codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

JOINT OFFICE: Viale delle Terme di Caracalla 00153 ROME Tel: 39 06 57051 www.codexalimentarius.net Email: codex@fao.org Facsimile: 39 06 5705 4593

**Agenda item 7** **CX/CF 09/3/7**
**March 2009**

**JOINT FAO/WHO FOOD STANDARDS PROGRAMME**

**CODEX COMMITTEE ON CONTAMINANTS IN FOODS**

**Third Session**

**Rotterdam, the Netherlands, 23 - 27 March 2009**

# PROPOSED DRAFT MAXIMUM LEVELS FOR TOTAL AFLATOXINS IN BRAZIL NUTS (N11-2008)

**Prepared by the Electronic Working Group led by Brazil**

## BACKGROUND

1. At its 1st Session, the Codex Committee on Contaminants in Food (CCCF) held in April 2007, agreed that the Discussion Paper on Aflatoxin Contamination in Brazil Nuts would be updated by the Delegation of Brazil, incorporating additional data that would become available on the contribution of the shell to aflatoxin contamination of Brazil nuts, for consideration at Second Session of the Committee (paragraph 66 - ALINORM 07/30/41).

2. At the 2nd CCCF, the Discussion Paper and a Project document were discussed. The 31st Session of the CAC endorsed the recommendation of the 61st Session of the Executive Committee and approved the new work proposal to discuss Maximum Levels for Total Aflatoxin in Brazil Nuts

3. At its Second Meeting, the Codex Committee on Contaminants in Food (CCCF) agreed to advance the Proposed Draft Sampling Plans for Aflatoxin Contamination in Ready-to-eat Treenuts and Treenuts Destined for Further Processing: Almonds, Hazelnuts, and Pistachios. The proposal was adopted at the 31st Session of the Codex Alimentarius Commission at Step 5/8, with the recommendation to omit Steps 6 and 7.

4. The CCCF agreed to amend the title by deleting reference to Brazil nuts, with understanding that, when Maximum Levels for Brazil nuts were established, the sampling plans would be revised to include an Annex covering these products, taking into account data on uncertainty and distribution sampling studies.

5. The present document has taken into account the 68th JECFA evaluation on the impact of different hypothetical limits of total aflatoxins (AFT) in tree nuts, including Brazil nuts, on the dietary intake.

6. This document was prepared by Brazil with contributions from the FAO, the Netherlands, European Community, Sweden, United Kingdom, United States, International Tree Nut and Dry Fruit Council Foundation (INC) and Confederation of the Food and Drink Industries of the EU (CIAA).

## INTRODUCTION

7. AF contamination can be a potential problem in tree nuts and other products such as maize, groundnuts, oilseeds, cocoa products, dried fruits and spices. This Paper is applicable only to Brazil nuts which is the only crop among the main traded tree nuts that involves the extractivism activity. Extractivism can be defined as a process of collecting and primarily handling of forest products that are intended for national or international trade (CAC/RCP 59-2005).

8. The Brazil nuts are the seeds of Bertholletia excelsa Humb. & Bompl., trees that grow wild in the Amazon rainforest. They can reach up to 60 meters in height, take 12 years to bear fruits and live up to 500 years. The trees occur in groves of 50-100 individuals and the distance between the groves can be up to 1 km; pollination occurs by wild large-bodied bees, especially *Euglossinae* bees (Wadt et al., 2005).

9. The Amazon rainforest consists of multiple ecosystems with a huge biodiversity. It has an important role in the global weather balance and it provides the shelter and sustenance for 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 extractivism represents an important activity for the native people in countries where the trees grow, stimulating a sustainable use of renewable natural resources while bringing together social development and conservation. It is an activity that neither destroys the forest nor threats the ecological balance and the environment. On the contrary, the Bertholletia Excelsa trees are essential in the rain forest as they help to maintain the equilibrium in the relationship between flora and fauna.

11. The number of collectors and processors living from the Brazil nut industry is about 1.2 million in Brazil, 600,000 in Bolivia and 200,000 in Peru. In 2006, the total world production reached 20100 metric tons, being about 64 % from Bolivia, 24 % from Brazil and 12 % from Peru (INC, 2007).

12. A Code of Practice for the Prevention and Reduction of AFT Contamination in Tree Nuts was adopted by the CAC at its 28$^{th}$ Session (CAC, 2001); an Appendix specifically for Brazil nuts was adopted by the CAC at its 29$^{th}$ Session. INC information indicated that industries and producers have been making considerable efforts in the last 15 years to minimize fungal growth and aflatoxin production in tree nuts. Particularly, in the case of Brazil nuts, the climatic conditions in the Amazonian environment and the characteristics of the extractivism activity (collecting and primarily handling) cannot be controlled, exerting direct or indirect effects on the toxigenic fungi and aflatoxin production

12. Processing/treatment that has been proven to reduce aflatoxin levels in Brazil nuts include shelling or sorting by size, specific gravity, colour or damage. Ready-to-eat Brazil nuts (RTE) are nuts which are not intended to undergo an additional processing/treatment before reaching the final consumer; those destined for further processing (DFP) are intended to undergo such a process. Brazil nuts can be traded shelled and in shell, for both ready-to-eat (RTE) and destined for further processing (DFP). Each of these commodities is defined as following:

Shelled Brazil nut ready-to-eat: are shelled nuts (kernels only) which can be directly marketed to the final consumer

Shelled Brazil nut destined for further processing: are shelled nuts (kernels only) that are intended to undergo a sorting process to reduce aflatoxin levels before being marketed to the final consumer.

In-shell Brazil nut ready-to-eat: are in-shell nuts which can be directly marketed to the final consumer

In-shell Brazil nut destined for further processing: are in-shell nuts that are intended to undergo sorting and/or shelling process to reduce aflatoxin levels before being marketed to the final consumer.

