codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

JOINT OFFICE: Viale delle Terme di Caracalla 00100 ROME Tel: 39 06 57051 www.codexalimentarius.net Email: codex@fao.org Facsimile: 39 06 5705 4593

Agenda Item 14 (e)

CX/FAC 04/36/22
December 2003

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON FOOD ADDITIVES AND CONTAMINANTS

Thirty-sixth Session
Rotterdam, The Netherlands, 22 – 26 March 2004

DISCUSSION PAPER ON AFLATOXINS IN TREE NUTS (OTHER THAN ALMONDS, HAZELNUTS AND PISTACHIOS), INCLUDING INFORMATION SUBMITTED ON AFLATOXINS CONTAMINATION AND METHODS OF ANALYSIS FOR THE DETERMINATION OF AFLATOXINS IN TREE NUTS

Governments and international organizations wishing to submit comments on the following subject matter are invited to do so **no later than 16 February 2004** as follows: Netherlands Codex Contact Point, Ministry of Agriculture, Nature and Food Quality, P.O. Box 20401, 2500 E.K., The Hague, The Netherlands (Telefax: +31.70.378.6141; E-mail: info@codexalimentarius.nl, with a copy to the Secretary, Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Viale delle Terme di Caracalla, 00100 Rome, Italy (Telefax: +39.06.5705.4593; E-mail: Codex@fao.org).

BACKGROUND

1. The 34th session of the Codex Committee on Food Additives and Contaminants (CCFAC) decided that a drafting group led by Iran would revise the discussion paper on aflatoxins in Tree Nuts for circulation, comment and further consideration at its current meeting. The 34th session of the CCFAC also agreed that information on aflatoxins in Tree Nuts as well as methods of analysis of the determination of aflatoxins in tree nuts would be requested on the Discussion Paper.

2. On the basis of the data presented in the document (CX/FAC 03/23), the 35th session of CCFAC agreed to the elaboration of maximum levels for aflatoxins in almonds, hazelnuts and pistachios. The remaining data for other varieties of tree nuts was considered insufficient for the elaboration of maximum levels. The Committee agreed that the delegation of Iran would revise the discussion paper for circulation, comments and further consideration at its next meeting, and that additional information would be requested on aflatoxin contamination in tree nuts other than almonds, hazelnuts and pistachios.

3. Information provided by China in response to CL 2003/13-FAC (point 18) is incorporated in the revised discussion paper.

4. Governments and interested international organizations are invited to comment on the revised discussion paper on *Aflatoxins in Tree Nuts (other than almonds, hazelnuts and pistachios), including Information submitted on Aflatoxins Contamination and Methods of Analysis for the Determination of Aflatoxins in Tree Nuts* as directed above. In view of the short time period between the submission of comments and the 36th Session of the Committee, comments received will be kept in original language.

INTRODUCTION

5. Aflatoxin contamination is a potential problem in tree nuts and other commodities. The incidence of contaminated nuts and the concentration of aflatoxins in contaminated nuts vary widely from location to location, from year to year and from cultivar to cultivar. Because of the variability associated with different nut crops, different production areas, and different agronomic practices, it is not possible to provide minute details concerning all tree nuts. This discussion paper is applicable to all tree nuts with edible kernel of both consumption and global trade interest, including but not limited to almond (*Prunus amygdalus*), Brazil nut (*Bertholletia excelsa*), cashew nut (*Anacardium occidentale*), hazelnut (*Corylus* Spp.), macadamia (*Macadamia integrifolia*), pecan (*Carya illinoensis*), pine nut (*Pinus parrayana*), pistachio (*Pistacia vera*) and walnut (*Juglans regia*).

6. The aflatoxins are a group of structurally related compounds produced by some strains of *Aspergillus flavus, A. parasiticus* and *A. nomius.* The naturally occurring aflatoxins are aflatoxins B_1, B_2, G_1, and G_2. Aflatoxin B_1 is normally predominant in amount in contaminated commodities; aflatoxins B_2, G_1, and G_2 are generally not reported in the absence of AFB_1.[1] Tree nuts and their varieties seem to differ in their susceptibility to aflatoxin contamination. Many of the apparent differences may be the result of environmental factors, different pest complexes as well as the technical ability to sort damaged and contaminated nuts during post-harvest processing.

