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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

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CODEX COMMITTEE ON FOOD ADDITIVES AND CONTAMINANTS

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DISCUSSION PAPER ON AFLATOXIN CONTAMINATION IN BRAZIL NUTS

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 (AFs) in Tree Nuts for circulation, comments and further consideration at its next meeting. The 34th session of the CCFAC also agreed that information on AFs in as well as methods of analysis 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 AFs in almonds, hazelnuts and pistachios. The remaining data for other varieties of tree nuts were considered insufficient to establish 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 AFs contamination in tree nuts other than almonds, hazelnuts and pistachios.

3. The 36th session of CCFAC agreed to address only Brazil nuts as the other tree nuts mentioned in the discussion paper (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.

4. The Committee agreed that the delegation of Iran would prepare a revised Discussion Paper on AFs Contamination in Brazil Nuts which should consider shelled, in-shell, peeled, or unpeeled Brazil nuts. The revision, for circulation, comments and consideration at the 37th meeting of committee, should be made on basis of the written comments submitted to and made at the current meeting and should take into account the ALARA principle with due consideration of JECFA assessment.

5. The 37th CCFAC meeting agreed to establish a new working group led by Brazil to prepare the Discussion Paper on Brazil nuts for the 38th meeting. The comments and data on AFs contamination in Brazil nuts presented by Brazil during the 37th meeting should be taken in consideration for the elaboration of the new document.

INTRODUCTION

6. AFs contamination is a potential problem in tree nuts and other commodities. 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 Brazil nut trees grow in their natural environment (CAC, 2005c).

8. Brazil nuts are seeds of the tree *Bertolethia excelsa Humb. & Bompl.*, which grows within the rain forest, frequently in the more dense areas of the Amazon forest. The trees which may grow up to 60 meters, take about 12 years to bear fruits and can -live up to 500 years. The Brazil nut tree is not evenly distributed, but instead often occurs in groves of 50-100 individual trees with each grove separated from another by distances up to 1 km. Brazil nut trees are not grown in orchard as they count on associated forest species for pollination and nut production. Brazil nut is pollinated by large-bodied bees especially *Euglossinae* bees (Wadt et al., 2005). .

9. The Amazon rainforest consists of multiple ecosystems where diverse nationalities and ethnicities live. Around 60 % of the forest is located in Brazilian lands with the remaining 40 % distributed among other South American countries. The equatorial climate in the region is hot and humid, with an average temperature of 26ºC. As the rainy season begins, the pods start falling and the risk of AFs contamination increases due to the high level of air humidity and warm temperature, which cannot be controlled.

10. The Amazon rainforest has been considered one of greatest environmental importance due to its role in the ecological balance and also because the region is the habitat of various native people and presents incredible biodiversity. Moreover, the Brazil nut industry comports with the principal objectives of international policies on development co-operation (poverty reduction linked with environmental protection) and forest conservation (maintaining forest cover).

11. In this context, the extractivism represents an important activity for the region by stimulating a sustainable use of renewable natural resources while conciliating social development with preservation. It should be stressed that Brazil nut extractivism does not involve the destruction of forests nor threaten the ecological balance and the environment. According to the IBGE (Brazilian Institute of Geography and Statistics), about a million of people’s livelihood depends on the Brazil nut production, and because of that, the inhabitants of the area have an interest in preserving the forest.

12. This discussion paper considers different aspects related to the contamination of Brazil nuts with AF and present data on AF occurrence and estimated intake.

CHEMICAL STRUCTURE

13. AFs are a group of structurally related compounds produced by some strains of *Aspergillus flavus*, *A. parasiticus* and *A. nomius* under favourable conditions. The 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. AFB_1 and AFG_1 are normally found in Brazil nuts at a ratio of approximately 1:1 w/w (CAC, 2005a).

14. Chemically AFs are highly oxygenated naturally occurring heterocyclic compounds and have closely related structures. All AFs essentially contain a coumarin nucleus fused to a bifuran. AFBs possess a pentanone structure attached to the coumarin nucleus while AFGs present a six-membered lactone instead (Salunkhe et al., 1987).

