### WHO FOOD ADDITIVES SERIES: 59

### Safety evaluation of certain food additives and contaminants

Prepared by the Sixty-eighth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)

AFLATOXINS (pages 305-356)

World Health Organization, Geneva, 2008

IPCS—International Programme on Chemical Safety

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#### AFLATOXINS: IMPACT OF DIFFERENT HYPOTHETICAL LIMITS FOR ALMONDS, BRAZIL NUTS, HAZELNUTS, PISTACHIOS AND DRIED FIGS

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#### 1. EXPLANATION

The aflatoxins (AFL) were evaluated by the Committee at its thirty-first, fortysixth, forty-ninth and fifty-sixth meetings (Annex 1, references 77, 122, 131 and 152). At the thirty-first meeting, the Committee considered AFL to be a potential human carcinogen and urged that dietary exposure to AFL be reduced to the lowest practicable levels, so as to reduce the potential risk as far as possible. The International Agency for Research on Cancer also concluded that naturally occurring AFL are carcinogenic to humans. At its forty-sixth meeting, the Committee considered estimates of the carcinogenic potency of AFL and the potential risk associated with their intake. In view of the value of such estimates, the Committee recommended that this task be continued at a subsequent meeting. At its forty-ninth meeting, the Committee analysed the effects of applying hypothetical standards for contamination in maize and groundnuts with AFL B1 (AFB1; 10 and 20 µg/kg) and concluded that reducing the standard from 20 to 10 µg/kg would not result in any observable difference in the rates of liver cancer. At its fifty-sixth meeting, the Committee concluded that the potency of AFL in hepatitis B virus surface antigen positive (HBsAg<sup>+</sup>) individuals is substantially higher than the potency in hepatitis B virus surface antigen negative (HBsAg<sup>-</sup>) individuals and 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.

The Codex Committee on Food Additives and Contaminants at its thirtyeighth session (Codex Alimentarius Commission, 2006) requested that the Committee conduct a dietary exposure assessment for total aflatoxins (AFT) from consumption of tree nuts (ready-to-eat)—in particular, almonds, Brazil nuts, hazelnuts and pistachios—and analyse the impact on dietary exposure of hypothetical maximum limits (MLs) of 4, 8, 10 and 15 µg/kg with consideration of the overall dietary AFT exposure, including consumption of maize and groundnuts. An additional request was received by the Committee to take into account in its assessment an additional hypothetical ML of 20 µg/kg.

In this evaluation, the sum of AFL B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> (AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub>) is referred to as AFT. The Committee agreed that this assessment applies to the edible parts of almonds (Codex food and feed classification number TN 0660) of cultivars grown from *Prunus amygdalus*, to Brazil nuts (TN 0662) ("white almonds") of the species *Bertholletia excelsa*, to the "common edible hazelnuts" (TN 0666) from *Corylus avellana* intended for direct consumption and to pistachio nuts (TN 0675) of cultivars grown from *Pistacia vera*. Additionally, the evaluation considered dried figs (DF 0297) from ripe fruits of cultivars grown from *Ficus carica* and intended for direct consumption. It does not apply to dried figs intended for processing.

AFL occurrence and concentration data, submitted from 22 European Union (EU) Member States for the European Food Safety Authority (EFSA) risk assessment requested by the European Commission (EC) in 2006, were available for this evaluation. Australia, Brazil, the Islamic Republic of Iran, Japan, Turkey, United Arab Emirates and the United States of America (USA) also submitted data on AFL contamination. In total, the Committee had access to over 100 000 data points for its analyses. Other data on contamination by these toxins have been taken from published literature, but they were not used to calculate dietary exposure because the disaggregated data were not available. Rather, they were used to reinforce the analysis made in the document.

The results of studies relevant to a toxicological evaluation, particularly metabolic and epidemiological studies, published since the last Joint FAO/WHO Expert Committee on Food Additives (JECFA) risk assessment of AFL, did not alter that assessment and indeed lent support to the conclusions reached in that assessment. They were not further considered in this current assessment.

#### 2. ANALYTICAL METHODS

#### 2.1 Chemistry

AFL are a group of related coumarin derivatives; the bifuran nucleus and the pentaheterocyclic arrangement lend rigidity to their structure. There are several known AFL (National Center for Biotechnology Information, 2007), but most of these are metabolites formed endogenously in animals and humans or AFL derivatives

formed during processing. The key AFL present in almonds, Brazil nuts, hazelnuts, pistachios and dried figs are (International Agency for Research on Cancer, 2002; European Food Safety Authority, 2007):

- AFB<sub>1</sub> (Chemical Abstracts Service [CAS] No. 1162-65-8), which has the synonyms 6-methoxydifurocoumarone; 2,3,6aα,9aα-tetrahydro-4-methoxycyclopenta[*c*]furo[3',2':4,5]furo[2,3-*h*][*I*]benzopyran-1,11-dione; and (6a*R-cis*)-2,3,6a, 9a-tetrahydro-4-methoxycyclopenta[*c*]furo[3',2':4,5]furo[2,3-*h*][*I*]benzopyran-1, 11-dione;
- AFB<sub>2</sub> (CAS No. 7220-81-7), which has the synonyms dihydroaflatoxin B<sub>1</sub>; 2,3,6aα,8,9,9aα-hexahydro-4-methoxycyclopenta[*c*]furo[3',2':4,5]furo[2,3-*h*][*I*]benzopyran-1,11-dione; (6a*R*-*cis*)-2,3,6a,8,9,9a-hexahydro-4-methoxycyclopenta[*c*]furo[3',2':4,5]furo[2,3-*h*][*I*]benzopyran-1,11-dione; and (6a*R*,9a*S*)-2,3, 6a,8,9,9a-hexahydro-4-methoxycyclopenta[*c*]furo[3',2':4,5]furo[2,3-*h*][*I*]benzopyran-1,11-dione (9CI);
- AFG<sub>1</sub> (CAS No. 1165-39-5), which has the synonyms 3,4,7aα,10aα-tetrahydro-5-methoxy-1*H*,12*H*-furo[3',2':4,5]furo[2,3-*h*]pyrano-[3,4-*c*][/]benzopyran-1,12-dione; (7a*R*-*cis*)-3,4,7a,10a-tetrahydro-5-methoxy-1*H*,12*H*-furo[3',2':4,5]furo-[2,3-*h*]pyrano-[3,4-*c*][/]benzopyran-1,12-dione; and (7a*R*,10a*S*)-3,4,7a,10a-tetrahydro-5-methoxy-1*H*,12*H*-furo[3',2':4,5]furo[2,3-*h*]pyrano[3,4-*c*][/]-benzopyran-1,12-dione (9CI);
- AFG<sub>2</sub> (CAS No. 7241-98-7), which has the synonyms dihydroaflatoxin G<sub>1</sub>; 3,4,7a $\alpha$ ,9,10,10a $\alpha$ -hexahydro-5-methoxy-1*H*,12*H*-furo[3',2':4,5]furo[2,3-*h*]-pyrano[3,4-*c*][/]benzopyran-1,12-dione; (7a*R*-*cis*)-3,4,7a,9,10,10a-hexahydro-5-methoxy-1*H*,12*H*-furo[3',2':4,5]-furo[2,3-*h*]pyrano[3,4-*c*][/]benzopyran-1,12-dione; and (7a*R*,10a*S*)-3,4,7a,9,10,10a-hexahydro-5-methoxy-1*H*,12*H*-furo-[3',2':4,5]furo[2,3-*h*]pyrano[3,4-*c*][/]benzopyran-1,12-dione; (72,3-*h*]pyrano[3,4-*c*][/]benzopyran-1,12-dione; (72,3-*h*]pyrano[3,4-*c*][/]benzopyran-1,12-dione; (73,2)-3,4,7a,9,10,10a-hexahydro-5-methoxy-1*H*,12*H*-furo-[3',2':4,5]furo[2,3-*h*]pyrano[3,4-*c*][/]benzopyran-1,12-dione (9CI).

Other information and chemical properties of some naturally occurring AFL and metabolites are included in previous evaluations (International Programme on Chemical Safety, 1979; Annex 1, references *77*, *122*, *131* and *153*).

#### 2.2 Description of analytical methods

AFL occurrence and concentration data, submitted from 22 EU Member States for the EFSA risk assessment requested by the EC in 2006, were available for this evaluation. In the EU, methods of analysis for the official control (enforcement, defence and referee purposes) of the levels of AFL and other mycotoxins in foodstuffs have to fulfil the analytical requirements laid down in Annex II of Commission Regulation EC No. 401/2006 (European Commission, 2006). These include, among others, criteria for laboratory blanks, recovery and precision and specify that the analytical result corrected for recovery shall be used for controlling compliance. Some details of the EU methodology can be found in European Food Safety Authority (2007).

Excellent reviews and descriptions of analytical methods for AFL are available (Gilbert & Anklam, 2002; Gilbert & Vargas, 2003, 2005; AOAC International, 2005; Krska et al., 2005; Krska & Molinelli, 2007). Specific references

on AFL analytical methodology not described in these reviews are, for tree nuts, Chan et al. (2004), Lee et al. (2004), Sapsford et al. (2006) and Aghamohammadi et al. (2007); and for dried figs, Stroka et al. (2000) and lamanaka et al. (2007).

Although there are several analytical methodologies described for AFL in tree nuts and dried figs, the submitted data were determined using only a few of them (Trucksess et al., 1994; VICAM, 1999; AOAC International, 2000a, 2000b; Ministério da Agricultura, Pecuária e Abastecimento, 2000; Stroka et al., 2000; Akiyama et al., 2001, 2002; R-Biopharm Rhône Ltd, 2002; Neogen, 2007). In the studies evaluated by the Committee at its present meeting, it was usually clear which AFL analytical method had been used.

In all of these methods, AFL are extracted from the samples with organic solvents, acetonitrile or methanol, in combination with small amounts of water. In the case of Brazil nuts or dried figs, instead of water, some samples are extracted with the organic solvent and an aqueous solution with potassium chloride or sodium chloride.

A major problem associated with most analytical methods for the determination of AFL is the presence of co-extractives with the potential to interfere with the analysis. Three different cleanup principles were used to assess AFL contamination in the submitted data. Formerly, liquid-liquid partitioning was the most commonly applied procedure for removing unwanted matrix components in the sample extract. This procedure, however, uses vast amounts of solvents, leads to losses and is time consuming. In the submitted data, only one country employed ammonium sulfate or copper(II) sulfate solutions to precipitate interfering coextractives before passage through celite and liquid-liquid partitioning with chloroform. Special attention has been given to the reduction of the use of chlorinated solvents by the employment of alternative extractants; as a consequence, new immunological or solid-phase cleanups have been developed. This could be the reason why in the other analytical methodologies, the extract interferences are eliminated, sometimes by passage through multifunctional columns (almonds, hazelnuts and pistachios), through a silica gel column before an immunoaffinity column (dried figs) or directly by immunoaffinity columns for all the matrices.

The extracts are then concentrated, usually by evaporation under nitrogen or vacuum. Afterwards, they are separated by thin-layer chromatography (TLC) or high-performance liquid chromatography (HPLC) or measured directly on a fluorometer (almonds, hazelnuts and pistachios) or a microwell reader (for all four tree nuts). AFL are visualized by TLC under ultraviolet (UV) light and quantified by visual comparison with known concentrations of standards. The methods using HPLC for AFL analyses are chosen because of their sensitivity and improved accuracy. The differences between methodologies used for the submitted data in this step are the type of detector used (UV light detectors or fluorescence detectors [FLD]) and the timing of derivatization (before or after passing through the HPLC column).

Recoveries of AFL in the different substrates sometimes affect the occurrence data more than the sensitivity of the quantification method. If the

extraction and cleanup steps are effective, it is also possible to obtain low limits of detection (LODs) or limits of quantification (LOQs) with low-cost methods such as TLC. During the JECFA meeting on several mycotoxins in 2001, mycotoxins considered for estimating intake were proposed to have recoveries greater than 60% at the LOQ level. It seems to be relatively easy to attain these levels with AFL, because all the available recoveries in the submitted data were greater than 70%, most of the recoveries being higher than 85%. Small recoveries could lead to underestimates of AFL exposure.

To define the contamination levels, one approach is to correct the results from the recoveries of the analytical methodology (commonly obtained by using one, two or three spiked levels), which may lead to error, as recoveries depend on the different contamination levels. To use this approach, it is necessary to estimate recoveries in more levels within the range of the analytical methodology. The Committee also concluded that it was better to restrict data used in the dietary exposure assessment to those with validated recoveries greater than 70% than to correct for lower recoveries.

It is important to point out that AFL recoveries for the analytical methodology chosen should be determined in the diverse raw matrices as well as in the products obtained from the different processes, as the variation in oil content in tree nuts and the different sugar concentrations in dried figs depend not only on the initial composition of the figs, but also on the drying process and sugar addition.

In the submitted data, LODs and LOQs for AFT were calculated in different ways. One method defined the LOD of AFT as twice the value of the LOD of AFB<sub>1</sub>, whereas the second used the sum of the LODs of AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub>. The Committee concluded that both definitions overestimate the LOD of AFT, resulting in conservative estimates of the exposure to AFL for the upper-bound estimate.

Quantification limits are sometimes called limits of reporting (LORs) or limits of determination, and detection limit is also called limit of determination. To avoid ambiguity, the following names and definitions are used in this monograph:

- *Limit of detection:* The LOD is the lowest concentration of a chemical that can be qualitatively detected using a specified laboratory method (i.e. its presence can be detected but not quantified).
- *Limit of quantification:* The LOQ is the lowest concentration of a chemical that can be detected and quantified, with an acceptable degree of certainty, using a specified laboratory method.

The Committee also noted that surveillance data should be accompanied by a clear description of the analytical method used; recoveries of the analytical methodology chosen should be specific to the food matrix tested; and LODs and LOQs should be provided with the definitions used to derive them. Efforts should be made to harmonize the nomenclature and the methodologies by which the LOD and LOQ are calculated.

Generally, for accurate exposure assessments, the LOD/LOQ should be as low as technically possible, since most foods will not contain detectable contamination and the value assigned to those samples will affect the estimated exposures. The LODs for AFL varied considerably between laboratories and different foods. The minimum LOD reported for AFB<sub>1</sub> in European Food Safety Authority (2007) was 0.0002  $\mu$ g/kg, and the maximum LOD was 10  $\mu$ g/kg, but usually the LOD was reported at around 0.1  $\mu$ g/kg.