13. This Paper considers many aspects related to the AFT in Brazil nuts, including occurrence, dietary intake estimation and proposes maximum levels for consideration by the Committee (see page 11). This paper also presents a proposal of a Sampling Plan in Annex I.

**TOXICOLOGICAL EVALUATION**

14. At its 49th Meeting, the JECFA (FAO/WHO, 1998) considered the carcinogenic potency of aflatoxins (AFs) and the potential risks associated with their intake. 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. No 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.

15. 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 (FAO/WHO, 1998).

## ANALYTICAL METHODS

16. There are a number of analytical methods available for the determination of aflatoxins in nuts. In general, the methods include the steps of sample preparation, extraction, clean up and quantification. After an effective homogenisation, a solvent extraction step is applied using a mix of acetonitrile or methanol and water. Sample clean-up uses either liquid-liquid partition or solid phase extraction (SPE), with sorbents such as silica, florisil, C18, aluminium oxide and immunosorbents as immunoaffinity column (Gilbert and Vargas, 2003). Methods for identification and quantification normally used are thin layer chromatography (TLC or HPTLC) or high performance liquid chromatography (HPLC), with fluorescence detection. Two methods for aflatoxin analysis in Brazil nuts were validated through collaborative studies using SPE (AOAC 994.08) and immunoaffinity column clean-up (Stroka et al., 2000), followed by HPLC-FL (Gilbert and Vargas, 2003). Liquid chromatography-tandem mass spectrometric with electrospray ionization or atmospheric pressure chemical ionization (LC-MS/MS) methods for determination and confirmation of AF contamination in different foodstuffs have been developed (Bacaloni et al., 2008; Spanjer et al., 2008). A method with direct injection into a LC-MS/MS after extraction with acetonitrile:water was developed by Spanjer et al. (2008 ) for 33 mycotoxins, including aflatoxins BG. Limit of detection (LOD) or quantification (LOQ) for each aflatoxin depends on the matrix, the clean-up procedure and the detection method and normally are within the 0.1 to 1 µg/kg range (Marklinder et al., 2005, Sobolev, 2007).

17. Antibody-based test kits for AF analysis are mostly used for screening purposes. The AOAC International website (AOAC, 2009) lists different kit formats for $AFB_1$ and AFT, with 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).

18. The CCFAC has established a performance criteria for methods of analysis for AFT in food (CX/CF 07/1/6). General method performance characteristics for aflatoxins have also been established by the European Committee for Standardization (CEN 1999) and European Union (EC, 2006) and adopted in the last documents of Codex for Peanuts and Tree nuts (almonds, hazel nuts, pistachios).

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

## FACTORS AFFECTING THE PRESENCE OF AFS IN BRAZIL NUTS

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

21. Olsen et al. (2008) have shown that the relation between aflatoxin $B_1$ and $G_1$ in most of the samples from 199 lots of in-shell Brazil nuts imported in Europe was about 50/50, indicating that the fungi with the major responsibility for aflatoxin production cannot be *Aspergillus flavus*, which produces solely B aflatoxins. Data collected over the last 5 years by industry members of the INC (Pino Calcagni, personal communication) show that the weighted average of the proportion of B1 to AFT is 66.3% varying from a low of 25% up to a maximum of 96.4%.

22. Toxigenic fungi growth and AF formation may occur at pre and post harvest (collection) stages. According to Johnson et al. (2008), preliminary infection of the Brazil nuts with aflatoxin producing fungi may take place at a very early stage as *A. flavus* was isolated from the exterior of aseptically pods still attached to the tree. The probability and rate of fungal growth and toxin formation was significantly related to the water activity ($a_w$), which is a measure of unbound water in the crop/food. The higher the $a_w$ the faster the growth. Baymam *et al.* (2002) have shown that most of the *Aspergillus* species infection in Brazil nuts is internal.

23. Fungus development and toxin production in Brazil nuts occurs mostly on the soil after falling down from the tree, during harvesting, transport and storage, and are favoured by the climatic conditions of the Amazon rainforest, mainly during the rainy season (Cartaxo et al., 2003; Wadt et al., 2005; Pacheco & Scusel, 2007).

24. *Aspergillus flavus* and *A. niger* were isolated in Brazil nuts collected up to 60 days after the pods had fallen in the forest (Cartaxo et al., 2003) and during processing (Souza et al., 2003). In addition to these species, *Aspergillus parasiticus* was also isolated from defective Brazil nuts (Brazil, 2003; unpublished data). *Aspergillus nomius* has recently been isolated from Brazil nuts lots and may be one of the most important producers of aflatoxins in these nuts (Olsen et al, 2008)

25. Arrus et al (2005b) conducted a study to better understand the origin of aflatoxins in Brazil nuts. Five Brazil nut pods were aseptically picked from trees located in each of three concessions of the Peruvian Amazon rainforest. The exteriors of nut pods did not contain *A. parasiticus,* and only pods from one concession yielded *A. flavus* isolates. All isolates tested were aflatoxigenic (630 to 915 µg/kg total aflatoxin). Whole, in-shell nuts obtained after opening the pods yielded no *A. flavus* or *A. parasiticus.* Aflatoxins were not detected (LOD of 1.75 µg/kg) in any of the nuts. In-shell and shelled nuts from various process operations were all positive for *A. flavus* but negative for *E. coli* and salmonellae.

26. The effects of relative humidity (RH) 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., 2005a). Maximum production of AFs was found in nuts stored at 25-30 ºC and 97% RH. Half kernels showed the highest level of $AFB_1$ (4483 µg/kg) and AFT (6817 µg/kg) while in-shell nuts contained the lowest $AFB_1$ and AFT levels (49 and 93 µg/kg, respectively). AFs were not produced at either 10 ºC (97% RH.) or at 30 ºC (75% RH.). This suggests that fungal growth after harvest can be prevented by the drying of the nut to safe moisture level or water activity as rapidly as possible. Additionally, an adequate control of temperature and RH during storage is an important strategy to prevent AF production in Brazil nuts.