TOXICOLOGICAL EVALUATION

15. AFs were evaluated by the JECFA at its thirty-first, forty-sixth, forty-ninth and its fifty-sixth meetings (AF M1 only). At its 49th meeting in 1997, JECFA considered estimates of the carcinogenic potency of AFs and the potential risks associated with their intake. At that meeting, no numerical TDI (Tolerable Daily Intake) was proposed since these compounds are genotoxic carcinogens, but the potency estimates for human liver cancer resulting from exposure to AFB_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 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 on peanuts (10 or 20 μg/kg) on sample populations and their overall risk. It was concluded that reducing the permitted quantity of AFB_1 in peanuts from 20 μg/kg to 10 μg/kg would not result in any observable difference in rates of liver cancer (JECFA, 1998).

16. In the evaluation at its 49th meeting, the JECFA 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 noted 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).

SAMPLING

17. Although the incidence of AF contamination in tree nuts is low, the AF levels are quite variable and high levels can be developed in a small percentage of nuts (Schade et al., 1975; Schatzki, 1995; Schatzki, 1996). As the distribution of AFs in tree nuts is very heterogeneous, an appropriate sampling plan is critical.

18. 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 peanuts, which have been the most evaluated commodity.

19. A sampling plan is uniquely defined by a test procedure and a sample acceptance level. The test procedure consists of specifying sample size, sample preparation method (including particle size and subsampling size) and analytical method.

20. The importance of sampling as a contribution to total variability has been well recognized for many years, and it has been shown that variability increases as AF concentration increases and this holds true for each step of the test procedure. For example, in testing a lot of peanuts using 1.1 kg samples and 50 g sub-samples, the total, sampling, sample preparation and analytical variances were 82.9 %, 73.1 %, 37.5 % and 10.7 % respectively (Johansson et al., 2000)**.**

21. One of the most important observations coming out of sampling is related to identification of the components of a lot associated with the highest risk of contamination. For peanut lots it was found that when examining sound mature kernels plus sound splits, other kernels, loose shelled kernels and damaged kernels, the latter three were associated with highest risk (Whitaker et al., 1998).This information was subsequently employed to evaluate the performance of AF sampling plans based on preferential analysis of sorted fractions (Whitaker et al., 1999). This approach is attractive in that it bridges the gap from sampling as a 'policing' measure to offering the possibilities of remedial action through sorting coupled to the sampling plan (Gilbert and Vargas, 2003). The distribution of AF in pistachios and almonds has been thoroughly investigated in the United States. Results from the investigations of the distribution of AF in pistachios and almonds in the United States indicate that sorting for quality removes a large part of the AF present in pistachios and almonds at harvest (Schatzki, 1995, 1996).

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

23. The 37th CCFAC decided that an electronic working group led by the United States would revise the proposed draft Sampling Plan for AF Contamination in almonds, Brazil nuts, hazelnuts and pistachios taking in consideration recent data presented at the meeting.

ANALYTICAL METHODS

24. The use of validated methods, the implementation of accreditation and the participation in proficiency testing have all been advocated as essential quality assurance measures for mycotoxin analysis. 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 (Gilbert, 1999). The AOAC Official Method 994.08 for AF in Brazil nuts, corn, peanuts, and pistachios nuts dates back to 1994 (AOAC, 2000), with performance characteristics with applicability range of 5-30 μg/kg, recovery of 70 to 85 % corrected by background levels and repeatability with relative standard deviation (RSDr) of 8,6 % at 30 μg/kg and reproducibility with relative standard deviation (RSD_R) ranging from 12 to 29.5 % in the applicability range.

25. General method performance characteristics for AFB_1 and AF total have been established by the European Committee for Standardization (CEN, 1999).

26. There are a number of analytical approaches that work well for determining AFs in Brazil nuts, although there is no formal validated analytical method. In developed countries most laboratories would employ solvent extraction, followed by sample immunoaffinity columns clean-up and HPLC (high performance liquid chromatography) with fluorescence detection. Recently immunoaffinity columns have become widely employed as they offer a simple attractive clean-up technique albeit one that is relatively expensive to undertake (Gilbert and Vargas, 2003).