With respect to the definition of the LOD of AFT, other methodological approaches should be tested, such as uniform, normal or lognormal distributions or methods based on quantiles (see, for example, Harter & Moore, 1966; Reid, 1981; Roger & Peacock, 1982; Gilliom & Helsel, 1986; Green & Crowley, 1986; Travis & Land, 1990; Hecht & Honikel, 1995; Vlachonikolis & Marriott, 1995; Giersbrecht & Whitaker, 1998; Korn & Tyler, 2001).

There is an increasing demand for screening techniques with quick and reliable results for field or industrial processors. Most of them are mentioned in the reviews of Gilbert & Vargas (2003) and Krska et al. (2005), although they are not fully validated for the products evaluated in this meeting. Progress was noted on the development of screening techniques for AFL using sometimes quite innovative approaches. Many of these techniques showed promise, such as lateral flow devices; these one-step tests take only 2–3 min to perform (Krska et al., 2005).

Continued progress on the development of improved sample cleanup techniques with good recoveries has been observed. An example of the application of a new cleanup column was an improvement of a fluorometric test kit that determines AFL in almonds, allowing it to be validated by the AOAC International Research Institute as a Performance Tested certified kit (Romer Labs, 2007).

The combination of liquid chromatography with mass spectrometry is one useful technique for the confirmation of the presence of AFL in foodstuffs. The improvement and availability of different types of mass spectrometers, such as quadrupole, ion-trap, time-of-flight instruments and combinations, not only allow the confirmation of the presence of mycotoxins, but will also lead to powerful multiresidue methods for mycotoxin analysis and also multisubstrate methods in the near future (Sulyok et al., 2006; Krska & Molinelli, 2007). A liquid chromatography/ atmospheric pressure chemical ionization tandem mass spectrometric method is described for the determination of AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub> in figs, but the percentage of AFL recovery from extracts spiked with AFL was lower than that observed in the submitted data using HPLC-FLD (Vahl & Jurgensen, 1998). As mycotoxins belong to different chemical families and have a broad range of polarities, sample pretreatment is a critical step; otherwise, significant losses may occur during extraction or cleanup. The number and types of mycotoxins analysed and their recoveries will be determined in part by the conditions during sample preparation and chromatographic separation. Although some methods are already implemented in routine analysis, the limited number of reference materials, high investment costs and lack of the required sensitivity could be a barrier to their use for AFL surveillance, because the LOD/LOQ should be as low as technically possible for accurate dietary exposure assessments. This is due to the fact that many foods that might be expected to contain AFL do not contain *detectable* AFL contamination, and the default value assigned to those censored samples will affect the estimated dietary exposures (upper-bound estimates only).

#### 3. SAMPLING PROTOCOLS

Almost all of the submitted data on AFL were derived using sampling plans designed for regulatory purposes (Institute of Standards and Industrial Research of Iran. 2000; Turkish Ministry of Agriculture and Rural Affairs and Ministry of Health, 2002, 2003; European Commission, 2006; Japanese Office of Imported Food Safety, 2006; Almond Board of California, 2007; United States Food and Drug Administration, 2007a, 2007b). The producing countries that submitted data to the Committee presented sampling plans similar to or the same as those following EC No. 401/2006 (European Commission, 2006) for the determination of AFL, which includes edible nuts and dried figs. It is not clear, however, how the sampling plan from the EC was derived, nor has the EU published the operating characteristic curves associated with this plan for the various commodities. Thus, producers and importers do not know the "producer risk" associated with operating this plan, nor it is clear to those concerned with food safety what the "consumer risks" are. It is probable that these sampling plans, which cover a range of commodities, such as dried figs, groundnuts and other nuts of very different sizes and for which in many instances little is known about AFL distribution, have all been derived by extrapolation from work on AFL distributions in lots of peanuts (Whitaker et al., 1995; Gilbert & Anklam, 2002), the most studied commodity. Adams & Whitaker (2004) derived the operating characteristic curves that predict the risk of misclassifying a lot associated with the EU sampling plans for raw shelled peanuts and ready-to-eat peanuts. Whitaker (2007) has also derived the operating characteristic curves for the EU plan for almonds, hazelnuts and pistachios.

It was observed that in some producing countries, there are two sampling plans: one for commodity to be exported to the EU, and the second for commodity to be exported to other countries with less strict regulations (Ministério da Agricultura, Pecuária e Abastecimento, 2002, 2004; Almond Board of California, 2007; United States Food and Drug Administration, 2007a, 2007b). There remains a need for harmonized sampling plans, both between different countries and within the same country.

For three of the products to be considered at the present meeting, the uncertainty evaluation was included in the Codex discussion paper CX/CF 07/1/9 on maximum levels of AFT in ready-to-eat almonds, hazelnuts and pistachios (Codex Committee on Contaminants in Food, 2007). It is interesting to point out that the uncertainty was not the same for the three analysed tree nuts (Codex Committee on Contaminants in Food, 2007: p. 46), and this result reinforces the need to evaluate the uncertainty in each particular product. It was proposed (Whitaker, 2006; Whitaker et al., 2006; Codex Committee on Contaminants in Food, 2007) that the performance of sampling plan designs for Brazil nuts be predicted using the distribution and uncertainty equations for almonds and adjusted using the count per unit mass for Brazil nuts. However, the counts per unit mass for shelled almonds,

#### AFLATOXINS (INTAKE FROM TREE NUTS AND DRIED FIGS)

hazelnuts and pistachios are 773, 1000 and 1600 kernels per kilogram, respectively, whereas those for Brazil nuts and dried figs are in the order of 220 and 45 per kilogram, respectively (Steiner et al., 1988; Whitaker, 2006; Whitaker et al., 2006). Besides the differences in the counts per unit mass, the type of contamination (i.e. the different relationship between AFB<sub>1</sub> and AFT found in almonds and Brazil nuts in European Food Safety Authority [2007: p. 27] and in the submitted data on dried figs) could also contribute to different uncertainties.

To evaluate the total uncertainty among sample test results, it is recommended that the procedure described by Ozay et al. (2006) and Whitaker et al. (2006) or a similar procedure be followed.

Dr Tom Whitaker (United States Department of Agriculture) and Dr Eugenia Vargas (Brazil Ministry of Agriculture) are evaluating the total variability associated with sampling, sample preparation and analytical test procedures for AFL in Brazil nuts, which will provide a base for statistically measuring the effectiveness of sampling plans in this nut. In connection with dried figs, only the contamination distribution is available, and research should be conducted to evaluate uncertainty in sample preparation and analytical test procedures.

The Committee noted that AFL sampling plans should be determined by data relating to contamination distributions and uncertainties within the particular foodstuff. The resulting knowledge of the uncertainty among sample test results should allow each country to refine its sampling plans using, for example, larger sample sizes and/or fewer analytical repetitions in order to meet harmonized criteria. The Committee noted that the data received for this analysis were robust.

The fact that the performance of a sampling plan is in part a function of the lot concentration at which the sampling plan is applied has led to a method by which the plan performance can be predicted by linking it to the AFL lot-to-lot distribution in the crop or the foodstuffs. With the submitted data, to analyse the lot-to-lot distribution of AFT and AFB<sub>1</sub> in relation to Brazil nuts, the log-transformed data on AFT only for values greater than the LOQ for the 2005 and 2006 Brazil nut productions were considered. The results suggest that to provide an adequate model for the lot-to-lot distribution, more data obtained using the same sampling methodology are needed.

It was not possible to identity the lot-to-lot AFL distribution for dried figs because of the small number of positive samples. Only 12.1% of the 53 692 (Turkey, Germany and the USA) analyses submitted tested positive.

There are only a few reports on AFL occurrence in processed products (Abdulkadar et al., 2002, 2004; Aycicek et al., 2005; Chun et al., 2007; Var et al., 2007), and there is a lack of distribution data on them. To improve sampling plans, it is necessary to consider the differences among product types (Samar et al., 2003; MacArthur et al., 2006). Foods are totally different in terms of mycotoxin distribution owing to the mixing effects during processing. There are processed products for which the raw material is ground, such as almonds, figs or hazelnut paste, and others for which it is not, such as hulva. The occurrence and distribution of AFL

contamination should be more homogeneous (probably a normal distribution curve) among packages in lots of pastes, for example, than in hulva lots.

#### 4. EFFECTS OF FOOD PROCESSING

Although AFL are highly stable, studies have indicated that they are degraded in contaminated food by heat treatment. The extent of AFL degradation achieved by roasting was analysed in different substrates, and the results showed that the extent of the reduction depends on the initial level of contamination, heating temperature, moisture content and heating duration (Rustom, 1997). Yazdanpanah et al. (2005) studied the effect of roasting on AFL in contaminated pistachio nuts. Roasting of pistachio nuts in two different ways, salted and unsalted, has traditionally been used to preserve and increase their shelf life. For example, the roasting of pistachio nuts at 150 °C for 30 min reduced AFL levels by 63% when the initial level was 44  $\mu$ g AFB<sub>1</sub>/kg, 24% when the initial level was 213  $\mu$ g AFB<sub>1</sub>/kg, 17% when the initial level was 21.9  $\mu$ g AFB<sub>1</sub>/kg and 47% when the initial level was 18.5  $\mu$ g AFB<sub>2</sub>/kg.

Among the submitted data on roasteries from the United Arab Emirates, a few samples in the last 6 months of 2006 were from pistachios, both roasted and unroasted. The roasted samples presented an average contamination of 7.6  $\mu$ g/kg, with a maximum of 70  $\mu$ g/kg (only one positive); the unroasted samples presented an average level of 127  $\mu$ g/kg (only one with a non-detected level), with a maximum of 430  $\mu$ g/kg.

Yazdanpanah et al. (2005) suggested that co-administration of some commonly used food additives with roasting may accelerate the destruction of AFL in pistachios even under more gentle roasting conditions. It was previously reported that boiling raw unshelled peanuts with 5% sodium chloride water solution can reduce AFL up to 80% (Farah et al., 1983). It has been suggested that the presence of water helps in opening the lactone ring in AFB<sub>1</sub> (by the addition of a water molecule to the ring) to form a terminal carboxylic acid. The terminal acid group thereafter undergoes heat-induced decarboxylation (Rustom, 1997). The presence of ionic salts will probably increase the extent of AFL degradation by heat in salted roasted nuts.

#### 5. LEVELS AND PATTERNS OF CONTAMINATION OF FOOD COMMODITIES

AFL concentration data for different food items, in particular tree nuts (edible portion) such as almonds, Brazil nuts, hazelnuts and pistachios, from 2001 to 2006 were evaluated for the current meeting from several producing and importing countries and regions (Australia, Brazil, EU, Islamic Republic of Iran, Japan, Turkey, United Arab Emirates and USA). The starting point for the compilation of the JECFA database from all data submitted by countries at this meeting was the EFSA EU monitoring database for AFL levels in food, compiled for the EFSA risk assessment requested by the EC in 2006 (European Food Safety Authority, 2007).

It was assumed that when information was missing from submitted data, the LOD for AFT was twice the LOD of AFB<sub>1</sub> and the LOQ was 3.3 times the LOD, as assumed in the European Food Safety Authority (2007) risk assessment.

Owing to the relatively low LOD obtained by Member States as reported in submitted data, the high number of data points reported below the LOD or LOQ for the four nuts included in this assessment (>60%) and the need to have understandable tables and figures, the upper-bound AFL concentration level was used in the dietary exposure estimates for reporting purposes (GEMS/Food-Euro, 1995).

#### 5.1 National surveillance data

#### 5.1.1 Australia

Australia submitted a report from the Department of Health (Government of Western Australia, 2005), with occurrence data for 109 individual samples of different nut samples prepared as ready-to-eat, for almond (19), Brazil nut (3), cashew (9), chestnut (4), hazelnut (15), macadamia (22), pecan (3), pine (3), pistachio (15) and walnut (16), based on food surveys of AFL conducted in 2003 and 2004. Sixteen per cent of these samples had quantified levels of AFL. AFL levels ranged between not detected (<2 µg/kg) and 11 µg/kg, with no level in excess of the 15 µg/kg limit in Australia.

#### 5.1.2 Brazil

Brazil submitted occurrence data for 329 individual results of Brazil nut lots to be exported to the EU (35 lots) and other countries (294 lots). The data refer to in-shell (processed and unprocessed) and shelled Brazil nuts, and in all cases only the edible portion (kernels) was analysed. AFL concentrations were reported as below the LOD for 85% of the exported nut lots. The most common LOD reported was 0.5–0.6  $\mu$ g/kg for AFB<sub>1</sub> and 0.3–0.5  $\mu$ g/kg for other AFL (AFB<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub>). Average concentration levels from Brazil nut lots were around 8.5  $\mu$ g/kg for AFB<sub>1</sub> and 20  $\mu$ g/kg for AFT (as shown in Table 4 below). The legal regulatory limit in Brazil is 30  $\mu$ g/kg for AFT for Brazil nut lots sold in the Brazilian market.

#### 5.1.3 European Union

A total of 49 748 analytical results for AFL were submitted from 22 EU Member States from 2000 to 2006 in response to a call for information issued by the EC.

After some data were discarded, as described in the EFSA scientific opinion (European Food Safety Authority, 2007), 34 326 analytical results were included in the EFSA analysis, submitted by Austria (1453), Belgium (434), Cyprus (212), Czech Republic (1464), Denmark (340), Estonia (349), Finland (1419), France (2719), Germany (5287), Greece (4847), Hungary (3750), Ireland (1765), Italy (6959), Latvia (549), Luxembourg (320), Slovakia (939), Slovenia (402), Spain (229), Sweden (211) and the United Kingdom (678). Most of these results came

from the official food control authorities in respective Member States and comprised both random and targeted sampling, thus introducing both uncertainty and variability. These analytical results comprised data for almonds (1768), Brazil nuts (622), hazelnuts (3163), pistachios (4069), figs (2067), other dried fruits (1472), other nuts (peanut, cashew, walnut, coconut) (9943), maize (961), cocoa products (248), oil of groundnut (peanut butter) (496), oil seeds (339), rice (541), other cereals (wheat, barley, oat, rye) (2304), spices (4704) and other foodstuffs (1028).

AFL concentration levels were reported as below the LOD for 25 451 of the European samples, whereas levels above the LOD were found in 8875 or 26% of the samples. The most common LOD reported was 0.1 or 0.2  $\mu$ g/kg for AFB<sub>1</sub> and 0.2 or 0.4  $\mu$ g/kg for AFT after some adjustment for missing values in relation to the LOD in some samples. The distribution of AFL contamination in foods in the EU is already described in the EFSA opinion (European Food Safety Authority, 2007).