7. The commodities with the highest risk of aflatoxin contamination include corn, peanuts, cottonseed, Brazil nuts, pistachio nuts, figs, spices and copra. The most important dietary sources of aflatoxins are maize and groundnuts and their products, which may form an essential part of the food diet in some countries.[1]

CHEMICAL STRUCTURE

8. Chemically, aflatoxins are highly oxygenated naturally occurring heterocyclic compounds and have closely related structures. All aflatoxins essentially contain a coumarin nucleus fused to a bifuran. A pentanone structure is attached to coumarin nucleus in case of aflatoxins of B series. This is substituted by a six-membered lactone in aflatoxins of G series.[2]

TOXICOLOGICAL EVALUATION

9. The aflatoxins were evaluated by JECFA at its thirty-first, forty-sixth, forty-ninth and its fifty-sixth meetings (aflatoxin M_1 only). At its forty-ninth meeting in 1997, JECFA considered estimates of the carcinogenic potency of aflatoxins and the potential risks associated with their intake. At that meeting, no numerical TDI was proposed since these compounds are genotoxic carcinogens, but the potency estimates for human liver cancer resulting from exposure to aflatoxin B_1 were derived from epidemiological and toxicological studies. JECFA reviewed a wide range of studies conducted with both animals and humans that provided qualitative and quantitative information on the hepatocarcinogenicity of aflatoxins. The JECFA evaluated the potency of these contaminants, linked those potencies to estimates of intake, and discussed the potential impact of 2 hypothetical standards on peanuts (10 or 20 mcg/kg) on sample populations and their overall risk.[1] Regarding tree nuts, similar information is required.

10. In the evaluation at its forty-ninth meeting, the JECFA noted that the carcinogenic potency of aflatoxin B1 is substantially higher in carriers of hepatitis B virus (about 0.3 cancers/year/100 000 persons/ng of aflatoxin B1/kg of body weight per day), as determined by the presence in serum of the hepatitis B virus surface antigen (HBsAg +individuals), than in HBsAg – individuals (about 0.01 cancers/year/100 000 persons/ng of aflatoxin B1/kg of body weight per day).[1] The JECFA also noted that vaccination against hepatitis B virus would reduce the number of carriers of the virus, and thus reduce the potency of the aflatoxins in vaccinated populations, leading to a reduction in the risk for liver cancer.[1]

11. Recent studies have shown presence of anti-mutagenic compounds (including linoleic acid) in corn, which inhibit the mutagenic potential of aflatoxin B1.[3-4] It is also reported that some tree nuts (walnut, pecan, pistachio, etc.) contain linoleic acid[5]. Therefore, further research is needed to verify possible inhibitory role of linoleic acid as an anti-mutagenic compound in these tree nuts.

SAMPLING

12. Although the incidence of aflatoxin contamination in tree nuts is low, aflatoxin levels can be quite variable and high levels can be develop in a small percentage of nuts.[6-7] The distribution of aflatoxin in pistachios and almonds has been thoroughly investigated in the United States.[7-15] The results from the investigations indicate that sorting for quality removes a large part of the aflatoxin present at harvest. Furthermore, these studies have also evaluated sampling methods and could form the basis for a Codex sampling plan for aflatoxins. The distribution of aflatoxins are very heterogeneous in tree nuts and consequently the out-line of the sampling plan is critical. Distribution data from other countries are needed before an attempt is made to develop an international sampling plan for tree nuts.

ANALYTICAL METHODS

13. Nowadays**,** for analysis of mycotoxins, there are adequate quality assurance means in place, both to assist laboratories to get accurate and reliable results and to check and demonstrate consistent satisfactory performance. Validated analytical methods are those for which performance characteristics have been established by interlaboratory collaborative trials and these are now widely accepted as being essential for monitoring and regulatory purposes. In addition to employing validated methods, internal quality control procedures need to be implemented in chemical laboratories – this normally implies accreditation, participation in proficiency testing and the proper use of control and reference materials.[16]