27. When implementing laboratory procedures in Brazil nut producing countries it should be considered that the analytical approaches used in developed countries are not necessarily the most appropriate. Due to high cost of HPLC and consumables, this technique is not always available and there is a real need for more feasible methods such as those based on TLC (thin layer chromatography) (Gilbert, 1999).

28. Extracts prepared using immunoaffinity columns are cleaner than those obtained by more conventional solid phase clean-up or liquid-liquid partition, and can be concentrated, thereby making TLC a far more attractive option for the chromatographic stage of the analysis (Gilbert and Vargas, 2003; Stroka et al., 2000b).

29. For AFs, the specificity incurred by their fluorescence has historically been thought to be adequate and the confirmation, apart from derivative formation with iodine or bromine (STROKA et al., 2000a), has not been routinely practiced. Electrospray LC/MS and full scan APCI spectra using LC/MS has been employed for confirmation of AFs (Tanaka et al., 2002; Trucksess and Wood, 1994).

30. There is a huge variety of choices in terms of commercial availability of antibody-based test kits for AFs. The AOAC International website (AOAC, 2005) lists some eleven different formats of test kits offered by different companies. The kits for AFB_1 and AFs total utilize antibodies coated variously onto cups, ELISA plates, columns, cards and tubes with different approaches being used for the read-out devices. Although in each case the manufacturers have made extensive claims as to the performance of these kits, few have been validated by a full interlaboratory collaborative study (Gilbert and Vargas, 2003).

31. The 36th CCFAC noted that there was no need for the Committee to work on the development of methods of analysis for the determination of AFs in tree nuts as some methods were already developed by the Codex Committee on Methods of Analysis and Sampling while the development of additional methods could be taken up by this committee upon request of CCFAC.

OCCURRENCE OF AFS IN BRAZIL NUTS

32. Several environmental factors are known to influence AF production, but temperature (T) and relative humidity (RH) are considered to be the most critical. Additional factors such as water activity, moisture, substrate composition, contact with soil, storage time, insect damage and the presence of a shell also influence fungal growth and AF production (Arrus et al., 2005). It was reported that Brazil nuts can be infected by *Aspergillus flavus* and *Aspergillus niger* since the moment the pod falls from the tree while *Fusarium sacchari, Fusarium oxysporum* and *Penicillium citrinum* were detected in Brazil nuts left in forest soil for 30 to 60 days (Cartaxo et al., 2003). *Aspergillus flavus*, *Aspergillus niger* and *Penicillium* sp. were also identified in Brazil nut during processing (Souza et al., 2003). *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus niger*, *Aspergillus ochraceus*, *Eurotium* sp. and *Penicillium* sp. have been isolated in defected Brazil nuts (Brazil – Ministry of Agriculture, 2003 – unpublished data).

33. The effects of relative humidity and temperature on aflatoxin production on previously dried Brazil nuts have been evaluated (Arrus et al., 2005). The study concluded that shelled half-nuts showed the highest level of B_1 and total aflatoxin (6817 μg/kg) while whole in-shell nuts contained the least total aflatoxin content. Table 1 shows the limiting moisture content and a_w values required to control aflatoxin production (< 4 ng/g).

Table 1 - Moisture content and water activity of Brazil nuts at 60 days, 30ºC and 80% relative humidity

Brazil nuts	Days	Storage Temperature (ºC)	Relative Humidity (%)	Nut Moisture Content (%)*	Nut Water Activity (a_w)*	Aflatoxin Production (µg/kg)
Shelled half	60	30	80	4.57	0.70	< 4.0
Shelled whole	60	30	80	4.50	0.68	< 4.0
Whole in-shell	60	30	80	5.05	0.75	< 4.0

* Mean of two values

34. Several Brazil nut producing and importing countries are concerned about and have been studying the occurrence of AFs in Brazil nuts. During 1993, of 176 Brazil nuts analyzed in the United States, 11 % were contaminated with AF total at levels between traces and 20 µg/kg, and 6 % were contaminated at levels higher than 20 µg/kg. The maximum level detected in that study was 619 µg/kg (Pohland, 1993).