#### 5.1.4 Islamic Republic of Iran

The Islamic Republic of Iran submitted the results of 6187 AFL monitoring data for 1849 pistachio nut lots (ready-to-eat) consigned to be exported from July 2004 to March 2007 (Secretariat of Iran Codex Committee on Contaminants in Food, 2007). The sensitivity of the analytical method was reported with an LOD of 0.2  $\mu$ g/kg for AFB<sub>1</sub> and 0.4  $\mu$ g/kg for AFT, with 24% of the data reported below the LOD. A linear regression coefficient of 1.13 (similar to the number reported in the EFSA opinion) was applied to estimate the level of AFT in a low number of samples (around 4%) in which AFB<sub>1</sub> data only were submitted.

#### 5.1.5 Japan

Using the Global Environment Monitoring System Food Contamination Monitoring and Assessment Programme (GEMS/Food) reporting format, Japan submitted aggregated monitoring results of inspection control for AFB<sub>1</sub> for pistachios (2342), walnuts (3427) and almonds (6706) and individual data for AFB<sub>1</sub> from a retail food survey for pistachios (159), almonds (103), walnuts (23) and hazelnuts (13). Sampling was performed during the 2000–2003 period for inspection control purposes and during the 1988–2006 period for the retail food survey (Sugita-Konishi et al., 2007). Both the LOD and LOQ ranged between 0.05 and 0.2  $\mu$ g/kg for AFB<sub>1</sub> and between 0.1 and 1  $\mu$ g/kg for AFT.

Results from monitoring data for AFB<sub>1</sub> for hazelnuts, almonds and pistachios were reported as 29.4, 9.4 and 7.4 µg/kg, respectively, with less than 3% of values below the reporting limits (LOD and LOQ), assumed to be zero for a lower-bound estimate. For the retail food survey, average AFB<sub>1</sub> and AFT levels for pistachios were reported to be 5.8 and 6.1 µg/kg, respectively, and for almonds, 0.02 and 0.03 µg/kg, respectively; the reporting limit values (LOD and LOQ) were assumed to be zero for a lower-bound estimate, where 96% of the results for pistachios and 92% of the results for almonds were less than the LOD or LOQ.

The present regulatory limit in Japan is 10  $\mu$ g/kg for AFB<sub>1</sub> in all foods.

#### 5.1.6 Turkey

Turkey submitted occurrence data for 37 622 samples of dried figs to be exported for the 2003–2006 period (Turkish Ministry of Agriculture and Rural Affairs, 2007). AFL concentrations were reported as below the reporting limit (LOD and LOQ) for 8% of the dried fig samples. The most common LOD reported for AFB<sub>1</sub> was 0.2–0.3  $\mu$ g/kg, and the LOD ranged between 0.1 and 0.4  $\mu$ g/kg for the other AFL (AFB<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub>). Average AFL concentrations for Turkish exported dried figs were around 0.6  $\mu$ g/kg for AFB<sub>1</sub> and 1.0  $\mu$ g/kg for AFT (as shown in Table 5 below).

#### 5.1.7 United Arab Emirates

AFL data submitted by the United Arab Emirates were the results of AFL tests (AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub>) in food and feed for 2005 conducted in the Food and Environment Laboratory of Dubai Municipality. All samples were from retailers (roasteries, general markets and hypermarkets). Ninety-nine per cent of imported samples of nuts available in the Emirate of Abu Dhabi in 2005 were from both the Dubai and Sharjah ports. The method of analysis was per SOP-FE-2320 using immunoaffinity column cleanup with HPLC-FLD based on AOAC International official methods (AOAC International, 2000a). The sensitivity of the analytical method was reported with an LOD of 0.7 µg/kg for AFB1 and 1.0 µg/kg for AFT. A total of 591 individual results for AFB<sub>1</sub> and AFT were provided for other nuts (groundnuts) (62%), pistachios (17%), sweet products containing nuts (19%), butter of Karité nut (5%), cocoa products (3%) and dried fruits (<1%). Forty per cent of those data were reported below the LODs. Average concentrations in AFB1 and AFT were reported at the upper-bound level to be 13.0 and 17.5  $\mu$ g/kg for other nuts, 49.1 and 53.4 µg/kg for pistachios, 11.6 and 12.8 µg/kg for sweet products, 8.3 and 10 µg/kg for butter of Karité nut, 3.7 and 4.7 µg/kg for cocoa products and 8.9 and 10.4 µg/kg for dried figs, respectively.

#### 5.1.8 United States of America

AFL levels in domestic and imported samples of tree nuts (almonds, Brazil nuts, hazelnuts and pistachios) and dried figs were provided at this meeting by the United States Food and Drug Administration (2006). Samples were collected during routine inspections of firms that stored and/or distributed domestic and imported foods during the 1996–2006 period. In total, 1310 results for tree nuts—almonds (44%, with more than 80% from domestic), Brazil nuts (15%), hazelnuts (16%) and pistachios (25%, with more than 65% from domestic)—and 103 for dried figs from domestic and imported samples were submitted. The LOQ for AFT was about 1  $\mu$ g/kg, with less than 10% of data reported to be below this limit. The present regulatory limit in the USA for AFT in tree nuts is 20  $\mu$ g/kg.

## 5.2 International surveillance data from countries importing tree nuts and dried figs

The Committee decided at this meeting to base the assessment of the impact of different MLs for AFT of 4, 8, 10, 15 and 20  $\mu$ g/kg on dietary exposure estimates on data submitted from countries that were producers of almonds, Brazil nuts, hazelnuts, pistachios and dried figs only, to best represent AFL contamination levels of these products prior to import restrictions.

#### 5.2.1 Almonds

The main almond producers in the world are the USA (42%), Spain (13%), the Syrian Arab Republic (8%), Italy (7%) and the Islamic Republic of Iran (5%) (FAOSTAT, 2007). The statistical distribution of AFB<sub>1</sub> and AFT levels (mean, coefficient of variation [CV] and percentiles) for almonds from producers is given in Table 1, as well as the impact of different MLs for AFT (4, 8, 10, 15 and 20  $\mu$ g/kg) on this distribution and the corresponding proportion of rejected samples from the world market for each scenario. Data were also submitted from the USA (568), EU (1766) and Japan (56) as importing countries. Mean concentration levels for AFT were not significantly different between the USA and other countries (1.6 vs 2.0  $\mu$ g/kg, *P* < 0.001), where 82% of data were reported to be below the reporting limits (LOD and LOQ).

Data in Table 1 show that the actual mean concentration of AFT in almonds from the main export country markets is around 2  $\mu$ g/kg. Setting MLs for AFT from 20 to 4  $\mu$ g/kg should result in mean concentrations of AFT approximately 2–3 times lower than the actual mean concentration of AFT (from 0.8 to 0.6  $\mu$ g/kg vs 2  $\mu$ g/kg). The proportion of rejected almond samples from the world market would be between 1% for an ML set at 20  $\mu$ g/kg and 3% for an ML set at 4  $\mu$ g/kg.

#### 5.2.2 Brazil nuts

The main producing region in the world is South America (Brazil, Bolivia, Ecuador and Peru) (International Tree Nut Council, 2002). The statistical distribution of AFB<sub>1</sub> and AFT levels (mean, CV and percentiles) for Brazil nuts from producers is given in Table 2, as well as the impact of different MLs for AFT (4, 8, 10, 15 and 20  $\mu$ g/kg) on this distribution and the corresponding proportion of rejected samples from the world market for each scenario. Data were submitted from Brazil (329) for the 2005–2006 period, where AFL concentrations were reported as below the LOD for 85% of the exported nut lots.

Data in Table 2 show that the actual mean concentration of AFT in Brazil nut lots for export is around 20  $\mu$ g/kg. Setting MLs for AFT going from 20 to 4  $\mu$ g/kg should result in mean concentrations of AFT approximately 10 times lower than the actual mean concentration of AFT (from 2.4 to 1.7  $\mu$ g/kg vs 20  $\mu$ g/kg). The proportion of rejected Brazil nut lots from the world market would be between 11% for an ML set at 20  $\mu$ g/kg and 17% for an ML set at 4  $\mu$ g/kg.

samples ti	om the	world m	arket											
Scenario	Type	No. of	Mean	CV (%) <sup>b</sup>	AFL c	ontent (	(pg/kg)							% of rejected samples
		aunues	(Ry/RH)		P5	P25	P50	P60	P75	P90	P95	P97.5	Max.	
All data	AFB <sub>1</sub>	2390	1.3	1189	0.1	0.1	0.2	0.2	0.5	0.5	1.5	4.1	575	0
	AFT		2.0	940	0.1	0.2	0.4	0.4	1.0	1.0	2.5	6.2	579	
ML 20 µg/kg	AFB <sub>1</sub>	2361	0.5	260	0.1	0.1	0.2	0.2	0.5	0.5	1.2	2.1	16	t
	AFT		0.8	206	0.1	0.2	0.4	0.4	1.0	1.0	1.8	3.3	18	
ML 15 µg/kg	AFB <sub>1</sub>	2354	0.4	234	0.1	0.1	0.2	0.2	0.5	0.5	1.1	2.0	13	0
	AFT		0.7	185	0.1	0.2	0.4	0.4	1.0	1.0	1.6	3.0	15	
ML 10 µg/kg	AFB <sub>1</sub>	2337	0.4	188	0.1	0.1	0.2	0.2	0.5	0.5	1.0	1.5	10	0
	AFT		0.7	143	0.1	0.2	0.4	0.4	1.0	1.0	1.4	2.4	10	
ML 8 µg/kg	AFB <sub>1</sub>	2325	0.3	152	0.1	0.1	0.2	0.2	0.5	0.5	0.9	1.3	9	б
	AFT		0.6	119	0.1	0.2	0.4	0.4	1.0	1.0	1.3	2.1	8	
ML 4 µg/kg	AFB <sub>1</sub>	2307	0.3	121	0.1	0.1	0.2	0.2	0.5	0.5	0.8	1.2	4	ε
	AFT		0.6	97	0.1	0.2	0.4	0.4	1.0	1.0	1.2	1.7	5 2	
Max., maxim	um; Px,	xth percent	tile.											

<sup>a</sup> Concentrations of less than the LOD or LOQ have been assumed to be equal to those limits.

<sup>b</sup> Coefficient of variation (standard deviation divided by mean, %).

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Table 2. Summary of the impact of different proposed MLs (4, 8, 10, 15 and 20 μg/kg) for AFT on the statistical distribution of
$\Lambda$ FB <sub>1</sub> and $\Lambda$ FT content (upper-bound scenario) in Brazil nuts for 2005–2006, including the predicted proportion of rejected
amples from the world market

Scenario	Type	NO. 01					(By/Brl)							von rejected adminited
		samples	"(pa/kg)		P5	P25	P50	P60	P75	P90	P95	P97.5	Мах.	
All data	AFB <sub>1</sub>	329	8.5	406	0.50	0.5	0.5	0.5	0.6	14.9	44.7	71.6	425	0
	AFT		19.8	399	1.60	1.6	1.6	1.6	1.7	21.9	114.9	161.1	873	
ML 20 µg/kg	1 AFB1	292	1.0	205	0.50	0.5	0.5	0.5	0.5	1.3	4.0	6.1	18	11
	AFT		2.4	119	1.60	1.6	1.6	1.6	1.6	3.0	6.9	12.1	19	
ML 15 µg/kg	1 AFB1	286	0.8	158	0.50	0.5	0.5	0.5	0.5	1.0	2.0	4.0	12	13
	AFT		2.1	87	1.60	1.6	1.6	1.6	1.6	2.5	3.9	6.9	15	
ML 10 µg/kg	1 AFB1	281	0.7	47	0.50	0.5	0.5	0.5	0.5	0.6	1.6	3.2	ъ 2	15
	AFT		1.9	27	1.60	1.6	1.6	1.6	1.6	1.7	3.1	4.2	6	
ML 8 µg/kg	AFB <sub>1</sub>	277	0.6	77	0.50	0.5	0.5	0.5	0.5	0.6	1.3	1.7	4	16
	AFT		1.8	32	1.60	1.6	1.6	1.6	1.6	1.7	3.0	3.5	9	
ML 4 µg/kg	AFB <sub>1</sub>	272	0.6	43	0.50	0.5	0.5	0.5	0.5	0.6	1.0	1.4	0	17
	AFT		1.7	21	1.60	1.6	1.6	1.6	1.6	1.7	2.5	3.0	4	

<sup>a</sup> Concentrations of less than the LOD or LOQ have been assumed to be equal to those limits. <sup>b</sup> Coefficient of variation (standard deviation divided by mean, %).

#### 5.2.3 Hazelnuts

Turkey is the primary producing country for hazelnuts, covering approximately 70% of the world market (FAOSTAT, 2007), but the Committee received no data on AFT levels in hazelnuts from Turkey; therefore, the Committee chose to use all of the submitted data supplied by the EU (3163), USA (215) and Japan (6) for its analyses on the distribution statistics for AFB<sub>1</sub> and AFT concentrations. Seventy-seven per cent of the data were reported to be below the reporting limits (LOD and LOQ).

The statistical distribution of AFB<sub>1</sub> and AFT levels (mean, CV and percentiles) for hazelnuts is given in Table 3, as well as the impact of different MLs (4, 8, 10, 15 and 20  $\mu$ g/kg) for AFT on this distribution and the corresponding proportion of rejected samples from the world market for each scenario. The actual mean concentration of AFT in hazelnuts from the main importing country markets was around 2  $\mu$ g/kg. Setting MLs for AFT for hazelnuts from 20 to 4  $\mu$ g/kg should result in mean concentrations approximately 2–4 times lower than the actual mean concentration of AFT in hazelnuts (from 1 to 0.6  $\mu$ g/kg vs 2  $\mu$ g/kg). The proportion of rejected hazelnut samples from the world market would be between 1% for an ML set at 20  $\mu$ g/kg and 7% for an ML set at 4  $\mu$ g/kg.

#### 5.2.4 Pistachios

The main country producer for which data were submitted was the Islamic Republic of Iran. This country accounts for around 65% of the world's export market for pistachios (FAOSTAT, 2007).