14. Various analytical methods for the identification and quantification of aflatoxins have been developed. For analysis of aflatoxins in tree nuts, thin layer chromatography (TLC), high performance liquid chromatography (HPLC), and immunochemical methods of analysis have been validated. Early methods were generally based on TLC.[17] TLC is still the widely used method for determination of aflatoxins in many developing countries. HPLC methods with fluorescence detection are most common in developed countries.[18-22] TLC and LC methods for determining aflatoxins in food are laborious and time consuming. Through advances in biotechnology, highly specific antibody-based test kits are now commercially available that can be used as a fast screening method for analysis of aflatoxins in foods. Only a few of these test kits have been evaluated by collaborative studies. It is believed that simple, specific, and rapid immunological methods will play a prominent role in monitoring tree nuts and other commodities for aflatoxins.[23] If the level of aflatoxins are higher than the accepted limits, the results should be confirmed using confirmatory tests (such as TLC).

OCCURRENCE OF AFLATOXINS IN TREE NUTS

15. *Aspergillus* fungi commonly grow on dead organic matter, including fallen blossoms and leaves as well as other dead plant material, that may be found on the ground in tree orchards. *Aspergillus* is only rarely able to infect healthy plant or nut tissue. *Aspergillus* infection and subsequent aflatoxin production depends on plant stress and/or insect/pest damage. Moist environments and optimal temperatures favour the proliferation of the fungi. Spores released by the fungi can be carried by the wind and by various insects into the foliage and nuts developing on trees with the possibility of producing aflatoxins. Whenever feasible, organic debris should be incorporated into the orchard soil during the early stages of nut development, a pest control program should be instituted early on, and irrigation (if needed) should be managed to reduce orchard floor dampness and high relative humidity during the maturation of the nuts. After maturation and harvest of the nuts, the common post-harvest procedures for most species of nuts include collecting, cleaning, drying, hulling, washing and/or drying. Operations specific to individual species of nuts include sorting, sizing, grading and testing for aflatoxin contamination. The shelf life of harvested tree nuts is expected to vary with the degree of processing and the conditions of storage.

a) Almond

16. Almonds are subject to damage by insects at the preharvest stage while still on the tree as well as during the postharvest stage when they are usually left for varying periods of time on the ground before they are moved to covered piles for storage.

17. HPLC analysis of eleven almond samples [with and without shells] imported to Qatar during 1997 revealed that none of them was contaminated with aflatoxins. The limit of detection (LOD) was 0.1 mcg/kg for each aflatoxins.[24]

18. Among 9719 samples of raw and semi-processed product of almonds were analyzed by HPLC method during 1993-1999 in USA, 8736 samples were not contaminated (<LOD), 611 samples contained total aflatoxins between LOD-4.9 mcg/kg, 212 samples contained total aflatoxins between 5-19.9 mcg/kg, 121 samples contained total aflatoxins between 20-99.9 mcg/kg and 39 samples contained total aflatoxins higher than 100 mcg/kg. The LOD of method was 0.1 mcg/kg. The maximum level detected was 1450 mcg/kg. Samples taken from damaged kernels (e.g. chipped and scratched, whole and broken, ungraded, blanching stock, etc.) showed the highest incidence of aflatoxins.[25]

19. The aflatoxins in 1547 samples of whole and/or broken natural almonds and in manufacturing stock almonds were analyzed for the 1993 crop. Analysis was carried out using HPLC. The LOD for total aflatoxins was 1 mcg/kg. Nineteen whole and/or broken natural almond samples, 84 samples of manufacturing stock and 8 ungraded samples contained aflatoxins at levels greater than 1 mcg/kg. The overall aflatoxin level was 0.67 mcg/kg.[15]

20. Two hundred and thirty three samples of in-shell and nutmeats were assayed for aflatoxins by TLC method in 1972. Aflatoxins were found in 10 of 74 samples of unsorted in-shell almonds and 9 of the 10 samples contained total aflatoxins less than 20 mcg/kg. The LOD of method was 1 mcg/kg. Sorting procedures were effective in removing most of contaminated nutmeats since none of the 26 samples of processed whole nutmeats contained aflatoxins.[6]