35. In Japan, among 74 Brazil nut samples analyzed, 70 (94.6 %) were not contaminated and only 2 samples contained AFs at levels higher than 10 µg/kg. The maximum detected level of AF total was 123 µg/kg (JECFA,1998).

36. Results from a survey of AFs in a variety of retail nuts and nut products was carried out by the UK Food Standard Agency between November 2003 and March 2004. The results from this survey showed that 4 out of 21 samples of Brazil nuts contained AFs above the EC and UK regulatory limits of 4 µg/kg for total aflatoxins (Food Standards Agency, 2004).

37. In Sweden a study conducted between December 2003 and March 2004 showed that the median and 95th percentile level of AFs total in 132 Brazil nut samples before panel sorting were 1.4 and 557 µg/kg and in the edible fractions after sorting 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. This study was designed to investigate the extent to which consumers can separate nuts with a high content of AF from sound nuts. The conclusion was that Brazil nuts in shell may be one of the few species of nuts that consumer may sort out by the means of visual examination and thus protect themselves from consuming high levels of AFs (Marklinder et al., 2005).

38. In a survey designed to estimate dietary exposure to mycotoxins in France, 25 samples of nuts and oil seeds collected in 2000-2001 showed no detectable levels of AFs (Leblanc, 2005).

39. At the 37th CCFAC meeting Brazil presented the results of an extensive study conducted in Brazil from 1998 to 2004 that analyzed 500 (302 shelled and 198 in-shell) Brazil nut samples collected at different sites in Brazil for AFB_1, AFB_2, AFG_1 and AFG_2.The study showed that the incidence of contamination of AFB_1 below the detection limit of the method (0.6 µg/kg) was 71.8 % and 41.4 % for shelled and in shell Brazil nuts (as sampled), respectively.AFB_1 levels were below 2 µg/kg and 10 µg/kg in 69.4 % and 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 below 4 µg/kg and 20 µg/kg. The distribution data of AFB_1 and AFs total in Brazil nuts (shelled + in-shell) are shown in Figure 1 and Figure 2 . As expected, the pattern of AF contamination does not follow a normal distribution. The median concentrations of AFs total were 1.85 and 0.8 (µg/kg) in inshell and shelled Brazil nuts samples as sampled, respectively. The study also concluded that the contamination in-shell Brazil nut was significantly higher when compared to shelled Brazil nut. The study indicates that the shell itself might have a contribution to the contamination of Brazil nuts with AFs, and the contamination can not be assumed to be only in the kernel. Therefore, additional studies need to be developed in order to elucidate and confirm these findings (CAC, 2005b).

