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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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**Agenda Item 15(d)** **CX/FAC 03/23**
**January 2003**

**JOINT FAO/WHO FOOD STANDARDS PROGRAMME**

**CODEX COMMITTEE ON FOOD ADDITIVES AND CONTAMINANTS**
**Thirty-fifth Session**
**Arusha, Tanzania, 17 - 21 March 2003**

# DISCUSSION PAPER ON AFLATOXINS IN TREE NUTS, INCLUDING INFORMATION SUBMITTED ON AFLATOXIN CONTAMINATION AND METHODS OF ANALYSIS FOR THE DETERMINATION OF AFLATOXINS IN TREE NUTS IN RESPONSE TO CL 2002/10-FAC

**Secretariat Note:** Due to time constraints, comments are not being requested on the attached document and therefore, comment summary paper CX/FAC 03/23-Add. 1 will not be issued.

## BACKGROUND

1. The 34th session of the CCFAC decided that a drafting group led by Iran would revise the Discussion Paper on Tree Nuts for circulation, comment and further consideration at its current meeting. The CCFAC also agreed that information on aflatoxin contamination in tree nuts as well as methods of analysis for the determination of aflatoxins in tree nuts wourd be requested.[1]

## INTRODUCTION

2. This discussion paper is applicable to all tree nuts with edible kernel of both consumption and global trade interest, including but not limited to almonds *(Prunus amygdalu*s), Brazil nut *(Bertholletia excels*a), cashew nut *(Anacardium occidental*e), hazelnut *(Corylus* Spp.), macadamia *(Macadamia integrifoli*a), pecan *(Carya illinoensi*s), pine nut *(Pinus parrayan*a), pistachio *(Pistacia ver*a) and walnut *(Juglans regi*a).

3. The aflatoxins are produced primarily by some strains of *Aspergillus flavus* and most, if not all, strains of *A. parasiticu*s. aflatoxin B1 is the most frequent one present in contaminated samples and aflatoxins B2 , G1 and G2 are generally not reported in the absence of AFB1. 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

4. 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

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1 ALINORM 03/12, para. 127.

## TOXICOLOGICAL EVALUATION

5. The aflatoxins were evaluated by JECFA at its thirty-first, forty-sixth, forty-ninth and its fifty-sixth meetings (aflatoxin M1 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 theses compounds are genotoxic carcinogens, but the potency estimates for human liver cancer resulting from exposure to aflatoxin B1 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 ppb) on sample populations and their overall risk.1 Regarding tree nuts, similar information is required.

6. 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

7. 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

8. Recent studies have shown presence of anti-mutagenic compounds (including linoleic acid) in corn, which inhibit the mutagenic potential of aflatoxin B1. 3-4 Considering the fact that some tree nuts (walnut, pecan, pistachio, etc.) contain anti-mutagenic compounds (such as linoleic acid)5 , further research is needed to verify their inhibitory role against mutagenic potential of aflatoxin B1.

## SAMPLING

9. Although the incidence of aflatoxin in tree nuts is low, however aflatoxin levels can be quite variable and high levels can be develop in small percentage of nuts.6-7 The distribution of aflatoxin in pistachios and almonds has been thoroughly investigated.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.

## ANALYTICAL METHODS

10. Various analytical methods for the identification and quantification of aflatoxins have been developed. For analysis of aflatoxins in tree nuts, thin layer chromatography (TLC), liquid chromatography (LC), and immunochemical methods of analysis have been validated. Early methods were generally based on TLC.16 Today, methods using HPLC with fluorescence detection are most common.17-21 TLC and LC methods for determining aflatoxins in food are laborious and time consuming. Through advances in biotechnology, highly specific antibody-based tests are now commercially available that can identify and measure Aflatoxins in food in less than 10 min. Only a few of these kits have been evaluated by collaborative studies. The simple, specific, and rapid immunoassays will play an increasing role in monitoring foods and feeds for mycotoxins.22

## OCCURRENCE OF AFLATOXINS IN TREE NUTS

### *a) Almond*

11. Eleven almond samples [with and without shells] were analysed for aflatoxins in Qatar during 1997. All of the samples were found free from aflatoxins.23