The statistical distribution of AFB<sub>1</sub> and AFT levels (mean, CV and percentiles) for pistachios from the Islamic Republic of Iran is given in Table 4, as well as the impact of different MLs (4, 8, 10, 15 and 20  $\mu$ g/kg) for AFT on this distribution and the corresponding proportion of rejected samples from the world market for each scenario. The actual mean concentration of AFB<sub>1</sub> or AFT in pistachios is around 50  $\mu$ g/kg. Setting MLs for AFT from 20 to 4  $\mu$ g/kg should result in mean concentrations approximately 10–50 times lower than the actual mean concentration (from 4 to 1  $\mu$ g/kg vs 50  $\mu$ g/kg). The proportion of rejected pistachio samples from the world be between 40% for an ML set at 20  $\mu$ g/kg and 60% for an ML set at 4  $\mu$ g/kg.

Table 3. Summary of the impact of different proposed MLs (4, 8, 10, 15 and 20 μg/kg) for AFT on the statistical distribution of
$\Lambda$ FB <sub>1</sub> and AFT content (upper-bound scenario) in hazeInuts for 1996–2007, including the predicted proportion of rejected
amples from the world market

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Scenario	Type	No. of	Mean	CV (%) <sup>b</sup>	AFL C	ontent	(hg/kg)							% of rejected samples
		samples	-(By/Brl)		P5	P25	P50	P60	P75	P90	P95	P97.5	Мах.	
All data	AFB <sub>1</sub>	3383	1.0	783	0.04	0.1	0.2	0.2	0.5	1.3	2.8	4.5	334	0
	AFT		1.9	714	0.08	0.2	0.4	0.4	1.0	2.7	6.0	9.9	630	
ML 20 µg/kg	AFB <sub>1</sub>	3340	0.5	241	0.04	0.1	0.2	0.2	0.5	1.1	2.3	3.4	17	1
	AFT		1.0	222	0.08	0.2	0.4	0.4	1.0	2.2	4.4	7.5	20	
ML 15 µg/kg	AFB <sub>1</sub>	3317	0.5	217	0.04	0.1	0.2	0.2	0.4	1.0	2.1	3.0	14	7
	AFT		0.9	198	0.08	0.2	0.4	0.4	0.9	2.1	3.9	6.1	15	
ML 10 µg/kg	AFB <sub>1</sub>	3285	0.4	194	0.04	0.1	0.2	0.2	0.4	1.0	1.8	2.9	6	ε
	AFT		0.8	178	0.08	0.2	0.4	0.4	0.8	1.9	3.6	4.9	10	
ML 8 µg/kg	AFB <sub>1</sub>	3246	0.4	171	0.04	0.1	0.2	0.2	0.4	0.8	1.6	2.1	7	4
	AFT		0.7	156	0.08	0.2	0.3	0.4	0.7	1.7	3.1	3.8	8	
ML 4 µg/kg	AFB <sub>1</sub>	3161	0.3	142	0.04	0.1	0.2	0.2	0.3	0.6	1.1	1.6	4	7
	AFT		0.6	126	0.08	0.2	0.3	0.4	0.6	1.2	2.2	3.0	4	
		:												

Max., maximum; Px, xth percentile.

<sup>a</sup> Concentrations of less than the LOD or LOQ have been assumed to be equal to those limits.  $^{\mathrm{b}}$  Coefficient of variation (standard deviation divided by mean, %).

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Scenario	Type	No. of	Mean	CV (%) <sup>b</sup>	AFL o	ontent	(hg/kg)							% of rejected samples
		samples	(pg/gg/		P5	P25	P50	P60	P75	P90	P95	P97.5	Max.	I
All data	AFB <sub>1</sub>	1849	49.2	216	0.2	1.2	8.6	17.8	46.8	133.6	243.7	331.0	1411.0	0
	AFT		54.4	212	0.4	1.2	9.6	19.7	51.2	150.9	269.5	368.1	1418.8	
ML 20 µg/kg	I AFB1	1112	3.95	120	0.2	0.2	2.0	2.9	5.9	11.7	14.7	16.4	19.2	40
	AFT		4.35	118	0.4	0.4	2.0	3.1	6.5	12.8	16.0	17.4	19.9	
ML 15 µg/kg	I AFB1	1036	3.07	116	0.2	0.2	1.6	2.5	4.7	8.9	10.6	12.0	14.3	44
	AFT		3.40	113	0.4	0.4	1.7	2.6	5.0	9.8	12.1	13.2	15.0	
ML 10 µg/kg	I AFB1	935	2.20	111	0.2	0.2	1.2	2.0	3.4	6.2	7.7	8.3	9.4	49
	AFT		2.43	106	0.4	0.4	1.3	2.1	3.6	6.8	8.4	9.0	9.9	
ML 8 µg/kg	AFB <sub>1</sub>	878	1.81	107	0.2	0.2	1.0	1.7	2.8	4.8	5.9	6.5	7.7	53
	AFT		2.01	101	0.4	0.4	1.0	1.8	3.0	5.3	6.5	7.1	8.0	
ML 4 µg/kg	AFB <sub>1</sub>	722	1.06	100	0.2	0.2	0.5	0.9	1.8	2.8	3.2	3.4	4.0	61
	AFT		1.20	86	0.4	0.4	0.6	1.0	1.9	2.9	3.4	3.6	4.0	
													z	

Max., maximum; Px, xth percentile.

<sup>a</sup> Concentrations of less than the LOD or LOQ have been assumed to be equal to those limits. <sup>b</sup> Coefficient of variation (standard deviation divided by mean, %). 323

#### 5.2.5 Dried figs

Turkey is the main country producing dried fruits, covering approximately 63% of the world market (Seker, 2007). The statistical distribution of AFB<sub>1</sub> and AFT levels (mean, CV and percentiles) for dried fruits from producers is given in Table 5, as well as the impact of different MLs (4, 8, 10, 15 and 20  $\mu$ g/kg) for AFT on this distribution and the corresponding proportion of rejected samples from the world market for each scenario. A large number of data (40 822 individual data) were provided for dried figs at this meeting by Turkey for the 2003–2006 period.

Data in Table 5 show that the actual mean concentration of AFT in dried figs from the main export country market is around 1.0  $\mu$ g/kg. Setting MLs for AFT going from 20 to 4  $\mu$ g/kg should result in mean concentrations approximately 2 times lower than the actual mean concentration of AFT (from 0.6 to 0.4  $\mu$ g/kg vs 1.0  $\mu$ g/kg). The proportion of rejected dried fruit samples from the world market would be between 1% for an ML set at 20  $\mu$ g/kg and 3% for an ML set at 4  $\mu$ g/kg.

# 5.3 Summary of aflatoxin occurrence and levels in food commodities and the potential effect of MLs in almonds, Brazil nuts, hazelnuts, pistachios and dried figs

AFL occurrence data on almonds, Brazil nuts, hazelnuts, pistachios and dried figs were obtained from both producing and importing countries. The Committee decided to base the assessment of the impact of different MLs (4, 8, 10, 15 and 20 µg/kg) for AFT for almonds, Brazil nuts, hazelnuts, pistachios and dried figs on data provided by producing countries, as these are more likely to represent the actual occurrence of AFL in the commodities. The primary producing countries or regions (FAOSTAT, 2007) were, for almonds, the USA (42% of the world market); for Brazil nuts, South America (100%); for hazelnuts, Turkey (70%); for pistachios, the Islamic Republic of Iran (65%); and for dried figs, Turkey (63% for dried fruits). Turkey is the primary producing country for hazelnuts, but the Committee received no data on AFT levels in hazelnuts from Turkey; therefore, the Committee chose to use all of the submitted data supplied by the EU, USA and Japan for its analyses.

The mean concentrations of AFT in nuts and dried figs in the main producing countries were, for almonds, 2  $\mu$ g/kg; for Brazil nuts, 20  $\mu$ g/kg; for hazelnuts, 2  $\mu$ g/kg; for pistachios, 54  $\mu$ g/kg; and for dried figs, 1  $\mu$ g/kg. The effects of the theoretical full enforcement of MLs (all samples above the ML would be excluded from the distribution) at 20, 15, 10, 8 and 4  $\mu$ g/kg are shown in Table 6. The reductions in mean AFT concentrations would be approximately 2- to 3-fold for almonds, 10-fold for Brazil nuts, 2- to 4-fold for hazelnuts, 10- to 50-fold for pistachios and 2-fold for dried figs. The corresponding proportion of rejected samples would be 1–3% for almonds, 11–17% for Brazil nuts, 1–7% for hazelnuts, 40–60% for pistachios and 1–3% for dried figs.

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samples fron	n the v	vorld	market											
Scenario Tyl	pe No.	of	Mean	CV (%) <sup>b</sup>	AFL of	ontent (	hg/kg)							% of rejected samples
	Sal	saidii	-(fix/firl)		P5	P25	P50	P60	P75	P90	P95	P97.5	Мах.	I
All data AF	B1 40 8	822	0.6	821	0.06	0.20	0.20	0.20	0.20	0.30	1.02	2.21	424	0
AF	F		1.0	689	0.07	0.40	0.40	0.40	0.40	0.56	1.63	3.40	424	
ML 20 µg/kg AF	B1 40 5	537	0.4	272	0.06	0.20	0.20	0.20	0.20	0.28	0.83	1.67	20	+
AF	F		0.6	230	0.07	0.40	0.40	0.40	0.40	0.56	1.27	2.59	20	
ML 15 µg/kg AF	B <sub>1</sub> 40.	431	0.3	230	0.06	0.20	0.20	0.20	0.20	0.28	0.77	1.54	15	+
AF	F		0.6	194	0.07	0.40	0.40	0.40	0.40	0.56	1.18	2.35	15	
ML 10 µg/kg AF	B <sub>1</sub> 40.	274	0.3	1031	0.06	0.20	0.20	0.20	0.20	0.28	0.69	1.60	10	-
AF	F		0.5	852	0.07	0.40	0.40	0.40	0.40	0.56	1.06	2.05	10	
ML 8 µg/kg AF	B1 40	167	0.3	168	0.06	0.20	0.20	0.20	0.20	0.28	0.64	1.20	8	2
AF	F		0.5	136	0.07	0.40	0.40	0.40	0.40	0.56	0.98	1.88	8	
ML 4 µg/kg AF	B <sub>1</sub> 39	731	0.3	113	0.06	0.20	0.20	0.20	0.20	0.28	0.51	0.85	ß	3
AF	F		0.4	89	0.07	0.20	0.40	0.40	0.40	0.56	0.75	1.30	4	

Max., maximum; Px, xth percentile.

<sup>a</sup> Concentrations of less than the LOD or LOQ have been assumed to be equal to those limits.

 $^{\mathrm{b}}$  Coefficient of variation (standard deviation divided by mean, %).

Scenario	Mean AFT	level, µg/kg (p	proportion of re	ejected sample	es, %)	
	No MLs	ML 20 µg/kg	ML 15 µg/kg	ML 10 µg/kg	ML 8 µg/kg	ML 4 µg/kg
Almonds	2.0 (0)	0.8 (1)	0.7 (2)	0.7 (2)	0.6 (3)	0.6 (3)
Brazil nuts	20 (0)	2.4 (11)	2.1 (13)	1.9 (15)	1.8 (16)	1.7 (17)
Hazelnuts	1.9 (0)	1.0 (1)	0.9 (2)	0.8 (3)	0.7 (4)	0.6 (7)
Pistachios	54 (0)	4.4 (40)	3.4 (44)	2.4 (49)	2.0 (53)	1.2 (61)
Dried figs	1.0 (0)	0.6 (1)	0.6 (1)	0.5 (1)	0.5 (2)	0.4 (3)

Table 6. Impact of different hypothetical ML scenarios for AFT on the mean AFT level and the corresponding proportion of rejected samples from the producing countries on the world market for tree nuts and dried figs

#### 5.4 Other foods contributing to total dietary aflatoxin exposure

Food sources other than tree nuts (almonds, Brazil nuts, hazelnuts and pistachios) and dried figs can contribute to the overall dietary exposure to AFT in humans. Occurrence data available from the last JECFA evaluation (Annex 1, reference *132*) and the EFSA opinion on AFL (European Food Safety Authority, 2007) were used with the corresponding amount of foods available for consumption from the GEMS/Food Consumption Cluster Diets to estimate AFT exposures (see section 6). In describing these contributing food sources, the Committee decided to focus mainly on food sources with detectable AFL levels that could be considered as being a contributing food source to average overall dietary exposure. This food prioritization was made by the Committee to avoid overestimating overall exposure to AFL. For example, for some cereals (except maize and rice), there were few detected AFL levels reported in EU data (below 5%) or too few data from only one country region (e.g. Brazil; Annex 1, reference *132*), which could not be extrapolated to other regions.

The majority of the data included in the estimation of dietary AFT exposure from other food sources came from the EU. The Committee noted that the European data do not reflect the actual mean concentration in other world regions for some foods considered here, as the mean concentration of AFT in the EU takes into account fewer highly contaminated samples as a result of existing EU MLs compared with regions with higher MLs or lack of enforcement.

Based on these considerations and submitted data in foods other than tree nuts and dried figs, the food commodities included in the average overall exposure were maize (961), groundnuts (i.e. peanuts, 9132) and other nuts (i.e. walnut, cashew, chestnut, macadamia, pecan, 1177), dried fruits other than figs (apricots, plums, grapes, dates and others, 1477), spices (4704), cocoa and cocoa products (cocoa mass, cocoa butter, cocoa powder, 266), rice (541), oil of groundnut (peanut butter, peanut cream, 496), oilseeds (339) and butter of Karité nut (29). A summary of the distribution of AFB<sub>1</sub> and AFT levels observed in these foods is given in Table 7. Most of the data considered in the dietary exposure assessment came from

#### AFLATOXINS (INTAKE FROM TREE NUTS AND DRIED FIGS)

the EU and the United Arab Emirates (for all data on butter of Karité nut, around 7% for cocoa products and 4% for other nuts). Because the European data do not reflect the actual mean concentration in other world regions for some foods, the mean upper-bound level has preferentially been used in the dietary exposure estimates in all 13 GEMS/Food Consumption Cluster Diets (see section 6).

This analysis indicates that the mean concentrations of AFB<sub>1</sub> and AFT were less than 1  $\mu$ g/kg for all foods except spices, cocoa products, groundnuts and butter of Karité nut, for which the mean levels ranged from 2 to 4  $\mu$ g/kg.