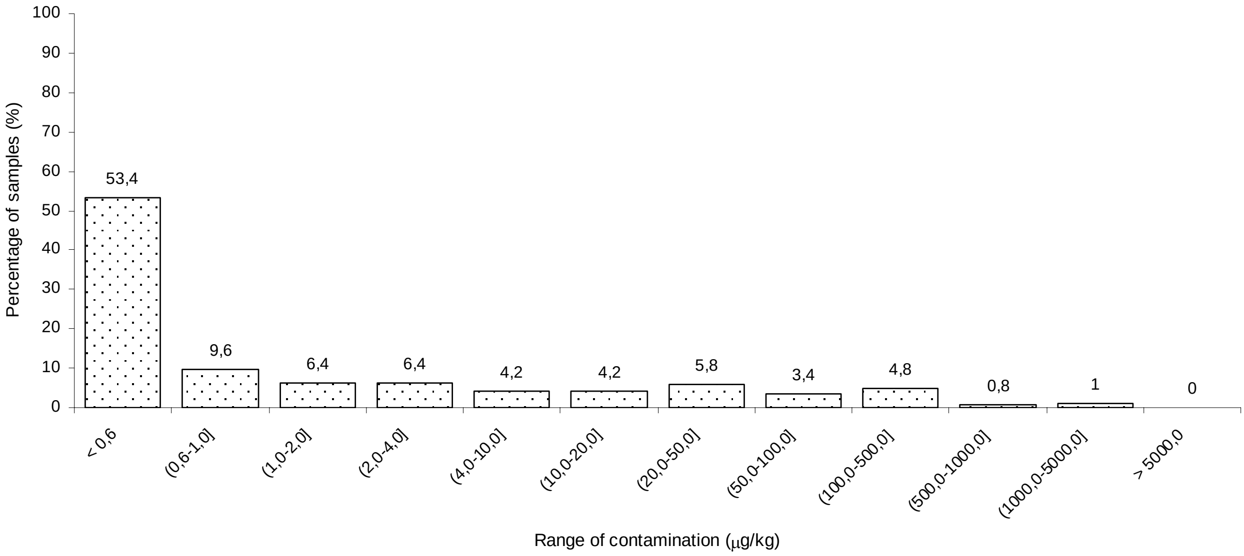

Figure 1 - Brazil nut sample distribution (%) per range of aflatoxin B_1 contamination

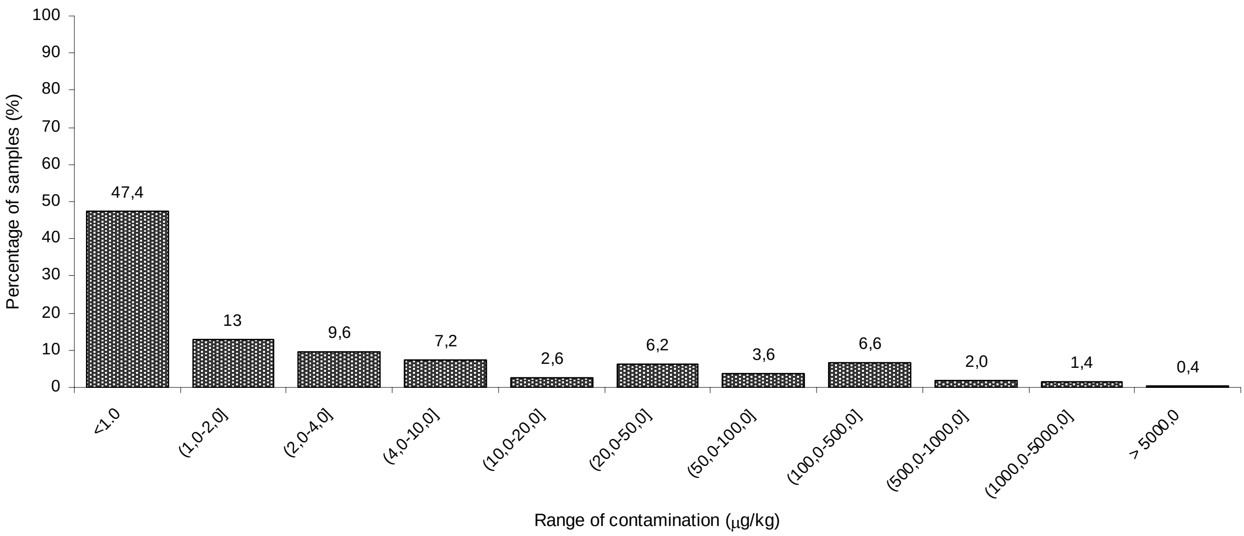

Figure 2 - Brazil nut sample distribution (%) per range of total aflatoxin contamination

40. AF contamination is a potential problem in tree nuts and other commodities. The incidence of contaminated nuts and the concentration of AFs in contaminated nuts depend upon environmental conditions, cultivar, agricultural/extractivistic and production practices as well as the technical capability to sort damaged and contaminated nuts during post-harvest processing.

41. It is well known that mould growth and toxin production requires a critical water activity in a particular matrix. Minimizing the period of time the commodity is present under these critical conditions therefore reduces the likelihood of contamination since it decreases the sources of initial fungal contamination. This means reducing the moisture content of the commodity to below that required for fungal activity in the shortest possible time.

42. Because of the carcinogenic and genotoxic effects of AFs in humans, such contamination is of major concern regarding public health. Ninety nine (99) countries have set up regulation for the presence of AF in food (FAO, 2004).

