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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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Agenda Item 7(e) **CX/CF 07/1/10**
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JOINT FAO/WHO FOOD STANDARDS PROGRAMME
CODEX COMMITTEE ON CONTAMINANTS IN FOODS
First Session
Beijing, China, 16 - 20 April 2007

DISCUSSION PAPER ON AFLATOXIN CONTAMINATION IN BRAZIL NUTS

Governments and international organizations are invited to submit comments on the following subject matters no later than 31 March 2007, preferably in electronic format, for the attention of Ms. Tanja Åkesson, the Netherlands Secretariat of the Codex Committee on Contaminants in Foods, Fax No.:+31 70 3786141; 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, 00153 Rome, Italy (Fax +39.06.5705.4593; E-mail: Codex@fao.org).

BACKGROUND

1. The 35th session of CCFAC, based on the Discussion Paper CX/FAC 03/23, agreed to establish maximum levels for total aflatoxins (AFs) in almonds, hazelnuts and pistachios. Data for other tree nuts were considered insufficient to establish maximum levels. The Committee agreed that the delegation of Iran would revise this paper for discussion in its next meeting, and that additional information would be requested on AFs contamination in tree nuts other than almonds, hazelnuts and pistachios.

2. The 36th session of the CCFAC agreed to address in the Discussion Paper only Brazil nuts, as the other tree nuts (e.g. cashew nut, macadamia, pecan, pine nut, walnut etc.) had a lower incidence of AFs contamination and their volume in international trade were not significant.

3. The CCFAC agreed that the delegation of Iran would prepare a revised Discussion Paper on Aflatoxins Contamination in Brazil nuts which should consider shelled, in-shell, peeled, or unpeeled Brazil nuts. The revision should consider the written comments submitted to and made at the current meeting and should consider the ALARA principle and the JECFA assessment.

4. The 37th CCFAC agreed to establish a new electronic Working Group led by Brazil to prepare the Discussion Paper on Brazil nuts for the 38th meeting.

5. The 38th CCFAC agreed to re-establish the electronic working group, led by Brazil, to revise the discussion paper (CX/FAC 06/38/24) for discucssion at the First Session of the Codex Committee on Contaminants in Foods. The working group includes United Kingdom, the United States and the INC. The working group prepared this discussion paper taking into account additional data on the occurrence of AFs in Brazil nuts, in particular on the contribution of the shell portion to the AFs content, as maximum levels apply to the edible portion.

INTRODUCTION

6. AFs contamination can be a potential problem in tree nuts and other products such as corn, barley, peanuts, dried figs, copra, cotton seed, paprika and chili pepper. This Discussion Paper is applicable only to Brazil nuts which is the only extractivistic crop among the main traded tree nuts.

7. Brazil nuts extractivism is the process of collection and primary handling of Brazil nuts in the Amazon rainforest, where the trees grow in their natural environment (CAC, 2005c).

8. Brazil nuts are seeds of *Bertholletia excelsa Humb. & Bompl.*, whose trees have the following characteristics: height up to 60 meters; 12 years to bear fruits; live up to 500 years; occurrence in groves of 50-100 individuals and the groves are separated by up to 1 km; pollination by wild large-bodied bees, especially *Euglossinae* bees (Wadt et al., 2005).

9. The Amazon rainforest has multiple ecosystems with a huge biodiversity, place an important role in the global weather balance and is the shelter of many native ethnicities. The equatorial climate is hot and humid, with an average temperature of 26ºC and relative humidity 80-95%.

10. The Brazil nuts tree pods fall during the rainy season having a risk of AFs contamination due to the extensive exposure to high humidity and temperature in the forest.

11. The Brazil nuts extractivism represents an important activity for the native people, stimulating a sustainable use of renewable natural resources while conciliating social development with preservation. It does not involve the destruction of forest nor threat the ecological balance and the environment. According to the IBGE (Brazilian Institute of Geography and Statistics), about a million people depend on the Brazil nuts production.

12. This Discussion Paper considers many aspects related to the AFs in Brazil nuts, including occurrence and dietary intake estimation.

CHEMICAL STRUCTURE

13. AFs are a group of structurally related compounds produced under favorable conditions by some strains of *Aspergillus flavus*, *A. parasiticus, A. nomius* and *A. pseudotamarii* (ITO et al., 2001). The most known naturally occurring AFs are named B_1, B_2, G_1, and G_2. AFB_1 occurs in the highest amounts in contaminated commodities; AFB_2, AFG_1, and AFG_2 are generally not reported in the absence of AFB_1.

