and in mice given a dose of 0.22 mg/kg of body weight per day in the diet for 71 weeks. The Committee noted that cats would be expected to be more susceptible to T-2 toxin than other species, in view of the demonstrated deficiencies in conjugation reactions in this species. Humans would not be expected to be similarly susceptible.

Little direct information was available on the toxicity of HT-2 toxin alone. The few comparative in vitro and in vivo data available on T-2 and HT-2 toxins indicate that they induce adverse effects at similar potencies. Furthermore, because T-2 toxin is rapidly converted to HT-2 toxin (and other metabolites common to T-2 and HT-2 toxins) in the gut, the toxicity of T-2 toxin in vivo can be considered to include that of HT-2 toxin. Hence, studies of T-2 toxin can be used as a basis for estimating the doses at which HT-2 toxin induces adverse effects.

Data on the toxicity of T-2 toxin were limited primarily to studies in which the mycotoxin was administered daily for less than 1 month. The immune system is a primary target of T-2 toxin, and the effects include changes in leukocyte counts, delayed hypersensitivity, depletion of selective blood cell progenitors, depressed antibody formation, allograft rejection and a blastogenic response to lectins. Either increased or decreased leukocyte counts were observed, depending at least in part on the dose and the time after administration of T-2 toxin that the leukocytes were counted. Similarly, both decreased and increased resistance to microbial infection have been observed in a number of studies. For example, in separate experiments in several laboratories, decreased resistance (leading in most cases to higher mortality rates) was observed in mice exposed to T-2 toxin at the time of infection with *Salmonella typhimurium*, *S. enteritidis*, *Mycobacterium bovis*, *Herpes simplex*, *Toxoplasma gondii* or *Listeria monocytogenes*. However, increased resistance (leading to a reduced mortality rate) was observed when mice were treated with T-2 toxin before infection with *L. monocytogenes*.

Feed refusal, reduced weight gain and changes in organ weights, which are sensitive end-points, have been observed in most dietary studies with T-2 toxin in which these parameters were recorded.

A 3-week study was conducted in which 7-week-old pigs were given T-2 toxin in the diet at a dose equal to 0.029, 0.062, 0.10 or 0.13 mg/kg of body weight per day. On the first and fourth days of administration, the pigs were given an intramuscular injection of horse globulin. The titre of antibodies to horse globulin was significantly lower in T-2 toxin-treated pigs than in controls at 14 and 21 days at all doses tested. The leukocyte count and the proportion of leukocytes represented by T-lymphocytes were lower in all treated groups. Decreased proliferative responses to phytohaemagglutinin and concanavalin A were observed at all doses of T-2 toxin at 21 days. A dose-related decrease in feed intake was observed in all treated groups, and decreased weight gain was observed in those treated at 0.062 mg/kg per day and above. The haemoglobin concentration was decreased in a dose-related manner at 0.062 mg/kg of body weight per day and above. A reduction in erythrocyte count was observed at 0.10 and 0.13 mg/kg of body weight per day; the erythrocyte volume fraction was also reduced at 0.13 mg/kg of body weight per day. The Committee noted that, as pair-fed animals were not used as controls, the potential confounding effects of feed intake and differences in weight gain on the observed endpoints could not be evaluated. A NOEL was not identified.

In a 71-week carcinogenicity study involving dietary administration of T-2 toxin to mice, the incidences of pulmonary adenomas and hepatic adenomas were statistically significantly increased at the end of the study in males at the highest dose, with no increase in tumour incidence in females. However, an increase in the incidence of benign tumours in the liver or lungs in mice of one sex in a single study constitutes, at most, weak evidence of carcinogenicity. A dose-related increase in heart weight was seen in treated males, but not in treated females. No other treatment-related changes were reported. Studies of cancer initiation and promotion in mice suggested that T-2 toxin is not likely to be a potent carcinogen. A working group convened by IARC in 1993 evaluated the same experimental data and concluded that T-2 toxin is not classifiable with regard to its carcinogenicity to humans (Group 3).