27. According to Arrus et al. (2005a), 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. The availability of water needed to allow for fungal growth is best expressed as $a_w$.

## OCCURRENCE OF AFS IN BRAZIL NUTS

28. 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 up to 20 µg/kg, and 6 % had levels above 20 µg/kg. The maximum level detected was 619 µg/kg (Pohland, 1993). In Japan, only 4 of the 74 Brazil nuts samples analysed were contaminated, with 2 samples containing AFs above 10 µg/kg (up to 123 µg/kg) (FAO/WHO, 1998).

29. 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 $G_1$ (Ioannou-Kakouri et al., 1999).

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

31. 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). About 70 % and 80 % of all samples had levels <4 µg/kg and <20 µg/kg, respectively. The median concentrations of AFT were 1.85 and 0.8 µg/kg in in-shell and shelled Brazil nuts, respectively (CAC, 2005a).

32. The Brazilian Ministry of Agriculture presented data referring to the occurrence of aflatoxins in Brazil nuts samples collected from lots to be exported and lots rejected by importing countries during the years of 2005 and 2006. In all cases only the edible portion (kernels) was analysed. About 85 % of the 294 samples (lots) analysed had no detectable levels of AFB1 (< 0.6 or 1µg/kg). AFT levels in positive samples (lower bound) ranged from 0.4 to 242 µg/kg and 13 samples (4.4 %) had levels > 20 µg/kg (Brazil, 2006; unpublished).

33. In a study conducted in Brazil, 79 out of 109 in-shell Brazil nuts samples analysed for AFs (72.4 %) 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).

34. The EFSA Scientific Panel evaluated the data on occurrence of AF in tree nuts and other products from 2000 to 2006 submitted by 22 EU Member States. The samples were related to import, market or company control and it was not specified which samples were ready-to-eat or for further processing. From 622 Brazil nuts samples analysed, 56.4% had AFT levels below the LOD (0.1 – 0.2 µg/kg), 22% between LOD and 4 µg/kg, 2.4 % between 4 and 10 µg/kg and 19.1 % above 10 µg/kg (EFSA, 2007).

35. 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 study concluded that Brazil nuts may be one of the few nuts that consumers may visually separate the edible from the inedible contaminated nut during the shelling process before eating and thus protect themselves from exposure to high levels of AFs.

36. At its dietary intake evaluation, the 68$^{th}$ JECFA used data on AF contamination from producing countries. The mean concentration of AFT in Brazil nuts (shelled) was 20 µg/kg (FAO/WHO, 2008).

37. In general, the shell represents about 50 to 60 % of the Brazil nut total weight. Marklinder et al (2005) have shown that in most cases, AFT levels were lower in shells than in kernels from the same sample. Pacheco and Scussel (2007) found a mean (n= 80) ratio of AFT in in-shell and shelled Brazil nuts of 1.1 when analysing samples collected directly from the silos, without any type of sorting.

38. De Mello & Scussel (2007) studied the external characteristics of in-shell Brazil nuts (dimensions, weight, chromaticity, and shell thickness), moisture content and aflatoxin contamination (analyzed by LC-MS/MS). According to their length, Brazil nuts were classified in three groups large, medium, and small sizes. Samples from the small size nut group presented a mean AFB1 level of 5.62 $\mu$g/kg. No aflatoxin was detected in samples from the other two groups. The authors concluded that Brazil nut external characteristics can help to distinguish healthy/safe and deteriorated nuts and could be useful for Brazil nut sorting and machine development. Currently, this machine is not available. Although UV light is in use for visual sorting and laser sorting machines are currently available, they are extremely costly (Pino Calcagni, personal communication). The efficacy of these sorting machines to reduce aflatoxins in in-shell Brazil nuts still needs to be determined.

39. In-shell and shelled Brazil nuts from the 2006 and 2007 seasons destined for export were analyzed by LC-MS/MS by Pacheco & Scussel (2009). Samples were collected from big bags (in-shell nuts) and sorting tables after size classification (shelled nuts) immediately after processing in a factory of Manaus, State of Amazonas. From the 171 samples analyzed, 8.7% (11 in-shell and 3 shelled) of the nuts had AFT levels >4 µg/kg; most of the in-shell nuts came from the 2006 season (9 samples).

40. The Brazilian Government – Ministry of Agriculture - submitted the results of the CONFORCAST Project (2005), presented in a workshop in Belém, Pará, Brazil, from 10th to 12th November, 2008. The main objectives of this project were to design sampling plans for shelled and in-shell Brazil nuts and to evaluate which Brazil nut categories (kernels and shells) could be most associated with the aflatoxin contamination in Brazil nut. Information was also obtained on the incidence of aflatoxins in Brazil nut lots ready to be marketed (shelled and in-shell). The samples were collected during the years of 2006 and 2008 in processing plants of the Pará and Acre states. In the sampling studies, 25 lots of shelled and 13 lots of in-shell Brazil (4 to 8 tons) were sampled according to unbalanced sampling designs.

41. All samples from the CONFORCAST Project (shells and kernels) were analyzed for aflatoxins $B_1$, $B_2$, $G_1$ and $G_2$ by HPLC-FL with post column derivatization using electrochemical cell, with performance criteria according to CEE 401/2006. For each sub-sample, the AFT levels for in-shell Brazil nuts were calculated from the levels found in the shells and kernels through mass balance.