14. AFs are highly oxygenated heterocyclic compounds and have closely related structures. AFs contain a coumarin nucleus fused to a bifuran: Aflatoxins B (AFB) contain a pentanone structure attached to the coumarin nucleus and Aflatoxins G (AFG) a six-membered lactone (Salunkhe et al., 1987).

TOXICOLOGICAL EVALUATION

15. At its 49^{th} meeting in 1997, the JECFA considered the carcinogenic potency of AFs and the potential risks associated with their intake. No TDI (tolerable daily intake) was proposed since these compounds are genotoxic carcinogens. The potency estimates for human liver cancer resulting from exposure to AFB_1 were derived from epidemiological and toxicological studies.

16. The JECFA reviewed a wide range of studies conducted with both animals and humans that provided qualitative and quantitative information on the hepatocarcinogenicity of AFs. The Committee evaluated the potency of these contaminants, linked those potencies to estimates of intake, and discussed the potential impact of two hypothetical standards for AF contamination on peanuts (10 or 20 µg/kg) on sample populations and their overall risk. It was concluded that reducing the permitted level of AFs in peanuts from 20 µg/kg to 10 µg/kg would not result in any observable difference in rates of liver cancer (JECFA, 1998).

17. The JECFA also noted that the carcinogenic potency of AFB_1 is substantially higher in carriers of hepatitis B virus (about 0.3 cancers/year/100,000 persons/ng of AFB_1/kg bw/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 AFB_1/kg bw/day). The JECFA also observed that vaccination against hepatitis B virus would reduce the number of carriers of the virus, and thus reduce the potency of the AFs in vaccinated populations, leading to a reduction in the risk for liver cancer (JECFA, 1998).

18. At its 38th Session, the CCFAC requested the JECFA to evaluate the impact to human health from a dietary exposure to AFs from the consumption of tree nuts (ready-to-eat), including Brazil nuts. Hypothetical standards of 4, 8, 10 and 15µg/kg of total AFs should be considered, as well as exposure from other sources and previous assessments on maize and groundnuts. This evaluation is scheduled for the JECFA's 68th meeting, to be held in June, 2007.

SAMPLING

19. Although the incidence of AF contamination in tree nuts is low, the AF levels are quite variable and high levels can be found in a small percentage of nuts (Schade et al., 1975; Schatzki, 1995; Schatzki, 1996). Furthermore, an appropriate sampling plan is critical.

20. Most of the sampling procedures in place for AF in tree nuts (CAC, 2001; EU, 1993; FAO, 1993) have been derived from sampling plans developed for AF in groundnuts, the most evaluated commodity.

21. A sampling plan is defined by a test procedure that consists of a specified sample size, sample preparation method (including particle size and subsampling size) and analytical method. These parameters are defined to comply with the sample accepted contamination level.

22. The 36th CCFAC agreed that a working group led by the United States, with the assistance of Argentina, Brazil, Iran, European Community and the INC, would prepare sampling plans for AFs in almonds, Brazil nuts, hazelnuts and pistachios.

23. The 37th CCFAC decided that the proposed draft Sampling Plan should be revised taking in consideration recent data presented at the meeting.

24. The 38th CCFAC agreed to retain the proposed draft Sampling Plan at Step 4 waiting for the outcome of the Discussion Paper on Maximum Levels of AFs in tree nuts. The Committee also agreed that the sampling plan for tree nuts should include Brazil nuts, unless the data indicated the need for another specific one for this product.

ANALYTICAL METHODS

25. The AOAC Official Method 994.08 for AF in Brazil nuts, corn, peanuts, and pistachios dates back to 1994 (AOAC, 2000). The method was validated at a range of 5-30 µg/kg, with recovery of 70 to 85%, corrected for background levels. Repeatability (RSD_r) was of 8.6% at 30 µg/kg and reproducibility (RSD_R) ranged from 12 to 29.5 %.

26. General method performance characteristics for AFB_1 and AF total have been established by the European Committee for Standardization (EC 401, 2006).

27. There are a number of analytical methods available for the determination of AFs in Brazil nuts, although most of them are not fully validated. In developed countries, most laboratories use solvent extraction, followed by sample immunoaffinity columns clean-up and HPLC (high performance liquid chromatography), with fluorescence or mass spectrometry detection (Gilbert and Vargas, 2003)

28. Due to high cost of HPLC and consumables, this technique is not always available in developing countries and there is a need for less costly methods, such as those based on thin layer chromatography (TLC) (Gilbert, 1999).

29. There is a variety of antibody-based test kits for AFs. The AOAC International website (AOAC, 2005) lists different kit formats for AFB_1 and AFs total that utilize antibodies coated onto cups, ELISA plates, columns, cards and tubes. However, few kits have been validated by a full interlaboratory collaborative study (Gilbert and Vargas, 2003) and are used mostly for screening purposes.