Tests for genotoxicity in microorganisms gave uniformly negative results with T-2 toxin. In cultured mammalian cells, however, low concentrations of T-2 toxin induced DNA strand breaks, unscheduled DNA synthesis, gene mutations, chromosomal aberrations and inhibition of intercellular communication across gap junctions. There was also evidence that T-2 toxin induced DNA strand breaks and chromosomal aberrations in vivo. It was unclear whether these effects were a consequence of interaction of T-2 toxin with genetic material or were secondary to inhibition of protein synthesis by this mycotoxin.

No embryotoxicity or gross fetal malformations were seen at intraperitoneal doses below 0.5 mg/kg of body weight per day. Continuous administration in the feed of concentrations equivalent to 0.22 and 0.45 mg/kg of body weight per day did not result in reproductive or gross developmental effects in CD-1 mice, although increased spleen weights were observed in male offspring of exposed dams at both doses.

The Committee noted that reduced feed intake is a potential confounder in studies of the toxicity of T-2 and HT-2 toxins. For example, in one study in mice in which pair-fed controls were used, changes in spleen weight, cell counts and lymphoproliferative response were observed that paralleled those in mice given T-2 toxin at 3 mg/kg of body weight per day. The spleen weight, cell counts and lymphoproliferative response were significantly lower in both the T-2 toxin-treated and pair-fed control groups than in control mice fed ad libitum.

Observations in humans

The available studies of adverse health effects in human populations were limited to a few investigations of outbreaks of acute poisoning, in which the reported effects included nausea, vomiting, pharyngeal irritation, abdominal pain and distension, diarrhoea, bloody stools, dizziness and chills. In subsequent investigations, analyses of limited numbers of suspected food or grain samples indirectly linked the outbreaks to T-2 toxin. The concomitant presence of T-2 toxin, deoxynivalenol, acetyl-deoxynivalenol and nivalenol was reported in one of these outbreaks, and the presence of these or other trichothecenes in other incidents could not be ruled out. A series of episodes of food-related poisoning referred to as alimentary toxic aleukia that occurred in 1931–47 in the former Soviet Union was associated with the ingestion of grain infected with moulds, in particular *F. poae* and *F. sporotrichioides*. The dominant pathological changes were necrotic lesions of the oral cavity, oesophagus and stomach and, in particular, pronounced leukopenia consisting primarily of bone-marrow hypoplasia and aplasia. The disease was lethal in a high proportion of cases. In investigations conducted three decades later, cultures implicated in the outbreak were shown to produce T-2 toxin.

Sampling protocols and analytical methods

No sampling guidelines for the determination of T-2 and HT-2 toxins in foods have been published, and details of the variation in sampling for these toxins have not been reported. Furthermore, no official

methods for the determination of T-2 and HT-2 toxins have been published, although some methods that have been validated in intra-laboratory studies have been reported.

The introduction of improved columns containing charcoal, alumina or modified diatomaceous earth for clean-up of samples before determination by chromatography has simplified and accelerated the analysis of T-2 and HT-2 toxins. Use of these columns in combination with gas chromatography and detection by electron capture or mass spectrometry after derivatization of the toxins is the most common technique for their quantification. These techniques allow determination of concentrations of a few nanograms per gram, even in complex food matrices. Effective clean-up of samples and derivatization are required for quantification by liquid chromatography with ultraviolet or fluorescence detection. The successful quantification of T-2 and HT-2 toxins by liquid chromatography with detection by mass spectrometry demonstrates the potential of this technique for direct and simultaneous determination of these mycotoxins; however, its high cost prohibits its use as a routine method. Thin-layer chromatography, particularly high-performance, can also be used for the determination of T-2 and HT-2 toxins. In addition, a few ELISA methods have been developed for screening of food commodities for T-2 toxin.