21. The occurrence of aflatoxins in 38 almond samples collected from the Spanish retail market was investigated by TLC method throughout 1986 and 1987. AFB1 and AFB2 were detected in 1 almond sample at the levels of 95 mcg/kg and 15 mcg/kg, respectively.[26]

b) Brazil nut

22. Among 416 Brazil nut (shelled and in shell) samples analysed using mostly TLC method during 1998-2002 in Brazil, 203 samples contained total aflatoxins lower than 0.8 mcg/kg, 60 samples contained total aflatoxins between 0.8-2 mcg/kg, 40 samples contained total aflatoxins between 2-4 mcg/kg, 38 samples contained total aflatoxins between 4-20 mcg/kg and 75 samples contained total aflatoxins higher than 20 mcg/kg. The contamination level ranged 0.4-10732 mcg/kg. The LOD of TLC method for aflatoxins B1, B2, G1 and G2 were 0.6, 0.3, 0.4 and 0.3 mcg/kg, respectively.[27]

23. During 1993, among 176 Brazil nuts analysed in the United States, 11% were contaminated at levels between trace to 20 mcg/kg, and 6% were contaminated at levels greater than 20 mcg/kg. The maximum level detected was 619 mcg/kg.[28]

24. Among 74 Brazil nut samples analyzed in Japan, 70 samples were not contaminated and only 2 samples contained aflatoxins higher than 10 mcg/kg. The maximum level detected was 123 mcg/kg.[1]

25. According to the FSA survey in four regions of the UK, among 12 Brazil nuts analyzed by HPLC, the level of total aflatoxins of all samples were below 1 mcg/kg (LOQ).[29]

c) Cashew nut

26. Three cashew nuts available in the local retail market in Lodz, Poland, were investigated for aflatoxins. One sample was contaminated with AFB1 at the level of 0.35 mcg/kg.[30]

27. Aflatoxins were not detected in 17 cashew nut samples analyzed by TLC method, in Osaka, Japan, during 1988-1992. The LOD was 0.1 mcg/kg for individual aflatoxins.[31]

28. According to the FSA survey, from 6 cashew nuts analyzed by HPLC, none of the samples was contaminated with aflatoxins. The LOQ for total aflatoxins was below 1 mcg/kg.[29]

d) Hazelnut

29. According to the FSA survey, the level of total aflatoxins in two hazelnut samples, analyzed by HPLC, were below 0.8 mcg/kg (LOQ).[29]

30. Two samples were analysed by HPLC in a survey of nuts in Qatar during 1997. None of the samples were contaminated with aflatoxins. The LOD for aflatoxin B1 was 0.1 mcg/kg.[24]

31. During 1998-2002, 13802 hazelnut samples were analyzed using TLC and HPLC in Turkey. The mean of AFB1 ranged 0.018-0.520 mcg/kg and the maximum level detected was 149.9 mcg/kg.[32]

e) Macadamia

32. Aflatoxins were not detected in 2 macadamia samples analyzed, by a TLC method in Osaka, Japan, during 1988-1992. The LOD was 0.1 mcg/kg for individual aflatoxins.[31]

f) Pecan

33. According to a survey in USA during 1972-1974 using a TLC method, only three out of the 48 samples contained total aflatoxins at levels greater than 8 mcg/kg (LOD).[33]

34. According to the FSA survey, the level of total aflatoxins in two pecan nuts, analyzed by HPLC, were below 0.8 mcg/kg (LOQ).[29]

g) Pine nut

35. According to the FSA survey, the level of total aflatoxins in 3 pine nuts, analyzed by HPLC, were below 0.8 mcg/kg (LOQ).[29]

36. Aflatoxin B1 was not detected in three pine nut samples analyzed by HPLC in china during February 2003. The lod was 5 ng/gr for aflatoxin B1. [42]

h) Pistachio

37. According to a report from Mexico, among 244 pistachio samples analysed during 1993-1996, 5 samples contained aflatoxins higher than 20 mcg/kg.[1]

38. Among 21 pistachio samples analyzed in Sweden between 1996 to 1998, 14 samples were not contaminated (<LOD), 5 samples contained AFB1 between LOD-2 mcg/kg and 2 samples contained AFB1 higher than 2 ppb. The maximum level detected was 1900 ppb and the LOD of the method was 0.005 mcg/kg.[34]

39. It was not detected any aflatoxins in 24 pistachio samples analyzed by TLC in Osaka, Japan from 1988 through 1992. The LOD was 0.1 mcg/kg for individual aflatoxins.[31]