30. The 36th CCFAC noted that there was no need for further development of methods of analysis for AFs in tree nuts as the Codex Committee on Methods of Analysis and Sampling (CCMAS) already developed some methods. Upon the request of the CCCF, the CCMAS could consider additional methods.

FACTORS AFFECTING THE PRESENCE OF AFS IN BRAZIL NUTS

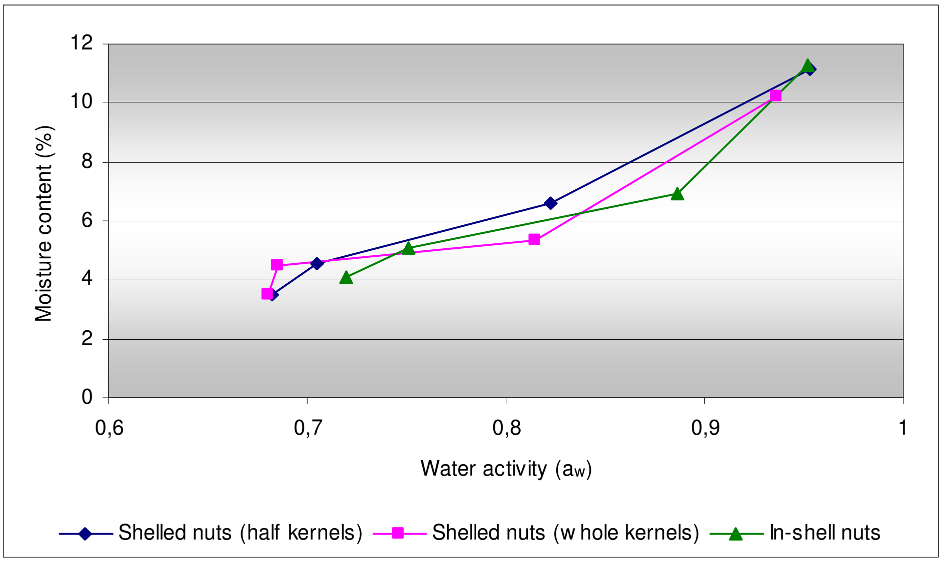
31. Several environmental factors influence fungal growth and AF production, but temperature and relative humidity are considered the most critical. Other factors include water activity, moisture, substrate composition, storage condition and insect damage (Arrus et al., 2005).

32 Aspergillus *flavus* and *A. niger* were isolated in Brazil nuts collected up to 60 days after the pods fall in the forest (*Cartaxo et al.,* 2003) and during processing (Souza et al., 2003). In addition to these species, *Aspergillus parasiticus* was also isolated in defective Brazil nuts (Brazil – Ministry of Agriculture, 2003 – unpublished data).

33. The effects of relative humidity (r.h.) and temperature on AF production were evaluated in Brazil nuts in-shell and shelled (whole and half kernels) inoculated with aflatoxigenic *Aspergillus* spp (Arrus et al., 2005). Maximum production of AFs was found in nuts stored at 25-30 ºC (97% r.h.). Half kernels showed the highest level of AFB_1 (4483 µg/kg) and total AF (6817 µg/kg) while in-shell nuts contained the lowest AFB_1 and total AFs levels (49 and 93 µg/kg, respectively). AFs were not produced at either 10 ºC (97% r.h.) or at 30 ºC (75% r.h.). This suggests that fungal growth after harvest can be prevented by an adequate control of temperature and water activity, which is an important strategy to prevent AF production in Brazil nuts.

34. According to Arrus et al. (2005), the limiting moisture content and water activity (a_w) required to control AF production (<4 µg/kg) at 30 ºC for up to 60 days of storage were: 4.57 % (a_w 0.70) for in-shell nuts and 4.50 % (a_w 0.68) and 5.05 % (a_w 0.75) for shelled nuts (whole and half kernels, respectively). Above these values, AF production may increase significantly. From the data of Arrus et al. (2005) the adsorption isotherms of Brazil nuts (in-shell, shelled) were obtained as shown in Figure 1. Fungal growth is always predicted by a_w and not by moisture content.

Fig. 1 Adsorption isotherms of in-shell and shelled (whole and half kernels) Brazil nuts at 30 ºC after 60 days of storage.



OCCURRENCE OF AFS IN BRAZIL NUTS

35. Several countries have been studying the occurrence of AFs in Brazil nuts. From 176 samples analyzed in the United States, 11 % were contaminated with AFs at levels ranging from traces to 20 µg/kg, and 6 % had levels above 20 µg/kg. The maximum level detected was 619 µg/kg (Pohland, 1993).