42. Twenty-five (25) lots of shelled Brazil nuts were sampled and twenty-five (25) samples of 200 kg were taken from each lot. From each sample, 20 sub-samples of 10 kg were taken for analysis. Table 1 shows the summary of the results obtained in the 500 sub-samples analyzed for AFT. In most sub-samples (67.8 %), the levels of aflatoxins found in the samples were below 0.39 µg/kg AFT; 4 % of them had levels higher than 10 µg/kg .

**Table 1:** Distribution of sub-samples of the aggregate samples from lots of shelled Brazil nuts classified by discrete range of contamination, in % of the 500 sub-samples analysed

| | **Range of total aflatoxin concentration*, µg/kg** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **≤0.39** | **> 0.39≤1.0** | **> 1≤2.0** | **> 2≤4** | **>4≤10** | **>10≤15** | **>15≤20** | **>20≤30** | **>30≤50** | **>50** |
| % of the sub-samples analysed | 67.8 | 14.0 | 5.8 | 3.4 | 5.0 | 0.8 | 1.0 | 0.4 | 1.0 | 0.8 |

*LOD = 0.11 µg/kg (MAPA, 2008)

43. Thirteen (13) lots of in-shell Brazil nuts were sampled and four hundred (400) kg were taken from each lot. One lot was excluded from the study due to missing samples. From each of the 12 lots, 10 sub-samples of 40 kg were taken, the nuts were shelled and, after visual inspection by trained personnel, segregated in good kernels and respective shells (with residue and without residue) and rotten kernels and respective shells. For 5 lots, this process resulted in 20 sub-samples of good kernels, 10 sub-samples of respectively shells, 10 sub-samples of rotten kernels and 10 sub-samples of respective shells (three sub-samples were excluded later due to technical problems). For each of the remained 7 lots, this process resulted in 20 sub-samples of good kernels and 1 sub-sample of respective shells (with residue and without residue), 10 sub-samples of rotten kernels of variable sample mass and 10 sub-samples of respective shells. A total of 54 sub-samples were derived in this process. A diagram with the sampling protocol is shown on Figure 2.

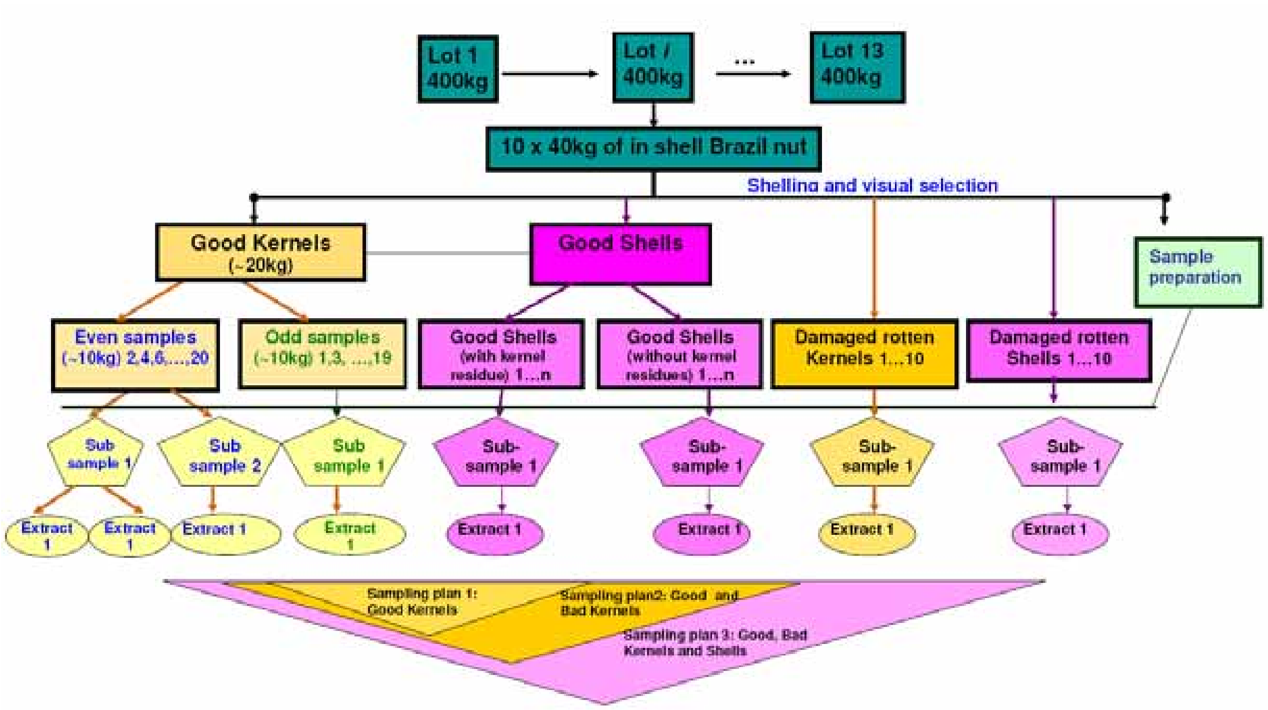


**Figure 2.** Procedures for in-shell Brazil nut preparation

44. Table 2 shows the distribution of AFT levels found in the 54 sub-samples of in-shell Brazil nuts analyzed. When all nuts were considered (good and rotten, including kernels and respective shells), 35.2 % of the sub-samples had AFT above 20 µg/kg, with 3.6% of them having levels above 50 µg/kg. If only the good nuts are considered (kernels and respective shells), over 90 % of the sub-samples had aflatoxin levels < 5 µg/kg and about 2 % of them had levels above 30 µg/kg.