40. According to the report of Japanese Ministry of Health, among 2422 pistachio samples analysed during 1972-1989, 2339 samples were not contaminated (<LOD), 35 samples contained aflatoxin B_1 between LOD-10 mcg/kg and 48 samples contained aflatoxin B_1 higher than 10 mcg/kg.[1] Among 47361 pistachio samples analysed, using mostly TLC and some HPLC, during March 1998-March 2001 in I. R. Iran, 28227 samples were not contaminated (<LOD), 7862 samples contained aflatoxin B1 between LOD-2 mcg/kg, 6583 samples contained aflatoxin B1 between 2-10 mcg/kg, and 4689 samples contained aflatoxin B1 higher than 10 mcg/kg. The maximum level detected was 1426 mcg/kg.[35-36]

41. In Food and Drug Control Labs of Iranian Ministry of Health, 7926 pistachio samples were analysed using mostly HPLC during March 2001-March 2002. The data indicated that 5390 samples were not contaminated (<LOD), 1324 samples contained aflatoxin B1 between LOD-2 mcg/kg, 451 samples contained aflatoxin B1 between 2-10 mcg/kg and 761 samples contained aflatoxin B1 higher than 10 mcg/kg. The maximum level detected was 261.8 mcg/kg. The LOD for aflatoxin B1 was 0.1 mcg/kg.[37]

42. During March 2001-March 2002, 3629 pistachio samples were analysed by Institute of Standards and Industrial Research of Iran affiliated Food Quality Control Labs using TLC methods. The results showed that 2286 samples were not contaminated (<LOD), 201 samples contained aflatoxin B1 between LOD-2 mcg/kg, 689 samples contained aflatoxin B1 between 2-10 mcg/kg, and 453 samples contained aflatoxin B1 higher than 10 mcg/kg. The maximum level detected was 710 mcg/kg.[38]

43. Among 2333 raw pistachio samples analysed in USA using HPLC between 1999 to 2002, 2118 samples were not contaminated (<LOQ), 107 samples contained total aflatoxins between 0.5-2 mcg/kg, 35 samples contained total aflatoxins between 2-5 mcg/kg, 24 samples contained total aflatoxins between 5-10 mcg/kg, 19 samples contained total aflatoxins between 10-15 mcg/kg, 4 samples contained total aflatoxins between 15-20 mcg/kg and 26 samples contained total aflatoxins higher than 20 mcg/kg. The maximum level detected was 169 mcg/kg. The LOQ of the method for total aflatoxins was 0.5 mcg/kg.[39]

44. During 1998-2002, 523 pistachio samples were analyzed using TLC and HPLC in Turkey. The mean of AFB1 ranged 1-3.78 mcg/kg and the maximum level detected was 113 mcg/kg.[32]

45. According to the FSA survey, among 52 pistachio samples analysed using HPLC, 44 samples were not contaminated (<LOQ), 2 samples contained total aflatoxins between LOQ-4 mcg/kg, 2 samples contained total aflatoxins between 4-10 mcg/kg and 4 samples contained total aflatoxins higher than 10 mcg/kg. The maximum level detected was 106.9 mcg/kg. The LOQ of method for total aflatoxins was below 0.8 mcg/kg.[29]

i) Walnut

46. Analysis of 40 in-shell walnut samples by TLC method in USA revealed that 14 samples were contaminated with aflatoxins and only 3 samples contaminated at levels greater than 1 mcg/kg. The maximum level detected was 10 mcg/kg. Several lots of walnuts, both in-shell and shelled, were unusual in that they contained AFB_2 in absence of AFB1. Positive identification of B_2 was made in a few cases by comparing the mass spectrum with that of a standard.[40]

47. HPLC analysis of 4 walnut samples [without shell] imported to Qatar during 1997 revealed that none of the samples was contaminated with aflatoxins. The LOD was 0.1 mcg/kg for each aflatoxins.[24]

48. Aflatoxins were not detected in 3 walnut samples analysed by a TLC method in Osaka, Japan, during 1988-1992. The LOD was 0.1 mcg/kg for individual aflatoxins.[31]