12. Among 9709 samples of raw and semi-processed product of almonds were tested to detect aflatoxins during 1993-1999 in USA, 90% were negative and 10% were positive and <1% tested less than 5 µg/kg. It is important to note, however, that despite the higher incidence of contamination in the raw almonds during 1998, only 1 sample of blanched almonds tested above 4 µg/kg.24

13. The aflatoxin distribution function in individual insect-damaged almonds obtained from an almond processor in California, USA, was determined. Substantially all almonds exhibited a positive aflatoxin level between 0.02 ng and 0.3 ng.14

14. The aflatoxin levels in 1547 samples of whole and/or broken natural almonds and in manufacturing stock almond were analyzed for the 1993 crop. The overall aflatoxinlevel was 0.67 ng/g, 33% from finely diced and ground almonds, 11% from slivered and sliced almonds and 49% due to natural almonds of unknown grade. A total of 1.7% of whole and/or broken natural almond 9.7% of manufacturing stock contained aflatoxin > 1ng/g.15

15. Aflatoxin was found in 14% of 74 samples of unsorted in-shell almonds in 1972 but it occurred at levels low than 20ppb in 90% of the contaminated samples. Commercial sorting procedures are effective in removing most contaminant nutmeats since none of 26 samples of processed whole nutmeats contained aflatoxin. In contrast, 13 of 27 samples of diced almond were contaminated but 9 of these contained <9 ppb. Only one of 25 samples of sliced nutmeats contained aflatoxin (4 ppb). Thus its incidence varies greatly with the category of finished product.6

***b) Brazil nut***

16. Among 416 Brazil nut (shelled and in shell) samples analysed during 1998-2002 in Brazil, 48.8% contained aflatoxin total lower than 0.8 ppb, 14.4% contained aflatoxin total between 0.8-2 ppb, 9.6% contained aflatoxin total between 2-4 ppb, 9.1% contained aflatoxin total between 4-20 ppb and 18.0% contained aflatoxin total higher than 20 ppb.25

17. Poland reported that among 176 brazil nut samples analysed, 17% were contaminated in the range of trace to 619 ppb.26

18. Among 74 brazil nut samples analyzed in Japan, 70% contained aflatoxins lower than limit of detection, and only 2% contained aflatoxins higher than 10 ppb.1

19. According to the FSA survey in four regions of the UK, from 12 consumer-size samples, none had aflatoxin levels more than 2 µg/kg.27

***c) Cashew nut***

20. Different kinds of raw and processed nuts available in the local retail market in Lodz, Poland, were investigated for aflatoxin content. The concentration of aflatoxin B1 in contaminated samples of cashew nuts was 0.35 µg/Kg.28

21. Aflatoxins were not detected in cashew nut samples analyzed in Osaka, Japan, during 1988-1992. 29

***d) Hazelnut***

22. According to the FSA survey, the level of contamination in hazelnut samples (Sample no =2) was below limit of detection.27 Similar results were obtained in a survey in Qatar (Sample no =2).23

***e) Macadamia***

23. Aflatoxins were not detected in cashew nut samples analyzed in Osaka, Japan, during 1988-1992. 29

***f) Pecan***

24. According to a survey in USA during 1972-1974, only three out of the 48 samples contained aflatoxins more than 8 ppb.30

***g) Pine nut***

25. [To Be Completed]

***h) Pistachio***

26. According to a report from Mexico, among 244 pistachios samples analysed during 1993-1996, 2 % contained aflatoxins higher than 20 ppb.1

27. Among 21 pistachios samples analyzed in Sweden between 1996 to 1998, more than 90% of samples contained aflatoxin B1 and total lower than 2 and 4 ppb (EU maximum limits for aflatoxins), respectively, although in two samples, much higher levels, up to 1900 and 2200 ppb, respectively, was reported.31

28. Taguchi et al. did not detect any aflatoxins in 24 pistachios samples analyzed in Osaka from 1988 through 1992. 29

29. According to the report of Japanese Ministry of Health, among 2422 pistachio samples analysed during 1972-1989, 96.5% contained aflatoxin B1 Lower than limit of detection and 2% contained aflatoxin B1 higher than 10 ppb.1