Although a variety of analytical methods are available for quantifying T-2 and HT-2 toxins, inter-laboratory comparisons have clearly shown that better analytical methods are needed for these mycotoxins, particularly with respect to their recovery, the accuracy and precision of their measurement, and their suitability for screening purposes. Wider availability of reference materials and regular international comparative studies are required for these mycotoxins in order to improve internal and external quality assurance.

Levels and patterns of contamination of food commodities

Data on the concentrations of T-2 and HT-2 toxins in food commodities were submitted by Brazil, China, Finland, Germany, Norway, Sweden and the United Kingdom, and were also obtained from the literature. The most frequently used technique for the quantification of T-2 and HT-2 toxins was gas chromatography with detection by electron capture, followed by thin-layer chromatography and ELISA, respectively.

Data from studies in which information on the sampling protocol or the analytical method was not provided were excluded from the evaluation. The remaining data were used only if the samples had been collected at random and if the analytical methods used were considered to have been adequate.

Data were available on 8918 grain samples, including barley, maize, oats, rice, rye and wheat, most of which had been collected in Europe. The incidence of contamination was 11% for T-2 toxin and 14% for HT-2 toxin; high concentrations of the two toxins were occasionally found together. Annual variations were reported in the levels of contamination of barley, oats and wheat. The mean concentrations in data sets in which positive samples of T-2 were found were 0.1–21 µg/kg in barley, 1.3–6.0 µg/kg for maize, 2.3–26 µg/kg for oats, 2.7–27 µg/kg for rice, 0.6 µg/kg for rye and 0.1–60 µg/kg for wheat. The mean concentrations in data sets in which positive samples of HT-2 were found were 0.4–15 µg/kg for barley, 2.4–14 µg/kg for maize, 3.7–20 µg/kg for oats, 26–100 µg/kg for rice, 0.03 µg/kg for rye and 0.2–20 µg/kg for wheat.

As T-2 and HT-2 toxins were not detected in a large proportion of samples from the United Kingdom, the distribution could not be derived. Use of distribution functions was also not practicable for the other data sets, mainly because of the way in which the data had been reported.

Food consumption and dietary intake assessment

The average intakes of T-2 and HT-2 toxins were estimated by multiplying the average concentrations in food commodities by the estimated average food consumption. For the latter, the GEMS/Food regional diets (*4*) were used. Most of the data on average concentrations of T-2 and HT-2 toxins that were available for the evaluation were pooled. Data on processed food products were excluded from the estimates of dietary intake. A total of 175 data points (representing 8410 individual samples) were included in the assessment, of which 147 were reported from countries in Europe; the remaining 28 data points represented only three commodities from the other four geographical regions. In view of the limited data on these toxins in regions other than Europe, the dietary intakes of T-2 and HT-2 toxins were estimated from the GEMS/Food European-type diet only, and the mean concentrations of T-2 and HT-2 toxins, weighted by sample size, were calculated for each commodity (barley, maize, oats, rice, rye and wheat) from data submitted by European countries. The intakes of T-2 and HT-2 toxins were estimated by multiplying the weighted mean concentrations in each commodity by the respective value for consumption in the European-type diet.

The total intake of T-2 toxin was estimated to be 7.6 ng/kg of body weight per day, wheat and barley being the major dietary sources. The total intake of HT-2 toxin was estimated to be 8.7 ng/kg of body weight per day, wheat, barley and oats being the most important

dietary sources. These estimates were based on the assumption that consumers choose foods randomly with respect to the distribution of concentrations of contaminants, which will approximate to the mean over time.

In general, more data on the occurrence of T-2 and HT-2 toxins in food commodities, particularly from geographical regions other than Europe, are required to make better estimates of intake. The Committee noted that the distribution of concentrations of contaminants for processed products might differ from that for raw cereals, given that contamination tends to be more homogeneous after processing. Despite the limited amount of data on T-2 and HT-2 toxin concentrations, the preliminary estimates of average contamination and dietary intake based on the GEMS/Food European-type diet proved to be useful. However, significant gaps were identified in the assessment, with respect to both the quality and geographical representativeness of the available data. Although it was not possible to estimate the intakes at high levels from the available data, such intakes may be approximated by multiplying the average intake by a factor of 2 for a single food commodity or 3 for the total diet.