**Table 2:** Distribution of sub-samples of the aggregate samples from 12 lots of in-shell Brazil nuts considering all nuts (good and rotten) and only good nuts, classified by discrete range of contamination, in % of 54 sub-samples analyzed.

| | **Range of total aflatoxin concentration*, µg/kg** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **≤5** | **>5 ≤10** | **> 10 ≤15** | **> 15 ≤20** | **> 20 ≤30** | **> 30 ≤50** | **>50** |
| All nuts | 1.9 | 11.1 | 27.8 | 24.1 | 25.9 | 5.6 | 3.7 |
| Only good nuts | 90.4 | 3.8 | 3.8 | 0.0 | 0.0 | 1.9 | 0.0 |

*LOD = 0.11 µg/kg (MAPA, 2008)

45. Table 3 shows the mass of nut (kg) and the content of AFT obtained in the 54 sub-samples analyzed, according to the design described in paragraph 43. Although the total mass (%) of kernels from good nuts weighted almost twice the shells, the levels of AFT in shell from good nuts were 3 times higher than what was found in kernels of good nuts. Rotten nuts represented about 6 % of all nut mass analysed, but contributed to almost 85 % of AFT mass (µg). AFT concentration in rotten nuts (268.7µg/kg) was 93 times higher than what is found in good nuts (2.89 µg/kg). While in good nuts the aflatoxins were more concentrated in the shells, shells from rotten nuts contributed more to the AFT found in rotten nuts. All defects, that included rotten kernels and all shells (38.5 %), contributed with about 94 % of AFT mass. Similar results were reported by Whitaker et al. (1998) that found that small mass of defect (18%) contributed to 93% of aflatoxin mass in peanuts.

**Table 3.** Sample mass (kg), aflatoxin mass (µg), and aflatoxin concentration (µg/kg) in all samples from 12 lots of Brazil nuts by category.

| Category | Mass (kg) | Mass (% of total) | AFT mass* (µg) | AFT massa[1] (% of total) | AFT concentration[1] (µg/kg) |
|---|---|---|---|---|---|
| Good nuts (kernel & shell) | 1,373.16 | 94.44 | 3,965.01 | 15.44 | 2.89 |
| Good kernels | 893.56 | 61.46 | 1,507.09 | 5.87 | 1.69 |
| Shells from good nuts[2] | 479.6 | 32.99 | 2457.92 | 9.57 | 5.04 |
| Rotten nuts (kernel & shell) | 80.84 | 5.56 | 21,723.29 | 84.56 | 268.72 |
| Rotten kernels | 38.83 | 2.67 | 13,653.32 | 53.15 | 351.62 |
| Shells from rotten kernels | 42.01 | 2.89 | 8,069.96 | 31.41 | 192.10 |
| All defects (rotten kernels & all shells) | 579.96 | 38.54 | 24,181.21 | 94.13 | 43.15 |
| All categories | 1,454.00 | 100.00 | 25,688.30 | 100.00 | 17.67 |

[1] samples containing AFT levels below the LOD (0.11 µg/kg) were considered to be at 0 µg/kg; [2] sum of what was found in shells with and without kernel residues (Figure 2); for the AFT concentration, the mean was taken.

46. The data presented on Table 3 clearly shows that aflatoxin is distributed in all parts of the nut, not only in the kernel (edible part of Brazil nut) or the rotten nuts. However, the rotten nut category was the one with the higher contribution to the overall AFT contamination.

47. The possibility of using aflatoxin mass and aflatoxin concentration in defects (shells and rotten kernels) to predict the aflatoxin concentration in in-shell Brazil nut sampled from a lot was also investigated in the CONFORCAST project. The best correlations was found between aflatoxin concentration in in-shell Brazil nut and the aflatoxin mass in rotten nuts ($R^2$=0.93; regression equation: C=0.0337M + 3.5687). The correlation with the AFT concentration and mass found in all defects were also high ($R^2$=0.74 and 0.73, respectively) (Table 4).

**Table 4.** Correlation from a linear regression between the AFT concentration in in-shell Brazil nut sample in a lot (µg/kg) and various parameters

| Parameter | $R^2$ | Parameter | $R^2$ |
|---|---|---|---|
| Aflatoxin mass, rotten nuts (µg) | 0.93 | Aflatoxin concentration, rotten kernels (µg/kg) | 0.50 |
| Aflatoxin concentration, all defects (µg/kg) | 0.74 | Mass of rotten nut (% of total mass) | 0.34 |
| Aflatoxin mass, all defects (µg) | 0.73 | Mass of rotten kernels (% of total mass) | 0.33 |
| Aflatoxin mass, rotten kernels (µg) | 0.64 | Mass of all defects (% of total mass) | 0.09 |
| Aflatoxin concentration, rotten nuts (µg/kg) | 0.63 | Mass of rotten nut (% of total mass)* | 0.084 |

*correlation with good nut aflatoxin concentration (µg/kg)

48. In many countries, when an in-shell Brazil nut lot enters the market, the nuts are analysed as such, without any type of processing. The results from the CONFORCAST project show that the levels found in the in-shell nuts cannot be used to assess the human exposure to aflatoxins from the consumption of Brazil nut, as the shells and the rotten nuts, which are not eaten, can be highly contaminated.

49. One approach to estimate the level of a substance, such as a contaminant, present in the edible food portion (relevant for exposure assessment) from results obtained from unprocessed food (that contains the inedible portion) is to estimate the processing factor (PF). When a set of data is available, PF can be estimated by dividing the concentration of the contaminant found in the processed food by the level found in the unprocessed food (also termed raw commodity) (CX/PR/07/39/8;ftp.fao.org/codex/ccpr39/pr39_08e.pdf). In the case of the Brazil nut, the processing procedures of interest are the shelling and the sorting. For a single contaminant/food/processing procedure, PFs can vary widely, and the recommendation is to use the best estimated value from the available data (CX/PR/07/39/8). This variability is especially true in the case of aflatoxins in Brazil nut, as the environmental conditions from harvest to market can influence the levels of fungal contamination and of aflatoxin in the kernel and the shell.