DIETARY INTAKE

43. The commodities with the highest risk of AF contamination include corn, peanuts, cottonseed, Brazil nuts, pistachio nuts, figs, spices and copra. The most important dietary sources of AFs are maize and groundnuts and their products, which may form an essential part of the food diet in some countries (CAC, 2005a). In many countries, tree nut consumption accounts for only a small percentage of the total food intake of consumers.

44. There are few data available on the intake of Brazil nuts and consequently on the intake of AFs from Brazil nuts .Dietary intake of AFs by the Swedish population was estimated as 0.6 and 0.7 ng/kg bw for average and high consumers (95th percentile), respectively (Thuvander, 2001). In that study the intake of Brazil nuts was estimated as approximately 0.3 g/ day for both the average and the 95th percentile consumer. In another study in Sweden in which a 70 kg consumer´s yearly consumption was assumed to be one normal sized bag of 300 g Brazil nuts the median AF intake from Brazil nuts in Sweden was estimated as 0.73 ng/kg bw and the 95th percentile 110 ng/kg bw (Marklinder et al., 2005).

45. In order to develop intake data for other regions an approach that can be undertaken is to estimate the contribution of Brazil nuts as a source of AFs in the diet using data on worldwide production of commodities in which AF contamination has been reported, and data on worldwide production of Brazil nuts with regard to the total production of tree nuts, and then combine these data with values for consumption of tree nuts in the five GEMS/Food regional diets, which were developed from balance sheets compiled by FAO. Since these sheets are available for most countries, they provide a set of data that are comparable across countries and regions of the world (WHO, 2000).

46. Table 2 presents data on the world production (2004) of commodities that can be considered potential sources of exposure to AFs and Table 3 shows the percentage of total tree nuts production (2004) represented by Brazil nuts.

Table 2 - Comparison of the worldwide production (2004) of tree nuts with regard to the production of other foods that can be potential aflatoxin source

Product	Production (Mt)*	%
Cereals	2,264,030,480	98.09
Peanuts in shell	35,723,285	1.55
Tree Nuts (total)	8,416,402	0.36
Total	2,308,170,167	100

Source: FAOSTAT, 2005
* metric ton

Table 3 - Comparison of the worldwide production (2004) of Brazil nuts with regard to the total production of tree nuts

Product	Production (Mt)*	%
Pistachio	549,837	6.53
Hazelnuts	680,580	8.08
Brazil Nuts	72,450	0.86
Other tree nuts	7,113,535	84.52
Total	8,416,402	100

Source: FAOSTAT, 2005
* metric ton

47. The data in Table 2 show that tree nuts production represent only 0.36 % of the total production of commodities that are potential sources of exposure to AFs while Table 3 indicates that 0.86 % of the total production of tree nuts corresponds to Brazil nuts.This clearly shows that the world production of Brazil nuts is much less than that of other major food commodities in which AFs have been identified.

48. According to data from the GEMS/FOODS regional diets (WHO, 2003) summarized in Table 4, the daily intake of tree nuts in the five regions varies from 1.1 g/person/day in Middle East to 17.8 g/person/day in Latin America, corresponding to 0.082 and 1.31 % of the total diet intake in the considered regions, respectively.

Table 4 - Tree nuts consumption (g/person/day) in comparison with total diet intake in different regions of the world

Commodities	Middle East	Far East	African	Latin America	European
Tree nuts	1.1	13.5	4.5	17.8	4.6
Total diet	1,342.5	1,083.5	1,018.1	1,355.5	1,896.4
Tree nuts/ total diet (%)	0.082	1.25	0.44	1.31	0.24

Source: GEMS/FOODS

49. Considering that Brazil nut production corresponds to only 0.86 % of the total tree nuts production, one can estimate that the intake of Brazil nuts in the five geographical regions would vary from 0.0095 g/person/day in Middle east to 0.153 g/person/day in Latin America.

50. In order to assess the intake of AFs from Brazil nuts, the median (0.8 μg/kg) concentration of AFs total in the 198 shelled Brazil nut samples were combined with the intake of Brazil nuts inferred from GEMS/FOOD database. As the concentration of AFs and the incidence of AFs contamination reflect those found in Brazil data, for the purpose of this analysis they were taken as representative of the Brazil nuts available in trade throughout the world.