For rice, different concentrations were reported in different regions (producing and non-producing countries). A survey of AFB<sub>1</sub> in rice was conducted on 108 food-grade rice samples randomly collected during July and August 2002 in Seoul, Republic of Korea; the mean level was of the same order of magnitude as that described for EU data (Park et al., 2004). Naturally occurring AFB1 was found in 5 out of 108 (6%) samples of rice, with a mean level of 4.8 ng/g for samples with detected levels only. The LOD gave a mean upper bound of around 1.2 µg/kg in rice marketed in Seoul. JECFA also reported an average concentration of around 2 µg/kg in rice from Brazil, with 10% of data below the LOD (Annex 1, reference 132). The EU database on AFL reported a mean AFT level around 0.6–1.0 µg/kg in the EU (see Table 7). Another survey from Qatar reported AFT levels around 0.1-0.2 µg/kg (Abdulkadar et al., 2004), and other countries, including Japan and Argentina, reported no detected levels (Broggi et al., 1999a, 1999b; Sugita-Konishi et al., 2006). High AFT levels, such as those for peanuts or maize, have never been reported in rice; the highest reliably reported levels are less than 10 µg/kg. Because of these uncertainties in the data, rice was not included in estimating overall dietary exposures to AFT for comparison with the contribution from almonds, Brazil nuts, hazelnuts, pistachios and dried figs. In regions where rice is a major component of the diet, any low levels of AFT in rice may lead to its being a major contributor to total dietary exposure to AFT, even though that exposure may be low when compared with that in other regions.

For maize, a publication review on AFT occurrence data by Williams et al. (2004) reported concentrations 10 times higher than those described in Table 7 (around 33  $\mu$ g/kg on average in Bangladesh). JECFA also reported a mean level of 35  $\mu$ g/kg in maize from Brazil, with 51% of data above the LOD. AFB<sub>1</sub> in barley-based food was also reported at an average level of 4.1  $\mu$ g/kg in the Republic of Korea (Park et al., 2004).

For peanut oil, mean AFT levels were reported to be 40  $\mu$ g/kg in Senegal (Williams et al., 2004).

For groundnuts, AFB<sub>1</sub> and AFT concentrations reported here are about 4 times lower than concentrations reported by JECFA previously (Annex 1, reference *132*): respectively 2.4 and 3.3  $\mu$ g/kg vs 7–8.3 and 13–14  $\mu$ g/kg.

other than tree nuts and figs contributing	
s of AFB <sup>1</sup> and AFT contents in foods (	
ole 7. Summary of the statistical distributions	he total dietary exposure for 2000–2006
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Commodity	Type	No. of	$n < LOR^a$ (%)	dean (	µg/kg)	C C C	AFL cor	ntent⁰ (µ	g/kg)						
		samples	. =	ρ <sub>ρ</sub>	ub°	- (o/ )-	P5	P25	P50	P60	P75	P90	P95	P97.5	Max.
Maize	AFB <sub>1</sub>	961	86 C	.1	0.3	194	0.04	0.10	0.14	0.20	0.20	0.50	0.70	1.02	8
	AFT		0	0.2	0.4	149	0.08	0.20	0.24	0.40	0.40	0.50	1.00	1.54	6
Rice	AFB <sub>1</sub>	541	86 C	).5	0.8	391	0.04	0.10	0.20	0.50	0.80	1.00	2.00	3.00	57
	AFT		0	).6	1.0	366	0.08	0.20	0.40	0.50	0.80	1.00	2.00	4.57	60
Cocoa	AFB <sub>1</sub>	266	68 1	с. -	1.9	587	0.04	0.10	0.20	0.35	0.50	0.76	1.86	7.31	120
products	AFT		-	4.	2.1	557	0.08	0.20	0.40	0.50	0.50	1.40	1.88	7.77	138
Oilseeds	AFB <sub>1</sub>	339	89 C	).3	0.4	441	0.02	0.10	0.10	0.10	0.20	0.23	0.91	1.29	52
	AFT		0	.4	0.6	396	0.03	0.20	0.20	0.20	0.40	0.46	1.02	2.63	25
Dried fruits	AFB <sub>1</sub>	1477	93 C	0.1	0.3	388	0.03	0.10	0.10	0.20	0.20	0.80	1.00	1.00	33
other than figs	AFT		C	0.2	0.6	481	0.06	0.20	0.30	0.40	0.40	1.00	2.00	2.00	06
Groundnuts	AFB <sub>1</sub>	9132	83 2	2.3	2.4	834	0.03	0.10	0.10	0.20	0.20	1.00	4.64	13.03	935
	AFT		IJ	3.0	3.3	719	0.04	0.20	0.20	0.40	0.40	2.00	6.72	21.79	985
Other nuts	AFB <sub>1</sub>	1177	93 C	.8	1.0	1356	0.04	0.10	0.10	0.10	0.20	0.23	1.00	1.20	385
	AFT		-	<u>-</u> .	1.3	1170	0.08	0.20	0.20	0.20	0.40	0.46	1.92	2.00	402
Butter of Karité	AFB <sub>1</sub>	29	28 C	.4	3.7	157	0.50	0.70	1.62	1.87	3.33	8.32	16.0	21.40	25
nut	AFT		4	1.3	4.7	147	0.57	1.40	1.86	2.21	4.03	9.81	18.6	24.85	30

AFLATOXINS (INTAKE FROM TREE NUTS AND DRIED FIGS)

Table 7. (contd)

Commodity	Type	No. of	<i>n</i> < LOR <sup>a</sup> (%)	Mean (	(hg/kg)	CV /o/ Jd	AFL col	ntent <sup>e</sup> (µ	g/kg)						
		saliplies		p₀	ub°	_(0/)_	P5	P25	P50	P60	P75	P90	P95	P97.5	Max.
Oil of	AFB <sub>1</sub>	496	62	0.5	0.6	284	0.05	0.10	0.20	0.21	0.50	1.07	2.03	3.22	53
groundnut	AFT			0.8	0.9	275	0.08	0.20	0.33	0.40	0.60	1.80	3.14	4.42	30
Spices	AFB <sub>1</sub>	4704	09	1.3	1.5	312	0.04	0.10	0.20	0.40	1.00	3.10	6.60	9.30	96
	AFT		-	1.6	1.9	277	0.08	0.20	0.40	0.60	1.52	4.00	7.80	11.3	96
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<sup>a</sup> Limits of reporting (LOD or LOQ).

<sup>b</sup> Lower bound: concentrations of less than the LOD or LOQ have been assumed to be equal to 0 or the LOD.

° Upper bound: concentrations of less than the LOD or LOQ have been assumed to be equal to those limits.

<sup>d</sup> Coefficient of variation (standard deviation divided by mean, %).

<sup>e</sup> All the statistical distributions for AFL content in the table are expressed for the upper-bound scenario.

#### 6. ESTIMATED DIETARY EXPOSURE

#### 6.1 National assessments of dietary exposure for aflatoxins

Dietary exposure estimates were reported at this meeting for EU Member States from the EFSA opinion (European Food Safety Authority, 2007), by Japan (Sugita-Konishi et al., 2007) and also from the scientific literature.

#### 6.1.1 European Union

The EFSA opinion assessed the influence of changes to the MLs (4, 8 and 10  $\mu$ g/kg) for almonds, hazelnuts and pistachios on the overall dietary exposure to AFL. In the EFSA opinion, dietary exposure estimates were based on food consumption data from individual dietary records for nuts from representative Member States and on food consumption data from the GEMS/Food Consumption Cluster Diets database of the World Health Organization (2006) for all other foods. Mean AFT occurrence data used in its calculations were those reported in the EFSA opinion.

Dietary AFT exposure estimates for adult high consumers (United Kingdom data for vegan and vegetarians included) of almonds, hazelnuts and pistachios (95th percentile) were derived from a range of individual consumption surveys for four Member States (France, Germany, Spain and the United Kingdom) and mean occurrence data. Dietary exposure estimates ranged between 0.93 and 2.45 ng/kg body weight (bw) per day for the lower- to upper-bound estimates. Almonds, hazelnuts and pistachios contributed between 16% and 22% of the overall dietary exposure to AFL, equivalent to 0.16–0.55 ng/kg bw per day.

#### 6.1.2 Japan

AFB<sub>1</sub> dietary exposure estimates were assessed in Japan based on food consumption data from the 2005 National Health and Nutrition Survey for 2 consecutive days (17 827 individuals). Surveillance data on AFB<sub>1</sub> concentration levels were available from a retail food survey, with samples purchased in a random manner from local supermarkets and small retail shops in all parts of Japan from the summer of 2004 to the winter of 2006 (Sugita-Konishi et al., 2007). Foods analysed included peanut, peanut butter, chocolate, pistachio, spices, almond, job's tears tea and buckwheat. A probabilistic approach was used to simulate the dietary exposure distributions in each age group with three different scenarios of MLs of AFT in tree nuts (10, 15 and 20  $\mu$ g/kg), following the same methodology as described previously for the EFSA opinion and assuming a lognormal distribution for occurrence data.

Dietary exposure estimates for  $AFB_1$  were reported to range from 0.26 to 0.29 ng/kg bw per day at the 99.5th percentile.

#### 6.1.3 Republic of Korea

A calculated probable daily intake of AFB<sub>1</sub> for people of the Republic of Korea reported by Park et al. (2004) was based on a survey for AFB<sub>1</sub> conducted on 88 food-grade rice samples randomly collected during July and August 2002 in Seoul and from a review of published data for food commodities from the Republic of Korea. Dietary exposure estimates for the lower- to upper-bound range were 1.19–5.79 ng/kg bw per day. It was concluded that the dietary exposure of people of the Republic of Korea to AFB<sub>1</sub> from rice was the major contributor to the overall dietary exposure estimate for AFB<sub>1</sub> in the Republic of Korea (from 75% to 93%).

#### 6.1.4 Other countries

A review by Williams et al. (2004) described variations in mean dietary AFL intakes between countries, largely as a function of diet. Data assembled by Hall & Wild (1993) indicated that dietary exposure to AFL was 3.5–14.8 ng/kg bw per day in Kenya, 11.4–158.6 ng/kg bw per day in Swaziland, 38.6–183.7 ng/kg bw per day in Mozambique, 16.5 ng/kg bw per day in Transkei (now a region of South Africa), 4–115 ng/kg bw per day in The Gambia, 11.7–2027 ng/kg bw per day in the southern Guangxi province of China and 6.5–53 ng/kg bw per day in Thailand, whereas the exposure in the USA was lower, at 2.7 ng/kg bw per day. The exposure in Ghana, as measured from peanut consumption alone, was estimated to be 9.9–99.2 ng/kg bw per day (Awuah, 2000).

#### 6.2 International estimates of dietary exposure from the 13 GEMS/Food Consumption Cluster Diets

International dietary exposure estimates were reported at the 1998 JECFA meeting on AFL (Annex 1, reference *132*). The evaluation reported at a later meeting in 2000 (Annex 1, reference *153*) assessed mean dietary exposure estimates for AFT for the five GEMS/Food diets from maize and groundnuts only for four ML scenarios (no ML where no samples excluded, ML of 10, 15 and 20 µg/kg for groundnuts and maize).

Dietary exposure estimates ranged from 0.56 ng/kg bw per day (in Latin America) to 11 ng/kg bw per day (in Africa) for AFT and from 0.82 ng/kg bw per day (in Europe) to 22.7 ng/kg bw per day (in Africa) for AFB<sub>1</sub> when no samples were excluded. When samples were excluded above 20 or 10  $\mu$ g/kg, dietary exposure estimates ranged from 0.13 ng/kg bw per day (in Europe) to 1.15 ng/kg bw per day (in Africa) for AFT and from 0.3 ng/kg bw per day (in Europe) to 6.7 ng/kg bw per day (in Africa) for AFB<sub>1</sub>.

In these estimates, AFT from groundnut consumption contributed from 0.14 to 2.7 ng/kg bw per day, and from maize consumption, from 0.35 to 8.3 ng/kg bw per day, when no samples were excluded; and AFT from groundnut consumption contributed from 0.03 to 0.18 ng/kg bw per day, and from maize consumption, from 0.17 to 1.1 ng/kg bw per day, when samples were excluded above 20 or 10  $\mu$ g/kg.

Of the scenarios considered, the Committee had concluded that the greatest relative impact on estimated mean AFL levels is achieved by establishing a programme that would limit AFL contamination to less than 20  $\mu$ g/kg. Depending upon assumptions made when looking at the distribution of residues, some small incremental reductions can be achieved by limiting AFL levels to no more than 15 or 10  $\mu$ g/kg.

The new descriptions of the 13 GEMS/Food Consumption Cluster Diets (World Health Organization, 2006) and the actual AFL levels in foods moving in international trade considered in this assessment together provide a refinement of dietary exposure assessments for tree nuts and the relative contribution of other food sources to overall dietary exposure to AFL, which makes the diets and ML scenarios more relevant than those used for the previous evaluation by the Committee.

Tables 1 to 6 above summarize the impact of different hypothetical MLs (4, 8, 10, 15 and 20  $\mu$ g/kg) for AFT on the statistical distribution of AFB<sub>1</sub> and AFT contents in almonds, Brazil nuts, hazelnuts, pistachios and dried figs from various export countries for 2001–2007; and the statistical distribution of AFB<sub>1</sub> in foods other than tree nuts contributing to the overall exposure for various countries from 2000 to 2006. These were the best available occurrence data for use in assessing international intake estimates at this meeting.

In general, the food items analysed were well characterized, and it was possible to match sources, contamination and consumption to the 13 GEMS/Food Consumption Cluster Diets (Table 8). Mean concentration data were multiplied by the total mean consumption of the corresponding food category or subcategory reported, to derive mean total dietary exposure estimates per cluster diet for AFB<sub>1</sub> and AFT from all food sources.

A summary of the international mean total dietary exposure estimates for AFB<sub>1</sub> and AFT from all tree nuts (almonds, Brazil nuts, hazelnuts and pistachios), dried figs and other contributing food sources, expressed in nanograms per kilogram body weight per day for the 13 GEMS/Food Consumption Cluster Diets, under different ML scenarios (no MLs, MLs at 4, 8, 10, 15 and 20 µg/kg) is presented in Tables 9, 10 and 11. The corresponding contributions of tree nuts, dried figs and other food sources to overall mean dietary AFT exposure (in % AFT) are also presented. In this assessment, mean lower- and upper-bound scenarios have been used in making the dietary exposure estimates employing the 13 GEMS/Food Consumption Cluster Diets.<sup>13</sup> The lower bound was calculated using 0 for non-detects or the LOD for trace values, whereas the upper bound was calculated using either the LOD or LOQ.

<sup>&</sup>lt;sup>13</sup> Country assignments to the 13 Consumption Cluster Diets may be found at http://www.who.int/entity/foodsafety/chem/countries.pdf.