50. Although the CONFORCAST project was not designed for this purpose, mean PFs for Brazil nuts can be estimated using the data shown on Table 3. Two scenarios were considered. The first one is related to a lot of in-shell Brazil nuts that will be marketed as ready-to-eat (good nuts); in this case, the estimated processing factor for shelling ($PF_{RTE}$) was 0.6 (1.69/2.89). The second scenario is related to a lot of in-shell Brazil nuts which will be marketed as destined for further processing (DFP - includes good and rotten nuts); in this case, visual sorting after shelling can be performed, and the estimated processing factor ($PF_{DFP}$) was 0.1 (1.69/17.69). These processing factors can be used to evaluate the safety of the regulatory levels of aflatoxin in in-shell Brazil nut on the aflatoxin dietary intake from the consumption of the shelled nut.

51. The Codex Alimentarius concept of Food Safety Objective (FSO) is the maximum frequency and or concentration of a hazard in a food at the time of consumption that provides or contributes to the appropriate level of protection (ALOP). This concept supports the approach of the PF to estimate the concentration of aflatoxin in Brazil nut at the time of consumption. Hence, the consumer can achieve the ALOP when shelling and sorting the Brazil nut.

52. It is recognized that the estimated $PF_{DFP}$ reflects the situation found within the CONFORCAST project, where Brazil nut DFP lots contained about 6 % of rotten nuts in mass. In lots with a higher or lower percentage of rotten nuts, $PF_{DFP}$ will be lower or higher, respectively.

**PRACTICE FOR THE PREVENTION AND REDUCTION OF AFLATOXIN CONTAMINATION IN BRAZIL NUTS**

53. A validation of good practices, with respect to the factors causing aflatoxin contamination in the Brazil nut production chain and the available methods of control, has recently been completed in the STDF[1] project SafeNut[2]. The findings from this project clearly indicate a need to update the current code of practice for the prevention and reduction of aflatoxin contamination in Brazil nuts (CAC/RCP 59 -2005)**.** The STDF-SafeNut final report will be available in early 2009.

**DIETARY INTAKE**

54. Cereals (mainly corn), groundnuts, oilseeds, tree nuts, dried fruits, spices and copra are the main products contaminated with AFs. The most important dietary sources of AFs are corn, groundnuts and their products, which form an essential part of the diet in some countries (CAC, 2005b).

55. At its 49$^{th}$ Meeting, the JECFA evaluated the potential impact of two hypothetical standards for AF contamination on peanuts (10 or 20 µg/kg AFB1) on sample populations and their overall risk. It was concluded that reducing the maximum permitted level (ML) of AFT in peanuts from 20 µg/kg AFB1 to 10 µg/kg AFB1 would not result in any observable difference in rates of liver cancer (FAO/WHO, 1998).

---

1 The Standards and Trade Development Facility (STDF) is a global programme in capacity building and technical co-operation established by the Food and Agriculture Organization of the United Nations (FAO), the World Organization for Animal Health (OIE), the World Bank, the World Health Organization (WHO) and the World Trade Organization (WTO).

2 http://stdf-safenutproject.com/. (STDF project 114)

56. The dietary intake of AFs by the Swedish population was estimated to be 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 conducted in Sweden, assuming a 70 kg b.w consumer and consumption of Brazil nuts of 0.3 kg during Christmas time, the median AFs intake was 0.73 ng/kg bw and the 95$^{th}$ percentile 110 ng/kg bw. Smoothing the consumption over a full year, the figures would be 0.002 and 0.3 ng/kg bw (Marklinder et al., 2005).

57. At its 68$^{th}$ Meeting, the JECFA evaluated the impact to human health from a dietary exposure to AFs from the consumption of the tree nuts edible parts (ready-to-eat) and dried figs (FAO/WHO, 2008). Using the 13 GEMs/Food Consumption Cluster diets (WHO, 2006) and assuming a body weight of 60 kg, the Committee evaluated the impact on dietary exposure to AFs of setting hypothetical MLs of 4, 8, 10, 15 or 20 µg/kg for AFT in almonds, Brazil nuts, hazelnuts, pistachios and dried figs.

58. According to the GEMs/FOOD data, the consumption of Brazil nuts is different from zero only in the E, K, and M cluster diets (0.1 g/person/day), that includes European, Latin American and North American countries, respectively. The consumption of other nuts are much higher, and can reach around 2 g/person/day of hazelnuts or almonds in cluster B, that includes the Mediterranean countries. Data from the INC indicates that the mean consumption of Brazil nut of the largest consuming countries is 0.082 g/person/day, which corresponds to 2.2 % of all four tree nuts together.

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

60. In the worst scenario, when no ML is in place, the intake of AFT from the consumption of tree nuts and dried figs contributed to more than 5% of the total dietary AFT exposure only for the GEMS/Food cluster diets B, C, D, E and M (24.6, 20, 45, 16.8 and 9.3 %, respectively).

61. If fully enforced, a ML at 20 µg/kg in almonds, Brazil nuts, hazelnuts, pistachios and dried figs would have an impact on the relative contribution to dietary AFT exposure only in these clusters, including high-level consumers of the tree nuts. This is due solely to the elevated AFT level in pistachios. For the tree nuts other than pistachios, the presence of a ML has no effect on total dietary AFT exposure.

62. The JECFA estimated that an enforced ML of 20, 15, 10, 8 or 4 µg/kg results in dietary exposures to AFT ranging from 0.12, 0.10, 0.08, 0.07 and 0.06 ng/kg bw per day in the cluster with the highest exposure (D) to 0.03, 0.02, 0.02, 0.02 and 0.01 ng/kg bw per day in the cluster with the lowest exposure (M).