51. When using median concentration values, estimates of intake of AFs total from Brazil nuts would range from 0.0072 ng/person/day in the Middle Eastern diet to 0.1224 ng/person/day in Latin American diet (Table 5).

Table 5 - Daily intake of Aflatoxins from Brazil nuts considering median values

Diet	Brazil nut intake (g/day)	Median* (μg/ Kg)	Aflatoxin intake	
			ng/person per day	ng/kg bw per day
European	0.039	0.8	0.0312	0.00052
Latin American	0.153	0.8	0.1224	0.00204
Far Eastern	0.116	0.8	0.0928	0.00155
Middle Eastern	0.009	0.8	0.0072	0.00012
African	0.039	0.8	0.0312	0.00052

*Source: Brazilian study (CODEX ALIMENTARIUS COMISSION, 2005b)

52. Using the current regulatory limits for AFs in Brazil nuts in different regions combined with the GEMS/FOOD regional diet, it is possible to explore different scenarios and estimate the corresponding intake (Table 6).

Table 6 – Comparison of aflatoxins intake from Brazil nuts in the five geographical regions considering different MLs

GEMS FOOD REGION Diet	Brazil nut intake (g/day)	Aflatoxin intake considering different MLs (ng/person per day)			
		4 μg/ kg	10 μg/ kg	20 μg/ kg	30 μg/ kg
European	0.039	0.156	0.390	0.78	1.17
Latin American	0.153	0.612	1.530	3.06	4.59
Far Eastern	0.116	0.464	1.160	2.32	3.48
Middle Eastern	0.009	0.036	0.090	0.18	0.27
African	0.039	0.156	0.390	0.78	1.17

53. Examining a scenario in which the Brazil nut intake data estimated for Latin American countries (the highest value) is combined with a concentration of AFs total of 20 μg/kg (80 % of the analysed Brazil nut samples presented level of contamination below this value, according to the distribution data of AFs concentrations) the intake of AFs would range from 0.18 ng/day/person in the Middle eastern diet to 3.06 ng/day/person in the Latin American diet (Table 6).

CONCLUSIONS & RECOMMENDATIONS:

54. The present Discussion Paper on AFs in Brazil Nuts leads to the following broad conclusions and recommendations for consideration at the 38th Session of the CCFAC:

I) Brazil nut production represents an important alternative economic activity for the Amazon rainforest area, contributing to its preservation.

II) The establishment of standards for AF contamination in Brazil nuts at levels below those needed for human health protection may cause a crash in the Brazil nut trade.

III) Although the contribution of Brazil nuts to the daily intake of AFs is low, there is a need for an international regulatory level for AFs in Brazil nuts because very restrictive levels of AFs have been imposed on Brazil nuts in some regions.

IV) A code of practice for the prevention and reduction of AF contamination in tree nuts was adopted by the CAC at its 28th session and an appendix to the code addressing additional measures for Brazil nuts is under development. The results of the implementation of the recommended measures will be available in the next few years and will certainly result in the decrease of the current levels of AF contamination in Brazil nuts.

V) Before a limit for AFs in Brazil nut is established, it is recommended that the CCFAC considers that:

a) Additional data on the occurrence of AF in Brazil nuts is necessary to supplement those presented in this document and to better clarify the influence of the shell in the analytical result. It is further recommended that new distribution curves be developed after the Good Extractivistic Practices currently in development are implemented.

b) Other foods in which AF-producing fungi commonly grow are widely used commodities and contribute more to the intake of AFs than Brazil nuts, nevertheless, they have maximum levels of AFs less restrictive than those currently applied to Brazil nuts.

c) There is a significant difference between the level of AF contamination in shelled and in shell nuts. According to the CODEX STAN 193 MLs should preferably be on the basis of the edible part of the product.

d) It is recommended that an international specific sampling plan for Brazil nuts be developed.

VI) In case that different MLs for AFs in Brazil nuts are proposed, it is recommended that the CCFAC asks JECFA to assess the impact of the hypothetical standards on the reduction in cancer and the magnitude of the reduction.

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