Table 8. Summary of the consumption data from 13 GEMS/Food Consumption Cluster Diets used for estimates of international dietary intake of AFB1 and AFT

Food commodities	Consu	mption (g	/person p	oer day)	for the 1	3 GEMS	/Food C	onsumpti	on Clust	er Diets			
	A	В	O	D	ш	ш	U	н	_	ſ	¥	_	X
Tree nuts	0.0	4.7	1.5	1.0	2.7	1.1	0.0	0.2	0.0	0.0	0.1	0.3	0.7
Almonds	0.0	1.9	1.0	0.0	1.0	0.8	0.0	0.1	0.0	0.0	0.0	0.3	0.3
Brazil nuts	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1
Hazelnuts	0.0	2.1	0.0	0.1	1.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.1
Pistachios	0.0	0.7	0.5	0.9	0.3	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.2
Dried figs	0.0	0.6	0.4	0.0	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.1
Dried fruits other than figs (grapes, apricots, plums, dates and others)	0.8	5.3	32.1	6.0	3.5	3.0	1.2	0.5	0.5	3.9	0.5	0.9	3.7
Maize	97.4	150.4	136.1	33.0	39.8	14.7	35.6	303.5	252.6	60.7	64.8	64.2	103.6
Groundnuts (shelled and in shell)	13.0	7.4	5.1	1.7	9.6	3.4	18.2	5.0	11.3	52.3	2.2	1.7	16.6
Other nuts (cashews, chestnuts, walnuts, pecans)	0.0	3.1	0.0	0.3	1.0	0.1	0.9	0.7	0.2	0.0	0.1	1.7	1.1
Oilseeds ( <i>lin, sesame, poppy,</i> melon, sunflower, rape)	15.3	61.5	31.3	51.2	57.4	33.2	17.6	17.3	18.4	9.9	3.9	61.1	21.7
Cocoa products (mass, powder, butter, other chocolate products)	1.0	4.4	1.3	1.8	10.4	9.9	1.0	3.0	1.1	0.9	2.1	2.9	7.5
Butter of Karité nut	1.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.3	2.8	0.0	0.0	0.0
Oil of groundnut	1.7	0.8	0.5	0.1	1.4	0.4	3.0	0.3	1.5	7.9	0.3	0.0	0.4
Spices	2.7	<del>1</del> .1	2.4	0.9	1.8	1.1	2.3	1.9	1.4	1.3	0.4	0.6	1.7

e to AFB <sub>1</sub> and AFT from all food sources for the 13 GEMS/Food Consumption	othetical ML scenarios for AFT (no MLs; MLs at 4, 8, 10, 15 and 20 µg/kg) in tree	al dietary AFT exposure
ire to AFB <sub>1</sub> and AFT from all food s	pothetical ML scenarios for AFT (n	otal dietary AFT exposure
ble 9. Mean estimates of dietary exposu	uster Diets taking into consideration hy	ts and the contribution of tree nuts to to

Scenario <sup>a</sup>		Mean di	etary ex <sub>l</sub>	oosure (r	ng/kg bw	per day)	for the -	13 GEMS	s/Food C	dunsuo	tion Clus	ter Diets		
		A	В	U	D	ш	ш	IJ	Т	_	ſ	¥		Σ
No ML	AFB <sub>1</sub>	0.9-1.2	1.7-2.3	1.1-1.7	1.2-1.4	1.3-1.7	0.6-0.8	1.0-1.1	1.0-1.9	1.1-1.8	2.3-2.8	0.3-0.5	0.6-0.9	1.3-1.8
	AFT	1.1-1.7	2.1-3.2	1.5-2.5	1.4-1.8	1.7-2.3	0.7-1.1	1.3-1.6	1.4-2.8	1.4-2.7	3.0-3.7	0.4-0.7	0.8-1.3	1.7-2.5
	All tree nuts (% AFT)	0.0	24.6	20.0	45.0	16.8	3.7	0.0	3.3	0.0	0.0	4.3	0.8	9.3
ML 20 µg/kg	AFB <sub>1</sub>	0.9-1.2	1.1-1.7	0.8-1.3	0.5-0.7	1.0-1.4	0.6-0.8	1.0-1.1	1.0-1.8	1.1-1.8	2.3-2.8	0.3-0.5	0.6-0.9	1.2-1.6
	AFT	1.1-1.7	1.5-2.5	1.1-2.0	0.7-1.1	1.3-2.0	0.7-1.1	1.3-1.6	1.3-2.7	1.4-2.7	3.0-3.7	0.4-0.7	0.8-1.3	1.5-2.3
	All tree nuts (% AFT)	0.0	4.7	2.6	6.3	3.2	1.6	0.0	0.3	0.0	0.0	0.6	0.3	1.1
ML 15 µg/kg	AFB <sub>1</sub>	0.9-1.2	1.1-1.7	0.7-1.3	0.5-0.7	1.0-1.4	0.6-0.8	1.0-1.1	1.0-1.8	1.1-1.8	2.3-2.8	0.3-0.5	0.6-0.9	1.2-1.6
	AFT	1.1-1.7	1.4-2.5	1.1-2.0	0.6-1.1	1.3-2.0	0.7-1.1	1.3-1.6	1.3-2.7	1.4-2.7	3.0-3.7	0.4-0.7	0.8-1.3	1.5-2.3
	All tree nuts (% AFT)	0.0	4.1	2.2	5.0	2.8	1.5	0.0	0.3	0.0	0.0	0.5	0.3	0.9
ML 10 µg/kg	AFB <sub>1</sub>	0.9-1.2	1.1-1.7	0.7-1.3	0.5-0.7	1.0-1.4	0.6-0.8	1.0-1.1	1.0-1.8	1.1-1.8	2.3-2.8	0.3-0.5	0.6-0.9	1.2-1.6
	AFT	1.1-1.7	1.4-2.5	1.0-2.0	0.6-1.1	1.3-2.0	0.7-1.1	1.3-1.6	1.3-2.7	1.4-2.7	3.0-3.7	0.4-0.7	0.8-1.3	1.5-2.3
	All tree nuts (% AFT)	0.0	3.3	1.7	3.6	2.3	1.4	0.0	0.2	0.0	0.0	0.4	0.2	0.7
ML 8 µg/kg	AFB <sub>1</sub>	0.9-1.2	1.1-1.7	0.7-1.3	0.5-0.7	1.0-1.4	0.6-0.8	1.0-1.1	1.0-1.8	1.1-1.8	2.3-2.8	0.3-0.5	0.6-0.9	1.2-1.6
	AFT	1.1-1.7	1.4-2.5	1.1-2.0	0.6-1.1	1.3-2.0	0.7-1.1	1.3-1.6	1.3-2.7	1.4-2.7	3.0-3.7	0.4-0.7	0.8-1.3	1.5-2.3
	All tree nuts (% AFT)	0.0	2.9	1.5	3.0	2.1	1.2	0.0	0.2	0.0	0.0	0.4	0.2	0.7
ML 4 µg/kg	AFB <sub>1</sub>	0.9-1.2	1.1-1.7	0.7-1.3	0.4-0.7	1.0-1.4	0.6-0.8	1.0-1.1	1.0-1.8	1.1-1.8	2.3-2.8	0.3-0.5	0.6-0.9	1.2-1.6
	AFT	1.1-1.7	1.4-2.5	1.1-2.0	0.6-1.1	1.3-2.0	0.7-1.1	1.3-1.6	1.3-2.7	1.4-2.7	3.0-3.7	0.4-0.7	0.8-1.3	1.5-2.3
	All tree nuts (% AFT)	0.0	2.3	1.1	1.9	1.7	1.1	0.0	0.1	0.0	0.0	0.4	0.2	0.5

<sup>a</sup> Lower- and upper-bound scenarios have been used in making the dietary exposure estimates for overall exposure and all tree nuts. The lower bound appropriate. "All tree nuts" includes dried figs, which contributed less than 0.3% of the dietary AFT exposure in all scenarios. % AFT is the contribution was calculated using 0 for non-detects or the LOD for trace values, whereas the upper bound was calculated using either the LOD or LOQ, as from almonds, Brazil nuts, hazelnuts, pistachios and dried figs to total dietary AFT exposure (upper-bound scenario only).

		Dietary ex	posure to A	FT (ng/kg b	w per day)	for 13 GE	MS/Food C	onsumptic	on Cluster	· Diets				
		A	В	C	D	Ш	ш	ŋ	н	_	ſ	¥	_	M
Overall exposu sources	ire from other	1.1-1.7	1.3-2.4	1.0-2.0	0.6-1.0	1.3-1.9	0.7-1.1	1.3-1.6	1.3-2.7	1.4-2.7	3.0-3.7	0.4-0.7	0.7-1.3	1.5-2.2
Mean dietary	Maize	0.2-0.7	0.4-1.0	0.3-0.9	0.1-0.2	0.1-0.3	0.04-0.10	0.1-0.2	0.8-2.1	0.6-1.7	0.2-0.4	0.2-0.5	0.2-0.4	0.3-0.7
exposure to	Groundnuts	0.7-0.7	0.4-0.4	0.3-0.3	0.1-0.1	0.5-0.5	0.2-0.2	0.9-1.0	0.3-0.3	0.6-0.6	2.6-2.9	0.1-0.1	0.1-0.1	0.8-0.9
AFT from individual food	Oilseeds	0.1-0.2	0.4-0.6	0.2-0.3	0.3-0.5	0.4-0.6	0.2-0.3	0.1-0.2	0.1-0.2	0.1-0.2	0.1-0.1	0.02-0.04	0.4-0.6	0.1-0.2
inuividuai 1000 Sources <sup>a</sup>	Cocoa products	0.02-0.04	0.1-0.2	0.03-0.1	0.04-0.1	0.2-0.4	0.2-0.4	0.02- 0.04	0.1-0.1	0.03-0.04	0.02-0.03	0.1-0.1	0.1-0.1	0.2-0.3
	Other nuts	0.0-0.0	0.04-0.1	0.0-0.0	0.0-0.01	0.01- 0.02	0.0-0.0	0.01- 0.02	0.01- 0.01	0.0-0.0	0.0-0.0	0.0-0.0	0.02-0.03	0.01-0.02
	Dried fruits other than figs	0.0-0.01	0.02-0.1	0.1-0.3	0.02-0.1	0.01- 0.03	0.01-0.03	0.0- 0.01	0.0-0.0	0.0-0.0	0.01-0.04	0.0-0.0	0.0-0.01	0.01-0.04
	Butter of Karité nut	0.01-0.02	0.0-0.0	0.0-0.0	0.0-0.0	0.0-0.0	0.0-0.0	0.0-0.0	0.0-0.0	0.0-0.0	0.0-0.02	0.0-0.0	0.0-0.0	0.0-0.0
	Peanut oil	0.02-0.03	0.01-0.01	0.01-0.01	0.0-0.0	0.02- 0.02	0.01-0.01	0.04- 0.05	0.0-0.0	0.02-0.02	0.1-0.1	0.0-0.0	0.0-0.0	0.01-0.01
	Spices	0.07-0.08	0.03-0.03	0.07-0.07	0.02-0.03	0.1-0.1	0.03-0.03	0.1-0.1	0.1-0.1	0.04-0.04	0.04-0.04	0.01-0.01	0.02-0.02	0.1-0.1
<sup>a</sup> Lower- and L	ipper-bound sc	cenarios hav	ve been ust	ed in makin	a the dieta	rv exposu	re estimate	s for over	all expos	ure and ind	ividual fooc	sources.	The lower b	ound was

calculated using 0 for non-detects or the LOD for trace values, whereas the upper bound was calculated using either the LOD or LOQ, as appropriate.

		Dietary	exposur	e to AFT (n	g/kg bw pei	day) for 1	3 GEMS/Fo	od Cons	sumption	Cluster	Diets			
		A	В	U	Δ	ш	ш	U	т	_	- -	¥	_	×
Overall expo other source	sure from s <sup>a</sup>	1.1-1.7	1.3-2.4	1.0-2.0	0.6-1.0	1.3-1.9	0.7-1.1	1.3-1.6	1.3-2.7	1.4-2.7	3.0-3.7	0.4-0.7	0.7-1.3	1.5-2.2
Scenario <sup>b</sup>														
No ML	All tree nuts	0.0-0.0	0.8-0.8	0.5-0.5	0.8-0.8	0.4-0.4	0.04-0.04	0.0-0.0	0.1-0.1	0.0-0.0	0.0-0.0	0.03-0.03	0.01-0.01	0.2-0.2
	Pistachios (% AFT)	0.0	20.1	18.4	44.8	12.0	0.0	0.0	3.2	0.0	0.0	0.0	0.0	7.4
	Other tree nuts (% AFT)	0.0	4.4	1.7	0.2	4.9	3.7	0.0	0.1	0.0	0.0	4.3	0.8	1.9
ML 20 µg/kg	All tree nuts	0.0-0.0	0.1-0.1	0.1-0.1	0.1-0.1	0.1-0.1	0.02-0.02	0.0-0.0	0.01- 0.01	0.0-0.0	0.0-0.0	0.0-0.0	0.0-0.0	0.03-0.03
	Pistachios (% AFT)	0.0	2.0	1.8	6.1	1.1	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.6
	Other tree nuts (% AFT)	0.0	2.7	0.8	0.2	2.1	1.6	0.0	0.0	0.0	0.0	0.6	0.3	0.5
ML 4 µg/kg	All tree nuts	0.0-0.0	0.1-0.1	0.02-0.02	0.02-0.02	0.03-0.03	0.01-0.01	0.0-0.0	0.0-0.0	0.0-0.0	0.0-0.0	0.0-0.0	0.0-0.0	0.01-0.01
	Pistachios (% AFT)	0.0	0.6	0.5	1.8	0.3	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.2

#### AFLATOXINS (INTAKE FROM TREE NUTS AND DRIED FIGS)

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	Dieta A	ry expos	ure to AFT C	ng/kg bw	per day) fc E	r 13 GEMS F	%Food Co	nsumptio	on Cluste	er Diets J	×	-	Σ
Other tree nuts (%	0.0	1.7	9.0	0.1	1.4	1.1	0.0	0.0	0.0	0.0	0.4	0.2	0.3
AFT)													
Mean concentration as	reported	d for othe	er contribui	ting food so	ources to th	e overall di	etary exp	osure to	AFT and	the corre	esponding	g food cons	umption from

the 13 GEMIS/Food Consumption Cluster Diets. aR

was calculated using 0 for non-detects or the LOD for trace values, whereas the upper bound was calculated using either the LOD or LOQ, as appropriate. <sup>b</sup> Lower- and upper-bound scenarios have been used in making the dietary exposure estimates for overall exposure and all tree nuts. The lower bound "All tree nuts" includes dried figs, which contributed less than 0.3% of the dietary AFT exposure in all scenarios. % AFT is the contribution from almonds, Brazil nuts, hazelnuts, pistachios and dried figs to the total dietary AFT exposure (upper-bound scenario only).