49. During 1998-2002, 51 walnut samples were analyzed using TLC and HPLC in Turkey. The mean and maximum level of AFB1 were 0.029 and 1.1 mcg/kg, respectively.[32]

50. HPLC analysis of thirty one walnut samples in February of 2003 in china revealed that none of them was contaminated with aflatoxin B1. The limit of detection (LOD) was 5 ng/gr for aflatoxin B1. [42]

DIETARY INTAKE

51. The primary route of potential human exposure to aflatoxins is ingestion of contaminated food. Grains, peanuts, tree nuts, and cottonseed meal are among the foods on which aflatoxin-producing fungi commonly grow. Meat, eggs, milk, and other edible products from animals that consume aflatoxin–contaminated feed are additional sources of potential exposure.[1] At present there is not enough data available on aflatoxin exposure due to consumption of tree nuts.

52. Tree nuts constitute a very small portion of daily food intake in different regions of the world. According to GEMS/FOODS regional Diets (1998) summarized in Table 1, the daily intake of tree nuts varies from 0 to 1.8 gram per person per day (g/person/day).[41] Based on this information, the percentage of tree nut to cereals consumption in the Middle Eastern and European countries can be estimated as 0.23 and 1.68%, respectively (Table 1).

Table 1: Tree nuts consumption (g/person/day) in comparison with cereals in different regions of the world.[41]

Commodities	Middle East	Far East	African	Latin America	European
Tree Nuts	1.0	13.5	3.4	17.5	3.8
Cereals	430.8	425.3	318.4	252.5	226.3
Tree Nuts /Cereals (%)	0.23	2.98	1.07	6.93	1.68
Cereals /Tree Nuts (Ratio)	430.80	33.50	93.65	14.43	59.55

53. Therefore, although commodities such as corn, peanuts, cottonseed, brazil nuts, pistachio nuts and copra are classified as commodities with the highest risk of aflatoxin contamination, their associated risk to humans vary due to difference in their dietary intake. These data may suggest that consumption of tree nuts is very low compared to cereals and that this lower consumption should be considered in setting aflatoxin tolerances.

54. The estimated aflatoxin intake in France, computed by JECFA (1998), presented in Table 2, clearly shows that more than 95 % of the aflatoxin intake comes from the consumption of cereals while nuts [including both tree nuts and groundnut] contribute only 1.6 % of aflatoxin intake.[1]

Table 2: Estimated aflatoxin intake in France (μg/day) [JECFA evaluation (1998)].[1]

Foodstuffs	**Mean of aflatoxin intake (μg/day)**	**Percent (%)**
Cereals	2.42	95.65
Nuts	0.04	1.58
Spices	0.01	0.40
Milk	0.06	2.37
Total	2.53	100

CONCLUSIONS & RECOMMENDATIONS:

55. The present Discussion Paper on Aflatoxins in Tree Nuts leads to the following broad recommendations for consideration at the 36th Session of the CCFAC:

I) Based on all the toxicological evidence available to date, levels of aflatoxins need to be as low as technologically feasible, taking into account economic and social factors. Some ways to reduce the exposure of consumers to aflatoxin contaminated tree nuts include:

1st) The implementation of good agricultural practices (GAP) that will result in eliminating or reducing the possible routes by which fungi may gain entry to various nuts, begin proliferating and producing aflatoxins during the preharvesting and harvesting periods.

2nd) The implementation of good manufacturing practices (GMP) and good storage practices (GSP) during post-harvest and processing operations.

C) Supporting research on the ecology of the Aspergillus fungi, the effects of various environmental factors, and fungal/pest interactions that might influence aflatoxin contamination of nuts on the tree and during storage with the objective being to reveal critical control points that might be used in developing a HACCP program for tree nuts in a given area.

D) Supporting additional research on methods and techniques to prevent fungal contamination in tree nuts during preharvest, harvesting, processing and storage.

II) It is recommended that the CCFAC request governments to provide additional survey data on the occurrence of aflatoxins in tree nuts to supplement the data presented in this document, so that the establishment of maximum levels may be considered. It is further recommended that governments be requested to submit any available data on the distribution of aflatoxins in tree nuts in their respective countries.

III) It is recommended that CCMAS should establish sampling plans and methods of analysis for aflatoxins in tree nuts.

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