Prevention and control

Preharvest measures to control or minimize infection by *Fusarium* spp. may also reduce the possibility of formation of T-2 and HT-2 toxins. Reducing the inoculum of *Fusarium* spp. in host debris and other reservoirs in the field appears to be an important control measure. Practices such as reduced tillage have been shown to increase the incidence of other trichothecenes and may also affect that of T-2 and HT-2 toxins. Good agricultural practice, such as immediate drying of grains after harvesting and proper storage, will prevent further contamination with T-2 and HT-2 toxins.

Physical, chemical and biological methods have been used to decontaminate grains containing trichothecenes, but few studies were available on any reduction in the concentration of T-2 or HT-2 toxins. Thermal processing is usually ineffective.

Evaluation

The Committee concluded that there was substantial evidence for the immunotoxicity and haematotoxicity of T-2 toxin in several species, and that these are critical effects after short-term intake. Only one long-term study was available, and this study alone was not suitable for establishing a tolerable intake. Nonetheless, on the basis of the critical effects in several short-term studies, the Committee concluded that the safety of food contaminated with T-2 toxin could be

evaluated from the LOEL of 0.029 mg/kg of body weight per day for changes in leukocyte and erythrocyte counts identified in the 3-week dietary study in pigs. This LOEL was the lowest LOEL for adverse effects in all of the available studies on T-2 toxin. It was considered to be close to a NOEL, as the reported effects were considered to be subtle and reversible. Furthermore, other studies in pigs showed no effects at this dose.

The Committee used this LOEL and a safety factor of 500 to derive a PMTDI of 60 ng/kg of body weight for T-2 toxin. A safety factor of 500 was used because there was no clear NOEL in the 3-week study in pigs and there were deficiencies in the database, including insufficient study of long-term administration of T-2 toxin and sex, species and individual variations in sensitivity.

The Committee further concluded that the toxic effects of T-2 toxin and its metabolite HT-2 toxin could not be differentiated and that the toxicity of T-2 toxin in vivo might be due, at least partly, to HT-2 toxin. Hence, HT-2 toxin was included in the PMTDI, resulting in a group PMTDI of 60 ng/kg of body weight per day for T-2 and HT-2 toxins, alone or in combination.

Recommendations

The Committee made the following recommendations:

- Further studies, preferably standard bioassays in rats and mice, with pair-fed controls, are needed to reduce the uncertainty in the evaluation of the carcinogenic potential of T-2 toxin.
- A longer-term study in pigs is needed in which a NOEL is identified, control groups are used to account for the potential effects of reduced feed consumption, and relevant, sensitive end-points of haematotoxicity and immunotoxicity are measured.
- Comparative studies on the toxicity and toxicokinetics of T-2 toxin in rodents, cats and pigs should be conducted, to clarify species differences in sensitivity.
- Studies are needed of the combined effects of T-2 and HT-2 toxins and other trichothecenes which contaminate foods consumed by humans.
- As little information on the concentrations of T-2 and HT-2 toxins in food commodities was available from geographical regions other than Europe, dietary intakes were estimated only on the basis of the GEMS/Food European-type diet. Dietary intakes in other geographical regions should be evaluated when more data on T-2 and HT-2 toxin concentrations become available. The average intake of T-2 toxin was estimated to be 8 ng/kg of body weight per day, and

that of HT-2 toxin was estimated to be 9 ng/kg of body weight per day. The total was therefore not expected to exceed the group PMTDI of 60 ng/kg of body weight per day. Nonetheless, more accurate information on human intake of T-2 toxin in various regions of the world, and improved analytical methods and reference materials for the determination of both toxins, are needed.