#### 6.2.1 Estimates of overall dietary exposure to aflatoxin-scenario with no MLs

International mean dietary exposure estimates for AFT from all sources were estimated to range from 0.4–0.7 ng/kg bw per day (cluster K) to 3.0–3.7 ng/kg bw per day (cluster J) for the 13 GEMS/Food Consumption Cluster Diets, by assuming a body weight of 60 kg and using the lower-bound/upper-bound approach. In these estimates, mean dietary AFB<sub>1</sub> exposures ranged from 0.3–0.5 ng/kg bw per day to 2.3–2.8 ng/kg bw per day for the same clusters (Table 9).

In these estimates, dietary exposures to AFT from maize, groundnuts, oilseeds and cocoa products made the greatest contribution to total exposure in all cluster diets (Table 10):

- Maize ranged from 0.04–0.10 ng/kg bw per day (cluster F) to 0.8–2.1 ng/kg bw per day (cluster H).
- Groundnuts ranged from 0.1 ng/kg bw per day (clusters D and L) to 2.6–2.9 ng/kg bw per day (cluster J).
- Oilseeds ranged from 0.02–0.04 ng/kg bw per day (cluster K) to 0.4–0.6 ng/kg bw per day (cluster B).
- Cocoa products ranged from 0.02–0.03 ng/kg bw per day (cluster J) to 0.2–0.4 ng/kg bw per day (clusters E and F).

#### 6.2.2 Dietary exposure estimates for tree nuts and dried figs

The mean contribution to dietary AFT exposure from consumption of almonds, Brazil nuts, hazelnuts, pistachios and dried figs ranged from 0 ng/kg bw per day (clusters A, G, I and J; nut consumption reported as zero for these clusters) up to 0.8 ng/kg bw per day (clusters B and D). In five cluster diets (B, C, D, E and M), the contribution from almonds, Brazil nuts, hazelnuts, pistachios and dried figs was higher than 5% of the overall dietary exposure to AFT (Table 9). Mean dietary exposures for all other cluster diets from tree nuts (including dried figs) were below 0.1 ng/kg bw per day.

Pistachios were the main contributor to dietary AFT exposure from tree nuts in these five cluster diets with higher than 5% contribution to overall dietary AFT exposure, ranging from 0.2 to 0.8 ng/kg bw per day, equivalent to 7–45% of the total AFT from all sources (Table 11). Almonds, Brazil nuts and hazelnuts contributed up to 0.1 ng/kg bw per day, and dried figs less than 0.01 ng/kg bw per day, in all Cluster Consumption Diets.

### 6.2.3 Effect of hypothetical MLs in almonds, Brazil nuts, hazelnuts, pistachios and dried figs on dietary exposure

The Committee evaluated the impact on dietary exposure to AFT of setting hypothetical MLs of 4, 8, 10, 15 or 20  $\mu$ g/kg for AFT in almonds, Brazil nuts, hazelnuts, pistachios and dried figs. For dried figs and tree nuts other than pistachios, the contribution to total dietary AFT exposure is less than 5%, regardless of whether an ML is in place or not. This is explained by the fact that the main part of the dietary exposure to AFT comes from other food sources (Tables 2, 3, 4 and 5).

Using the five cluster diets where almonds, Brazil nuts, hazelnuts, pistachios and dried figs contribute more than 5% to dietary AFT exposure (clusters B, C, D, E and M), and assuming a body weight of 60 kg, the Committee estimated that an enforced ML of 20, 15, 10, 8 or 4  $\mu$ g/kg results in dietary exposures to AFT ranging from 0.12, 0.10, 0.08, 0.07 and 0.06 ng/kg bw per day in the cluster with the highest exposure (B) to 0.03, 0.02, 0.02, 0.02 and 0.01 ng/kg bw per day in the cluster with the lowest exposure (M).

United Kingdom food consumption data for vegetarians and vegans showed that for high-level consumers of almonds, Brazil nuts, hazelnuts and pistachios, enforcing an ML of 20  $\mu$ g/kg reduces total dietary AFT exposure when compared with no ML. Setting a lower ML would have little impact compared with the ML of 20  $\mu$ g/kg. The dietary exposure from tree nuts assuming no ML was estimated to be 5.8 ng/kg bw per day. The estimate with an ML of 20  $\mu$ g/kg would be 0.5 ng/kg bw per day and with an ML of 4  $\mu$ g/kg would be 0.2 ng/kg bw per day.

In these analyses, the contribution from tree nuts to the total dietary AFT exposures in all five cluster diets, whatever the ML scenario (4, 8, 10, 15 or 20  $\mu$ g/kg), will remain below a dietary exposure of 0.1 ng/kg bw per day compared with less than 0.8 ng/kg bw per day for the scenario with no MLs. The highest decrease in AFT exposure results from the contribution from pistachios to total dietary AFT exposure when setting an ML at 20  $\mu$ g/kg in comparison with no MLs.

The Committee also noted that in all these different ML scenarios, dried figs were included (dietary exposures not shown in tables). However, the contribution of dried figs (less than 0.01 ng/kg bw per day) to total dietary AFT exposure estimates in all Consumption Cluster Diets, whatever the ML scenario, would be less than 0.3% of the overall dietary AFT exposure.

The Committee noted the previous assessments of exposure to AFT made by JECFA in 1998 (Annex 1, reference *132*) and EFSA in 2007 (European Food Safety Authority, 2007). The estimates made at this meeting for EU dietary exposures—0.7–2.5 ng/kg bw per day for clusters B, E and F (with ML scenario from 4 to 20 µg/kg for tree nuts)—were in the range of those reported in the EFSA opinion: 1.0–2.5 ng/kg bw per day (with ML scenario from 4 to 10 µg/kg for tree nuts, and including high-level consumers of these nuts). These estimates can be compared with the JECFA estimate of 0.8 ng/kg bw per day (with ML scenario from 10 to 20 µg/kg in groundnuts) (Annex 1, reference *132*). In these estimates, groundnuts and maize were the main contributors to AFT exposure, ranging from 0.2 to 1.4 ng/kg bw per day at the current meeting, compared with 1.1–2.0 ng/kg bw per day in the JECFA assessment (Annex 1, reference *132*) and 0.03–1.0 ng/kg bw per day in the EFSA opinion (European Food Safety Authority, 2007).

#### 7. PREVENTION AND CONTROL OF AFLATOXIN PRODUCTION

A prevention programme to reduce and control AFL contamination should be established, considering various steps from cultivation through harvesting, postharvesting, processing, storage, transportation and marketing (Campbell et al., 2003, 2005; Kabak et al., 2006).

#### 7.1 Aflatoxin-producing fungi

AFL are found in different tree nuts as a result of fungal contamination both pre- and post-harvest, with the rate and degree of contamination dependent on tree or shrub species, geographical location, meteorological conditions, and different harvest, drying, processing and storage conditions, among others (Pitt, 2006).

AFL production has been reported in a large number of fungi, but Aspergillus *flavus* and *A. parasiticus* were the only species reliably reported to accumulate AFL. Aspergillus flavus is ubiquitous, favouring the aerial parts of plants (leaves, flowers), and produces B AFL. Aspergillus parasiticus produces both B and G AFL, is more adapted to a soil environment and has more limited distribution. Later, A. nomius, A. toxicarius, A. pseudotamarii, A. flavus var. columnaris, A. flavus var. parvisclerotigenus, A. zhaogingensis and A. bombycis from section Flavi were also reported as AFL producers (Klich et al., 2000; Ito et al., 2001; Peterson et al., 2001; Frisvad et al., 2006; Pitt, 2006). Other species in Aspergillus and one of its teleomorphs, Emericella, but also species in Monocillium, Chaetomium, Bipolaris and Humicola, are able to produce sterigmatocystin, an AFL precursor (Frisvad & Samson, 2004; Frisvad et al., 2004, 2005). These species were screened for AFL, but AFL were not found in these other genera. However, AFL were discovered in the section Ochraceorosei in Aspergillus (A. ochraceoroseus and A. rambellii) and in three species of *Emericella*: *E. astellata*, *E. venezuelensis* and *E. olivicola* (Klich et al., 2003; Cary et al., 2005). The latter five species accumulate both AFB1 and sterigmatocystin, in contrast to species in section Flavi, which accumulate AFL only and are particularly efficient producers of 3-O-methylsterigmatocystin. None of the latter five species, with the possible exception of E. olivicola, seems to be of significance for food safety. This leaves members of Aspergillus section Flavi as the important AFL producers in foods and foodstuffs. In some cases, the nomenclature was updated, and the following species are considered to be important AFL producers in special situations: A. parvisclerotigenus, A. nomius, A. toxicarius, A. pseudotamarii and A. bombycis (Cotty & Cardwell, 1999; Freire et al., 2000; Ehrlich et al., 2007). Several papers on the molecular biology of AFL producers indicate that more species exist in the section *Flavi* (Färber et al., 1997; Pitt & Samson, 2000; Cary & Ehrlich, 2006).

The occurrence of these fungal species varies by food commodity and geographically (Abdel-Hafez & Saber, 1993; Doster & Michailides, 1994, 1995; Doster et al., 1996; Freire et al., 2000; Moretti et al., 2000; Hua & McAlpin, 2001; Bayman et al., 2002; Simekş et al., 2002; Logrieco et al., 2003; Iamanaka et al., 2005, 2007; Gürses, 2006; Codex Committee on Contaminants in Food, 2007a). Brazil nuts and dried figs seemed to have a different AFL profile from the rest of the analysed food products. The calculated relationships from the submitted data seem to indicate that there are atypical *Aspergillus* isolates on dried figs, as reported by Steiner et al. (1988).

#### 7.2 Pre-harvest control

The main ways to reduce AFL contamination are to control the presence of insects such as the orangeworm, *Amyelois transitella*, in almonds and in pistachio

nuts and other pests in the orchard, minimize early split nut formation and avoid late harvesting (Schatzki & Ong, 2000, 2001; Doster et al., 2001b; Michailides, 2005). Research in the use of sex pheromones for insect control to replace pesticides is increasing in response to food safety concerns (Campbell et al., 2005). Additional non-pesticidal approaches include augmenting the constitutive natural products, as, for example, in the case of almonds, which deter insect feeding (Dicenta et al., 2002), or the use of natural antifungal products (Campbell et al., 2005; Kabak et al., 2006). Pistachio shell splitting is sensitive to irrigation deficits; therefore, it should be carefully monitored (Ferguson et al., 2005). Delaying harvest allows more time for AFL-producing fungi, and it can also increase insect attack (Campbell et al., 2003; Bentley et al., 2005; Michailides, 2005). In Iran, the most effective ways to reduce the AFL content of pistachio nuts were the introduction of early harvest and keeping the harvesting period and drying time as short as possible.

A possible preventive treatment is the application of microorganisms (Doster et al., 2001a; Hua, 2002, 2004; Palumbo et al., 2006). In the last few years, experiments have been performed using atoxigenic strains of *A. flavus* to control AFL contamination in pistachios (Michailides, 2005) and in figs (Doster et al., 2001a). The potential of saprophytic yeast as a biocontrol agent has been investigated by Hua (2002, 2004). It is evident, though, that further research is needed to determine if these suggested practices are able to reduce mycotoxin contamination.

A major reservoir of *Aspergillus* spores in tree nuts can occur in the leaf, hull and unharvested litter surrounding the trees. This type of litter presents a special problem to tree nuts in general, but especially to Brazil nuts when they are harvested after they have fallen to the jungle floor. In many cases, they are in direct contact with the soil for several days prior to collection (Doster & Michailides, 1984; Arrus et al., 2005). It would be interesting to try to develop an improved way of collection aimed at reducing the inoculum sources and AFL contamination as much as possible and minimizing the risk to collectors when remaining pods fall down after the crop season.

#### 7.3 Genetic improvement

Some procedures used to reduce and prevent AFL production include a selection of resistant varieties. Progress has been made in all crops, and genetic improvement offers considerable potential. Current status and prospects for the future are discussed by Mehlenbacher (2003) for each tree nut crop, including efforts at mapping, marker-assisted selection and transformation (Gradziel & Dandekar, 2001), and information on fig selection by Doster et al. (2001a). The small number and size of breeding programmes are major limitations to genetic improvement of tree nuts.

#### 7.4 Post-harvest control

Different oil contents of tree nuts emphasize the necessity of using water activity as a conservation parameter instead of moisture content (Bianco et al., 2001) and should be carefully controlled during storage. Collection of useful data for future modelling that integrates technological and practical achievements requires knowledge of not only fungal distribution in each product and in the different steps of the food-chain, but also the fungal growth and AFL production kinetics during the storage period in relation to weather conditions or the storage parameters.

Some results showed that pistachio AFL contamination in storage can be controlled by oxygen exclusion (Iqbal et al., 2006).

#### 7.5 Physical decontamination

Removal of AFL-contaminated nuts or figs by means of physical segregation is the most effective control measure for reducing levels of AFL in a lot to an acceptable level.

Some adsorbents can bind AFL and thus remove them from aqueous solutions. Natrolite  $(Na_{16}[(AIO_2)_{16}(SiO_2)_{24}]\cdot 16H_2O)$  was recently shown to decontaminate pistachio nuts, reducing AFB<sub>1</sub> with a slurry of 5% concentration, but its efficacy against AFB<sub>2</sub> is proving to be limited (Fooladi & Farahnaky, 2003).

The complete elimination of AFL contamination in the evaluated products is currently not realistically achievable, and research should be improved to develop further detoxification strategies.

#### 8. COMMENTS

#### 8.1 Analytical methods

In the studies evaluated by the Committee at its present meeting, it was usually clear which AFT analytical method had been used. However, in the submitted data, detection and quantification limits for AFT were calculated in different ways. One method defined the LOD of AFT as twice the value of the LOD of AFB<sub>1</sub>, whereas the second used the sum of the LODs of AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub>. The Committee concluded that both definitions overestimate the LOD of AFT, resulting in conservative estimates of the exposure to AFT for the upper-bound estimate. The Committee also concluded that it was better to restrict data used in the dietary exposure assessment to those with validated recoveries greater than 70% than to correct for lower recoveries. The Committee also noted that surveillance data should be accompanied by a clear description of the analytical method used; recoveries of the analytical methodology chosen should be specific to the food matrix tested; and LODs and LOQs should be made to harmonize the nomenclature and the methodologies by which the LOD and LOQ were calculated.