36. In Japan, 70 of the 74 Brazil nuts samples analysed were not contaminated and only 2 samples contained AFs above 10 µg/kg. The maximum detected level was 123 µg/kg (JECFA, 1998).

37. From 51 Brazil nuts samples analysed in the Republic of Cyprus, 10 samples were contaminated with AFs at levels ranging from 8.3 to 20 µg/kg for B_1, ND to 1.1 µg/kg for B_2 and 2.3 to 9.4 µg/kg for G_1 (Ioannov-Kakairi et al., 1999).

38. A survey was conducted by the UK Food Standard Agency between November 2003 and March 2004 for AFs in a variety of nuts and nut products. Four of the 21 analysed samples of Brazil nuts contained levels above the EC and UK regulatory limits of 4 µg/kg for total AFs (Food Standards Agency, 2004).

39. A survey conducted in Brazil from 1998 to 2004, analysed 500 (302 shelled and 198 in-shell) Brazil nut samples. No AFs were detected (<0.6 µg/kg) in 71.8 % of the shelled and 41.4 % of the in-shell nuts analysed. AFB1 levels were <2 µg/kg in 69.4 % and <10 µg/kg in 80 % of the samples (shelled + in-shell), respectively. As for AF total, 70 % and 79.8 % of the samples (shelled + in-shell) were contaminated, respectively, with levels <4 µg/kg and <20 µg/kg. The median concentrations of AFs total were 1.85 and 0.8 µg/kg in in-shell and shelled Brazil nuts, respectively (CAC, 2005b).

40. A study to evaluate the consumers' ability to discriminate AF contaminated in-shell Brazil nuts was carried out in Sweden (Marklinder et al., 2005). The median and 95^{th} percentile level of AFs in the edible portion of 132 samples collected before panel sorting were 1.4 and 557 µg/kg, respectively. After sorting these levels were 0.4 and 56 µg/kg, respectively. The AF total levels in most cases were lower in-shells than in kernels from the same samples. The study concluded that in-shell Brazil nuts may be one of the few nuts that consumers may visually select and thus protect themselves from exposure to high levels of AFs.

41. In Brazil, from 109 in-shell Brazil nuts samples analysed for AFs, 9 samples were contaminated at levels ranging from 183.4 to 924.2 µg/kg. No AFs were detected in 30 shelled samples analysed (Pacheco et al., 2006).

42. In 2005, the Brazilian government analysed 212 shelled Brazil nuts samples and found the following results for AFs: 87.7% below 4 µg/kg, 8.2% above 20 µg/kg, with a mean concentration of 14.1 µg/kg and a median of 0.8 µg/kg. For AFB_1, 86.8% were below 2 µg/kg and 6.6% were above 20 µg/kg, with mean concentration of 6.3 µg/kg and median of 0.2 µg/kg (Brazil unpublished data, 2005).

DIETARY INTAKE

43. Cereals (mainly corn), groundnuts, cottonseed, Brazil nuts, pistachio, dried figs, spices and copra are the main products contaminated with AFs. The most important dietary sources of AFs are corn, groundnuts and their products, which may form an essential part of the diet in some countries (CAC, 2005a).

44. The dietary intake of AFs by the Swedish population was estimated to be of 0.6 and 0.7 ng/kg bw for the average and the high consumers (95^{th} percentile), respectively (Thuvander, 2001). The estimated Brazil nuts consumption was 0.3 g/day for both the average and the 95^{th} percentile consumers. The body weights of the two populations were not reported. In another study in Sweden, for a 70 kg b.w consumer, yearly consumption was assumed to be a 300 g bag of Brazil nuts, the median AFs intake was estimated as 0.73 ng/kg bw and the 95^{th} percentile 1.10 ng/kg bw (Marklinder et al., 2005).

45. Table 1 shows the consumption data, according to the 13 GEMS/Food Consumption Cluster Diets (WHO, 2006), of potential sources of exposure to AFs. The consumption of Brazil nuts is 0 g/person/day in A, B, C, D, F, G, H, I, J and L Diets. For the E (Western Europe), K (North of South America, including Brazil, Central America and Caribe) and M (Argentina, Chile, Canada, USA, Australia and New Zeland) Diets the consumption was 0.1 g/person/day. This consumption represents 0.2, 1.2 and 0.1 %, respectively, of the total consumption of food with the potential to be contaminated with AFs.