63. The JECFA noted that the estimates for European clusters B, E and F, with MLs from 4 to 20 µg/kg for tree nuts were in the range of those reported in the EFSA opinion with MLs from 4 to 10 µg/kg for tree nuts, including high-level consumers.

64. The JECFA concluded that enforcing an ML of 15, 10, 8 or 4 µg/kg would have little further impact on the overall dietary exposure to AFT in all five of the highest exposed population groups, compared with setting an ML of 20 µg/kg. When the impact of the theoretical full enforcement of MLs scenarios for AFT was evaluated, the proportion of rejected samples by establishing a ML of 20 µg/kg for Brazil nuts was 11 %. This value increased to 17 % for a ML of 4 µg/kg.

65. The scientific Panel on Contaminants in the Food Chain (CONTAM) of the European Food Safety Authority was asked to advise on the potential increase in risks to consumer health associated with an increase in current EU maximum levels for almonds, pistachios and hazelnuts, taking into account the consumption patterns of these nuts in the EU. In its opinion N° EFSA-Q-2006-174 the Panel concluded that changing the maximum levels for total aflatoxins from 4 to 8 or 10 µg/kg AFT would have minor effects on the estimates of dietary exposure and cancer risk.

**CONCLUSIONS & RECOMMENDATIONS:**

66. The present Paper on AFs in Brazil Nuts leads to the following conclusions and recommendations for consideration at the 3rd Session of the CCCF:

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

II) The consumption of Brazil nuts in the world is lower than that of other tree nuts. Unlikely the other tree nuts, the extractivist characteristic of the Brazil nut limits a high increase in consumption, even if there is an increase in demand by the market.

III) At its last Session, the JECFA has concluded that enforcing an ML of 15, 10, 8 or 4 µg/kg would have little further impact on the overall dietary exposure to AFT through the consumption of almonds, Brazil nuts, hazelnuts and pistachios in all five of the highest exposed population groups, compared with setting a ML of 20 µg/kg. Furthermore, for the tree nuts other than pistachios, the presence of a ML has no effect on dietary AFT exposure.

IV) The levels of aflatoxins in good (edible) shelled Brazil nuts in lots to be exported from Brazilian plants as ready-to-eat are normally very low, mostly ranging from below 0.39 µg/kg up to 10 µg/kg.

V) In many importing countries, in-shell Brazilian nuts are analysed as such at the point of entry without any sorting to separate good from rotten nuts. Results from the CONFORCAST project has shown that the levels of AFT in no-sorted in-shell Brazilian nuts ready to be exported (which could be classified as destined for further processing) was higher than 50 µg/kg in 3.7 % of the lots. This lot should undergo a processing/treatment, such as shelling and/or sorting to decrease aflatoxin levels, before marketed to the final consumer.

VI. The CONFORCAST project has shown that the AFT levels found in in-shell good nuts was mostly below 5 µg/kg. However, it should be pointed out that in the project, the good nuts were selected after inspection of the whole kernel after shelling, which is a procedure that is not practical in a routine basis in a laboratory.

VII. Good in-shell Brazil nuts can be segregated from the rotten nuts in the analytical sample by visual inspection at the laboratory by cutting the nut in half in order to estimate the AFT contamination in the Brazil nut lot. This procedure is routinely performed by the exporters when buying the nuts from the producers and when they want to sort a lot to be exported as ready-to-eat.

VIII. Based on the incidence data of aflatoxins in Brazil nuts evaluated at this paper, including the data reported by the CONFORCAST project, the following maximum limits (ML) of total aflatoxins in the various Brazil nut commodities are recommended to be used in international trade:

| **Brazil nut commodity** | **AFT ML, µg/kg** |
|---|---|
| Shelled, ready to eat | 15 |
| Shelled, destined for further processing | 20 |
| In-shell, ready-to eat | 20 |
| In-shell, destined for further processing | 50 |

IX) Aflatoxin levels in rotten in-shell Brazil nuts are considerably higher than the levels found in good in-shell nuts, selected by visual sorting after shelling or other available processing that has proven to reduce aflatoxin levels. Additionally, the shells contribute significantly to the aflatoxin concentration in the whole nut, even when the nut is visually classified as good nut. Studies have shown that consumers may visually separate the edible from the inedible (highly contaminated) Brazil nut kernels after the shelling process and thus protect themselves from exposure to high levels of AFs.

X) The processing factors estimated in this paper (para 50) ($PF_{RTE}$ of 0.6 and $PF_{DFP}$ of 0.1) can be used to evaluate the impact of the ML recommended for in-shell Brazil nut ready-to-eat and destined for further processing, on the dietary intake of aflatoxins. Using these factors, we can estimated that at a ML of 20 and 50 µg/kg, the consumers would be exposed to nuts that have levels at 12 and 5 µg/kg, respectively.

XI) In cases where a in-shell DFP lot has a % of rotten nuts lower than 6% in mass (found in the CONFORCAST project), the $PF_{DFP}$ will be higher, but it is unlikely that at a ML of 50 µg/kg, the consumer will be exposed to unsafe levels of aflatoxins in edible Brazil nuts.

XII) It is recognized that Brazil nuts are included in many products, e.g. breakfast cereals, snack products, cereal bars and ready-to-eat nut products, including pastes. The human exposure to aflatoxins from their content in the Brazil nut used to prepare these products is lower than the exposure from the Brazil nut consumption itself due to dilution factors. It is clear that the commodities used to prepare Brazil nut based food should be controlled to guarantee the safety of consumers.