The combination of liquid chromatography with mass spectrometry is one useful technique for the confirmation of the presence of AFL in foodstuffs. Although some methods are already implemented in routine analysis, the limited number of reference materials, high investment costs and lack of the required sensitivity could be a barrier to use for AFL surveillance, because it was noted that for accurate dietary exposure assessments, the LOD/LOQ should be as low as technically possible. This is due to the fact that many foods that might be expected to contain AFL do not contain *detectable* AFL contamination, and the default value assigned to those censored samples will affect the estimated dietary exposures (upper-bound estimates only).

#### 8.2 Sampling protocols

Almost all of the submitted data on AFT were derived using sampling plans designed for regulatory purposes. The producing countries that submitted data to the Committee presented sampling plans similar to or the same as those following EC No. 401/2006 for the determination of AFL, which includes edible nuts and dried figs. It was observed that in some producing countries, there are two sampling plans: one for commodity to be exported to the EU, and the second for commodity to be exported to other countries with less strict regulations. There remains a need for harmonized sampling plans, both between different countries and within the same country. The Committee noted that AFL sampling plans should be determined by data relating to contamination distributions and uncertainties within the particular foodstuff. The resulting knowledge of the uncertainty among sample test results should allow each country to refine its sampling plans using, for example, larger sample sizes and/or fewer analytical repetitions in order to meet harmonized criteria. The Committee noted that the data received for this analysis were robust.

#### 8.3 Effects of processing

Although AFL are highly stable, studies have indicated that they are degraded in contaminated food by heat treatment. For example, the roasting of pistachio nuts at 150 °C for 30 min reduced AFT levels by 63% when the initial level was 44  $\mu$ g AFB<sub>1</sub>/kg, 24% when the initial level was 213  $\mu$ g AFB<sub>1</sub>/kg, 17% when the initial level was 21.9  $\mu$ g AFB<sub>1</sub>/kg and 47% when the initial level was 18.5  $\mu$ g AFB<sub>2</sub>/kg.

# 8.4 Aflatoxin occurrence and levels in food commodities and the potential effect of MLs in almonds, Brazil nuts, hazelnuts, pistachios and dried figs

AFL occurrence data on almonds, Brazil nuts, hazelnuts, pistachios and dried figs were obtained from both producing and importing countries. The Committee decided to base the assessment of the impact of different MLs for AFT for almonds, Brazil nuts, hazelnuts, pistachios and dried figs (4, 8, 10, 15 and 20 µg/kg) on data provided by producing countries, as these are more likely to represent the actual occurrence of AFL in the commodities. The primary producing countries (FAOSTAT, 2007) were, for almonds, the USA (42% of the world market); for Brazil nuts, Latin America (100%); for hazelnuts, Turkey (70%); for pistachios, the Islamic Republic of Iran (65%); and for dried figs, Turkey (63% for dried fruits). Turkey is the primary producing country for hazelnuts, but the Committee received no data on AFT levels in hazelnuts from Turkey; therefore, the Committee chose to use all of the submitted data supplied by the EU, the USA and Japan for its analyses.

The mean concentrations of AFT in nuts and dried figs in the main producing countries were, for almonds, 2 µg/kg; for Brazil nuts, 20 µg/kg; for hazelnuts, 2 µg/kg; for pistachios, 54 µg/kg; and for dried figs, 1 µg/kg. The effects of the theoretical full enforcement of MLs (all samples above the ML would be excluded from the distribution) at 20, 15, 10, 8 and 4 µg/kg are shown in Table 6. The reductions in mean AFT concentrations would be approximately 2- to 3-fold for almonds, 10-fold for Brazil nuts, 2- to 4-fold for hazelnuts, 10- to 50-fold for pistachios and 2-fold for dried figs. The corresponding proportion of rejected samples would be 1-3% for almonds, 11-17% for Brazil nuts, 1-7% for hazelnuts, 40-60% for pistachios and 1-3% for dried figs.

#### 8.5 Assessment of dietary exposure

At the regional level, published studies reported that estimated mean dietary exposures to AFT for the general population from all food sources were 0.93–2.4 ng/kg bw per day in Europe, 3.5–180 ng/kg bw per day in Africa, 0.3–53 ng/kg bw per day in Asia and 2.7 ng/kg bw per day in the USA.

In this assessment, mean lower- and upper-bound scenarios have been used in making the dietary exposure estimates employing the 13 GEMS/Food Consumption Cluster Diets (Tables 8, 9, 10 and 11). The lower bound was calculated using 0 for non-detects or the LOD for trace values, whereas the upper bound was calculated using either the LOD or LOQ, as appropriate.

The Committee employed the 13 GEMS/Food Consumption Cluster Diets to make international estimates of dietary AFT exposure from all sources. These were estimated to range from 0.4–0.7 ng/kg bw per day (cluster K) to 3.0–3.7 ng/kg bw per day (cluster J), by assuming a body weight of 60 kg and using the lower-bound/ upper-bound approach. The mean total dietary exposure to AFT from maize, groundnuts, oilseeds and cocoa products made the greatest contribution to total exposure in all cluster diets (Table 10). Dietary AFB<sub>1</sub> exposure ranged from 0.3–0.5 ng/kg bw per day to 2.3–2.8 ng/kg bw per day for the same clusters (Table 9).

#### 8.5.1 Almonds, Brazil nuts, hazelnuts, pistachios and dried figs

The mean contribution to dietary AFT exposure from consumption of almonds, Brazil nuts, hazelnuts, pistachios and dried figs ranged from 0 ng/kg bw per day (clusters A, G, I and J; nut consumption reported as zero for these clusters) up to 0.8 ng/kg bw per day (clusters B and D). In five cluster diets (B, C, D, E and M), the contribution from almonds, Brazil nuts, hazelnuts and pistachios was higher than 5% of the overall dietary exposure to AFT (Table 9).

Pistachios were the main contributor to dietary AFT exposure from tree nuts in all five cluster diets, ranging from 0.2 to 0.8 ng/kg bw per day, equivalent to 7–45% of the total AFT from all sources (Table 11). Almonds, Brazil nuts and hazelnuts contributed up to 0.1 ng/kg bw per day, and dried figs less than 0.01 ng/kg bw per day, in all Consumption Cluster Diets.

#### 8.5.2 Foods other than tree nuts and dried figs

In order to evaluate the relative contribution of tree nuts and dried figs to the overall AFT exposure, the Committee considered other foods known to contribute to the overall exposure to AFT in humans. Occurrence data and dietary exposures to AFT from these other foods were described. Food commodities included in the mean overall exposure were maize, groundnuts (i.e. peanuts) and other nuts (i.e. walnuts, cashews, chestnuts, macadamia nuts, pecans), dried fruits other than figs (apricots, plums, grapes, dates and others), spices, cocoa and cocoa products (cocoa mass, cocoa butter, cocoa powder), peanut butter, peanut cream, oilseeds and butter of Karité nut.

The majority of the data included in the estimation of dietary AFT exposure from other food sources came from the EU. The Committee noted that the European data do not reflect the actual mean values in other world regions for some foods considered here, as the mean concentration of AFT in the EU takes into account fewer highly contaminated samples due to existing EU MLs compared with regions with higher MLs or lack of enforcement.

The mean concentrations of AFB<sub>1</sub> and AFT were less than 1  $\mu$ g/kg for most foods, except spices, cocoa products, groundnuts and butter of Karité nut, where mean levels ranged between 2 and 4  $\mu$ g/kg.

The Committee noted that different concentrations in rice were reported in different regions (producing and non-producing countries), with mean AFT levels around 0.6–1.0  $\mu$ g/kg in the EU, 0.2–1.2  $\mu$ g/kg in the Republic of Korea and 0.1–0.2  $\mu$ g/kg in Qatar, with no reports of detected levels in other regions, including Japan and Argentina. High AFT levels, such as those for peanuts or maize, have never been reported in rice; the highest reliably reported levels are less than 10  $\mu$ g/ kg. Because of uncertainties in the data, rice was not included in estimating overall dietary exposures to AFT for comparison with the contribution from almonds, Brazil nuts, hazelnuts, pistachios and dried figs. In regions where rice is a major component of the diet, any low levels of AFT in rice may lead to its being a major contributor to total dietary exposure to AFT, even though that exposure may be low when compared with that in other regions.

## 8.6 Effect of hypothetical MLs in almonds, Brazil nuts, hazelnuts, pistachios and dried figs on dietary exposure

The Committee evaluated the impact on dietary exposure to AFT of setting hypothetical MLs of 4, 8, 10, 15 or 20  $\mu$ g/kg for AFT in almonds, Brazil nuts, hazelnuts, pistachios and dried figs. For dried figs and tree nuts other than pistachios, the contribution to total dietary AFT exposure is less than 5%, regardless of whether an ML is in place or not. This is explained by the fact that the main part of the dietary exposure to AFT comes from other food sources (Tables 2–6).

Using the five cluster diets where almonds, Brazil nuts, hazelnuts, pistachios and dried figs contribute more than 5% to dietary AFT exposure (clusters B, C, D, E and M), and assuming a body weight of 60 kg, the Committee estimated that an enforced ML of 20, 15, 10, 8 or 4  $\mu$ g/kg results in dietary exposures to AFT ranging

from 0.12, 0.10, 0.08, 0.07 and 0.06 ng/kg bw per day in the cluster with the highest exposure (D) to 0.03, 0.02, 0.02, 0.02 and 0.01 ng/kg bw per day in the cluster with the lowest exposure (M).

United Kingdom food consumption data for vegetarians and vegans showed that for high-level consumers of almonds, Brazil nuts, hazelnuts and pistachios, enforcing an ML of 20  $\mu$ g/kg reduces total dietary AFT exposure when compared with no ML. Setting a lower ML would have little impact compared with the ML of 20  $\mu$ g/kg. The dietary exposure from tree nuts assuming no ML was estimated to be 5.8 ng/kg bw per day. The estimate with an ML of 20  $\mu$ g/kg would be 0.5 ng/kg bw per day, and with an ML of 4  $\mu$ g/kg would be 0.2 ng/kg bw per day.

In these analyses, the contribution from tree nuts to the total dietary AFT exposures in all five cluster diets, whatever the ML scenario (4, 8, 10, 15 or 20  $\mu$ g/kg), will remain below 0.1 ng/kg bw per day, compared with <0.8 ng/kg bw per day for the scenario with no MLs. The highest decrease in AFT exposure results from the contribution from pistachios to total dietary AFT exposure when setting an ML at 20  $\mu$ g/kg in comparison with no ML.

The Committee also noted that in all these different ML scenarios, dried figs were included (dietary exposures not shown in tables). However, the contribution of dried figs (<0.01 ng/kg bw per day) to total dietary AFT exposure estimates in all Consumption Cluster Diets, whatever the ML scenario, would be less than 0.3% of the overall dietary AFT exposure.

The Committee noted the previous assessments of exposure to AFT made by JECFA in 1998 (Annex 1, reference *132*) and EFSA in 2007 (European Food Safety Authority, 2007). The estimates made at the present meeting for EU dietary exposures (0.7–2.5 ng/kg bw per day for European clusters B, E and F, with MLs from 4 to 20 µg/kg for tree nuts) were in the range of those reported in the EFSA opinion, where AFT exposures ranged from 1.0 to 2.5 ng/kg bw per day (with MLs from 4 to 10 µg/kg for tree nuts, and including high-level consumers of these nuts), compared with 0.8 ng/kg bw per day reported by JECFA in 1998 (with MLs from 10 to 20 µg/kg in groundnuts). In these estimates, groundnuts and maize were the main contributors to AFT exposure, ranging from 0.2 to 1.4 ng/kg bw per day at the current meeting, compared with 1.1–2.0 ng/kg bw per day in the 1998 JECFA evaluation and 0.03–1.0 ng/kg bw per day in the EFSA opinion.

#### 9. EVALUATION

The Committee noted that the majority of data included in the estimation of dietary AFT exposure from foods other than almonds, Brazil nuts, hazelnuts, pistachios and dried figs came from the EU and that these data do not reflect the actual mean values in other world regions. This probably results in an underestimate of dietary AFT exposure and overstates the relative contribution of dietary AFT exposure from tree nuts. The Committee decided to base the assessment of the impact of different MLs for AFT on data provided by producing countries, noting that these better represent the materials in commerce and result in a robust estimate of dietary AFT exposure from tree nuts.

The Committee calculated that the consumption of almonds, Brazil nuts, hazelnuts, pistachios and dried figs contributes greater than 5% of the dietary AFT exposure in only five cluster diets (clusters B, C, D, E and M). If fully enforced, an ML at 20 µg/kg in almonds, Brazil nuts, hazelnuts, pistachios and dried figs would have an impact on the relative contribution to dietary AFT exposure only in these clusters, including high-level consumers of the tree nuts. This is due solely to the elevated AFT level in pistachios. For the tree nuts other than pistachios, the presence of an ML has no effect on dietary AFT exposure.

Moreover, the Committee concluded that enforcing an ML of 15, 10, 8 or 4  $\mu$ g/kg would have little further impact on the overall dietary exposure to AFT in all five of the highest exposed population groups, compared with setting an ML of 20  $\mu$ g/kg. The proportion of rejected samples from the world market would be between 1% (ML 20  $\mu$ g/kg) and 3% (ML 4  $\mu$ g/kg) for almonds, 11% and 17% for Brazil nuts, 1% and 7% for hazelnuts and 40% and 60% for pistachios, respectively.

Based on the large data sets on AFT concentrations in dried figs submitted at this meeting by Turkey, the most important producing country for dried figs (>40 000 data points), the Committee concluded that whatever the hypothetical ML scenario applied (no ML, 4, 8, 10, 15 or 20  $\mu$ g/kg) to dried figs, there would be no impact on the overall dietary exposure to AFT (below 0.03%, equivalent to a dietary exposure of <0.01 ng/kg bw per day), and that the proportion of rejected samples from the world market could range between 1% and 3% for MLs at 20  $\mu$ g/kg and 4  $\mu$ g/kg, respectively.

The Committee noted that the reduction of dietary AFT exposure is an important public health goal, particularly in populations that consume high levels of any potentially AFT-contaminated food.

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