4. Recommendations

1. In view of the large number of food additives, food ingredients and contaminants requiring evaluation or re-evaluation, the important role that the recommendations of the Committee play in the development of international food standards and of regulations in many countries, and the need for maintaining consistency and continuity within the Committee, it is strongly recommended that meetings of the Joint FAO/WHO Expert Committee on Food Additives continue to be held at least once yearly to evaluate these substances.
2. The data on the health effects of mycotoxins in humans exposed by ingestion and inhalation are incomplete. The Committee recommended that appropriate research programmes be undertaken to elucidate the role of mycotoxins in human and animal disease. The outcome would be expected to improve risk assessments for individual mycotoxins.
3. From the data available to the Committee at its present meeting, it was clear that several mycotoxins may exist in many contaminated foods and food products. Moreover, contaminated feeds are frequently more toxic than the pure toxin in animals and humans, indicating possible interactions. The Committee therefore recommended further studies on mycotoxins occurring concomitantly in foods, their possible interactions, and how the toxicological significance of such interactions could be assessed.

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Annex 1
Reports and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on Food Additives

1. *General principles governing the use of food additives* (First report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 15, 1957; WHO Technical Report Series, No. 129, 1957 (out of print).
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Monographs containing summaries of relevant data and toxicological evaluations are available from WHO under the title:

Safety evaluation of certain mycotoxins in food. WHO Food Additives Series, No. 47, 2001; FAO Food and Nutrition Paper, No. 74, 2001.

INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

The preparatory work for toxicological evaluations of food additives and contaminants by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is actively supported by certain of the Member States that contribute to the work of the International Programme on Chemical Safety (IPCS).

The International Programme on Chemical Safety (IPCS) is a joint venture of the United Nations Environment Programme, the International Labour Organization, and the World Health Organization. One of the main objectives of the IPCS is to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment.

SELECTED WHO PUBLICATIONS OF RELATED INTEREST

Evaluation of certain food additives and contaminants.
Fifty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives.
WHO Technical Report Series, No. 901, 2001 (117 pages)

Safety evaluation of certain food additives and contaminants.
WHO Food Additives Series, No. 46, 2001 (392 pages)

Evaluation of certain food additives and contaminants.
Fifty-third report of the Joint FAO/WHO Expert Committee on Food Additives.
WHO Technical Report Series, No. 896, 2000 (136 pages)

Safety evaluation of certain food additives and contaminants.
WHO Food Additives Series, No. 44, 2000 (539 pages)

Evaluation of certain food additives and contaminants.
Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives.
WHO Technical Report Series, No. 884, 1999 (104 pages)

Safety evaluation of certain food additives and contaminants.
WHO Food Additives Series, No. 40, 1998 (538 pages)

Evaluation of certain food additives and contaminants.
Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives.
WHO Technical Report Series, No. 868, 1997 (77 pages)

Fumonisin B_1.
WHO Environmental Health Criteria, No. 219, 2000 (169 pages)

Human exposure assessment.
WHO Environmental Health Criteria, No. 214, 2000 (405 pages)

Principles for the assessment of risks to human health from exposure to chemicals.
WHO Environmental Health Criteria, No. 210, 1999 (130 pages)

Further information on these and other WHO publications can be obtained from Marketing and Dissemination, World Health Organization, 1211 Geneva 27, Switzerland.

This report presents the conclusions of a Joint FAO/WHO Expert Committee convened to assess the risks associated with the consumption of food contaminated with certain mycotoxins. The first part of the report contains a general discussion of the principles for evaluating mycotoxins in food, including those concerning analytical methods, sampling, data on food consumption and dietary intake, and prevention and control. The second part provides a summary of the Committee's evaluations of toxicological data on specific mycotoxins, including aflatoxin M_1, fumonisins B_1, B_2 and B_3, ochratoxin A, deoxynivalenol, and T-2 and HT-2 toxins.

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