Table 1 – Several Aflatoxin source products consumption (g/person/day) in the 13 GEMS/Food Consumption Cluster Diets

Product	GEMS/Food Consumption Cluster Diets (g/person/day)												
	A	B	C	D	E	F	G	H	I	J	K	L	M
CORN	82.7	148.4	135.9	31.8	33.3	7.5	35.2	298.6	248.1	57.4	63.1	58.6	85.5
TREE NUTS	4,2	21,5	3,9	3,0	5,5	10,2	16,3	15,7	9,7	1,9	19,1	29,0	5,6
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
NUTS PREPARED	0,0	0,4	0,1	0,1	0,6	0,9	0,1	0,1	0,1	0,0	0,0	0,4	0,1
PECAN NUTS	0,0	0,0	0,0	0,0	0,1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1
GROUNDNUTS IN-SHELL	7,6	4,3	3,0	1,0	5,6	2,0	10,6	2,9	6,6	30,5	1,3	1,0	9,7
GROUNDNUTS SHELLED	5,4	3,1	2,1	0,7	4,0	1,4	7,6	2,1	4,7	21,8	0,9	0,7	6,9
SPICES	2,7	1,1	2,4	0,9	1,8	1,1	2,3	1,9	1,4	1,3	0,4	0,6	1,7
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
TOTAL	102.6	179.4	147.8	37.5	51.2	23.3	72.1	321.3	270.6	112.9	84.9	90.3	109.8

46. The intake of AFs from the consumption of Brazil nuts was calculated by multiplying the mean and the median concentrations found in shelled samples analysed in Brazil in 2005 (Brazil, 2005) for the E, K and M cluster diet consumption data (Tables 2).

47. The estimated AFs intakes were 0.02 and 0.001 ng/bw/day considering the mean and the median concentrations, respectively (Table 2).

Table 2. Daily intake of aflatoxins from Brazil nuts consumption for the E, K and M GEMS/Food Cluster diets

Brazil nuts consumption (g/day)	Total Aflatoxin level (µg/ kg)		Mean intake		Median intake	
	Mean	Median	ng/person/ day	ng/kg bw/day*	ng/person/ day	ng/kg bw/day*
0.1	14.1	0.8	1.41	0.02	0.08	0.001

* considering a 60 kg body weight

48. AF dietary intakes were also estimated using the Brazil nuts consumption data for E, K and M Cluster Diets and different hypothetical standard concentrations of AFs. The intakes ranged from 0.007 to 0.034 ng/kgbw/day (Table 3). The carcinogenic potency of AFB_1 evaluated by the JECFA (1998) is 0.3 and 0.01 cancers/year/100,000 persons/ng AFB_1/kgbw/day for $HBsAg^+$ and $HBsAg^-$ individuals, respectively.

Table 3 – Comparison of aflatoxins intake from the consumption of Brazil nuts in the E, K and M Clusters considering different hypothetical standards

Brazil nuts intake (g/day)	Aflatoxin intake (ng/bw per day*)				
	4 µg/ Kg	8µg/ Kg	10 µg/ Kg	15 µg/ Kg	20 µg/ Kg
0.1	0.007	0.014	0.017	0.025	0.034

* considering a 60 kg body weight

CONCLUSIONS & RECOMMENDATIONS:

49. The present Discussion Paper on AFs in Brazil Nuts leads to the following conclusions and recommendations for consideration at the 1st Session of the CCCF:

I) Brazil nuts production represents an important economic activity for the Amazon population, contributing to the rainforest preservation.

II) Although the consumption of Brazil nuts leads to a low dietary daily intake of AFs, restrictive standard levels have been set in some countries. Furthermore, there is a need for an international regulatory level, based on scientific evidence, aiming at the protection of human health with a minimum economical impact on international trade.

III) A Code of Practice for the Prevention and Reduction of AFs Contamination in Tree Nuts was adopted by the CAC at its 28th Session and an Appendix specific for Brazil nuts was adopted by the CAC at its 29th Session. The results of the implementation of the recommended measures will be available in the next few years.

IV) Before establishing a limit for AFs in Brazil nuts, it is recommended that the CCCF should consider:

a) The results of the JECFA dietary exposure assessment on tree nuts (ready-to-eat), including Brazil nuts, and the impact on exposure to human health taking into account hypothetical standards for AF contamination.

b) This assessment should also include the hypothetical standard of 20 µg/kg, considering the low consumption of Brazil nuts and the social and economical importance of this product for the producing countries.

c) There is a significant difference between the level of AF contamination in shelled and in-shell nuts. According to the CODEX STAN 193 the maximum limits should preferably be based on the edible portion.

d) It is necessary additional data to clarify the influence of the shell in the final AF contamination level on the edible portion of Brazil nuts.

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