30. Among 47361 pistachios samples analysed during March 1998-March 2001 in I. R. Iran, 59.6% contained aflatoxin B1 lower than limit of detection (LOD), 16.6% contained aflatoxin B1 between LOD-2 ppb, 13.9% contained aflatoxin B1 between 2-10 ppb, and 9.9% contained aflatoxin B1 higher than 10 ppb.32-33

31. In Food and Drug Control Labs of Iranian Ministry of Health, 7926 pistachio samples were analysed during March 2001-March 2002. The data indicated that 68 % of samples contained aflatoxin B1 lower than limit of detection (LOD), 16.7% contained aflatoxin B1 between LOD-2 ppb, 5.7 % contained aflatoxin B1 between 2-10 ppb and 9.6% contained aflatoxin B1 higher than 10 ppb.34

32. During March 2001-March 2002, 3629 pistachio samples were analysed by Institute of Standards and Industrial Research of Iran affiliated Food Quality Control Labs. The results showed that 63 % contained aflatoxin B1 lower than LOD, 5.5% contained aflatoxin B1 between LOD-2 ppb, 19.0 % contained aflatoxin B1 between 2-10 ppb, and 12.5% contained aflatoxin B1 higher than 10 ppb.35

33. Among 2333 raw pistachio samples analysed in USA between 1999 to 2002, 90.8% contained total aflatoxins below 0.5 ppb, 4.6% contained total aflatoxins between 0.5-2 ppb, 1.5% contained total aflatoxins between 2-5 ppb, 1% contained total aflatoxins between 5-10 ppb, 0.81% contained total aflatoxins between 10-15 ppb, 0.17% contained total aflatoxins between 15-20 ppb and 1.1% contained total aflatoxins higher than 20 ppb.36

***i) Walnut***

34. Analysis of 40 in shell walnut samples in USA revealed that only 3 samples contained >1 ppb of aflatoxins. Several lots of walnut both in shell and shelled were unusually contaminated with AFB2 (without AFB1).37

35. Analysis of 4 samples of walnuts [without shell] imported to Qatar during 1997 revealed that none of them was contaminated with aflatoxins.23

36. Aflatoxins were not detected in walnut samples analysed in Osaka, Japan, during 1988-1992. 29

**DIETARY INTAKE**

37. 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 most of tree nuts.

38. 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).38 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.38**

| **Commodities** | **Middle East** | **Far East** | **Africa** | **Latin America** | **Europe** |
|---|---|---|---|---|---|
| Tree Nuts | 1.0 | 13.5 | 3.4 | 17.5 | 3.8 |
| Cereals | 430.8 | 45.3 | 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 |

39. Therefore, although commodities such as corn, peanuts, cottonseed, brazil nuts, pistachio nuts and copra all classified as commodities with the highest risk of aflatoxin contamination, but their associated risk to human varies due to difference in their dietary intake. These data may suggest that the risk associated with consumption of aflatoxin contaminated tree nuts is negligible as compared to cereals.

40. The estimated aflatoxin intake in France, computed by JECFA (1998), presented in Table 2 clearly shows that more than 95 % of aflatoxin intake comes from the consumption of cereals while nuts [including both tree nuts and groundnut] contribute only 1.6 % of aflatoxin intake.1 Therefore, tree nuts despite of their higher contamination levels than cereals have a much less important role in aflatoxin exposure.

**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:**

41. The present Discussion Paper on Aflatoxins in Tree Nuts leads to the following broad recommendations for consideration at the 35 th 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. The ultimate way to protect the consumer from the toxic effect of aflatoxins, is to encourage and ensure a good agricultural practice by:

i. revealing the critical points where the fungi starts growing and producing aflatoxins during the agricultural production.
ii. applying GAP principles in production
iii. improving the training of individuals involved at all stages of production.
iv. supporting research on methods and techniques to prevent fungal contamination in the orchard and during harvesting, processing and storage.

II) It is recommended that the Committee asks governments to provide data, to supplement the data presented in this document, so that the establishment of maximum levels can be considered.

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

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