**REFERENCES**


1. AOAC International. Available in: http://www.aoac.org/testkits/testedmethods.html. Access at: February 2009.
2. AOAC Official methods 994.08. Aflatoxins in corn, almonds, Brazil nuts, peanuts and pistachio nuts. AOAC – Official Methods of Analysis (2000), 17th Ed., Vol. II, AOAC International, Gaithrsburg, MD, USA, Chapter 49, 26-27.
3. Arrus. K.; Blank, G.; Abramson, D.; Clear, R.; Holley, R.A. Aflatoxin production by Aspergillus flavus in Brazil nuts. *Journal of Stored Products Research*, 41: 513-527. 2005a.
4. Arrus, K.; Blank, G.; Clear, R.; Holley, R.A.; Abramson, D. Microbiological and aflatoxin evaluation of Brazil nut pods and the effects of unit processing operations. *Journal of Food Protection* 68, 1060-65, 2005b.
5. Bacaloni, A., Cavaliere, C., Cucci, F. Foglia, P. Samperi, R., Laganà, A., Determination of aflatoxins in hazelnuts by various sample preparation methods and liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A*, 1179 182–189, 2008.
6. Bayman, P.; James,L.; Mahoney,N. E. Aspergillus on tree nuts: incidence and associations. *Mycophatologia,* 155:161-169. 2002.
7. BRAZIL. Ministry of Agriculture, Data on Brazil nuts. 2006 (unpublished data).
8. CAC - Codex Alimentarius Commision. 24th Session of Codex Comission. Alinorm 01/41, paragraph 138. 2001.
9. CAC - Codex Alimentarius Commission, Codex Committee on Food Additives and Contaminants, 37th Session of the Codex Committee on Food Additives and Contaminants CRD 17, Data on the occurrence of aflatoxins in Brazil nuts, in Brazil, from 1998-2004, 2005a.
10. CAC - Codex Alimentarius Commission, Codex Committee on Food Additives and Contaminants, 37th Session. Discution Paper on aflatoxins in Brazil nuts. CX/FAC 05/37/24, December 2004, The Hague, the Netherlands, 25-29, April, 2005b.
11. CAC - Codex Alimentarius Commission, Codex Committee on Food Additives and Contaminants, 37th Session of the Codex Committee on Food Additives and Contaminants, ALINORM 05/28/12, 2005c.
12. Cartaxo, C. B. C.; Souza, J. M. L.; Corrêa, T. B.; Costa, P.; Freitas-Silva, O. Occurrence of aflatoxin and filamentous fungi contamination in brazil-nuts left inside the forest. In: IV Congreso Latinoamericano de Micotoxicología. Anais eletrônicos. Havana, Centro Nacional de Sanidad Agropecuária, 2003.
13. CONFORCAST. Ferramentas Analíticas para Capacitação do Brasil na Garantia da Conformidade da Castanha-Do-Brasil (Bertholletia Excelsa) quanto ao Perigo aflatoxina. Projeto nº 1.265/05, Aprovado pela FINEP na Chamada Pública, "Ação Transversal - TIB - 06/2005 - Linha 1". MAPA. Minist~erio da Agricultura, pecuária e do Abasteciento. Secretaria de Defesa Agropecuária - DAS, Departamento de Inspeção de Produtos de Origem Vegetal – DIPOV. Coordenação-Geral de Apoio Laboratorial – CGAL, Laboratório Nacional Agropecuário – LANAGRO/MG, United States Department of Agriculture (Thomas Whitaker and Andy Slate).
14. EC. European Commission. European Regulation (EC) No 401/2006 of 23 February 2006. Methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. *Official Journal of the European Union,* 2006.

15. EFSA, Opinion of the Scientific Panel on Contaminants in the Food Chain on a Request from the Commission Related to the Potential Increase Of Consumer Health Risk By A Possible Increase of the Existing Maximum Levels for Aflatoxins in Almonds, Hazelnuts and Pistachios and Derived Products - Question N° EFSA-Q-2006-174. *The EFSA Journal* 446, 1 – 127. 2007
16. FAO/WHO. Safety evaluation of certain food additives and contaminants. WHO Food Additive Series, No. 40, 1998.
17. FAO/WHO. Safety evaluation of certain food additives and contaminants (Sixty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Food Additives Series: 59, p.305. 2008. Available at http://whqlibdoc.who.int/publications/2008/9789241660594_eng.pdf. Accessed on January 2009.
18. Food Standard Agency, 2004. Analysis of Aflatoxin $B_1$ and total aflatoxins in edible nuts and nut products. Summary available at: http://www.food.gov.uk/multimedia/pdfs/summarynuts.pdf
19. Frisvad, J. C., Thrane, U., Samson, R. A. and Pitt, J.I. (2006) Important mycotoxins and the fungi which produce them. In: Hocking. A. C., Pitt, J.I., Samson, R. A. and Thrane, U. (Eds) *Advances in Food Mycology*. Springer, New York, pp.3-31.
20. Gilbert, J. and Vargas, E.A. Advances in Sampling and Analysis for Aflatoxins in Food and Animal Feed. *Toxin Reviews* (formerly Journal of Toxicology: Toxin Reviews), 22(2&3): 381-422. 2003.
21. INC. International Nut and Dried Fruit Council Foundation. Document prepared for the Electronic Working Group "Discussion Paper on Maximum Levels for Total Aflatoxins in Ready-to-eat Almonds, Hazelnuts and Pistachios" led by the European Community, 2007.
22. Ioannou-Kakouri E, Aletrari M, Christou E, Hadjioannou-Ralli A, Koliou A, Akkelidou D. Surveillance and Control of Aflatoxins B1, B2, G1, G2 and M1 in Foodstuffs in the Republic of Cyprus: 1992-1996. *J. of AOAC International*, 82(4): 883 – 892. 1999.
23. Ito, Y.; Peterson, S.; Wicklow, D.T.; Goto, T. *Aspergillus pseudotamarii*, a new aflatoxin producing species in Aspergillus section flav. *Mycological Research*, 105(2): 233-239. 2001.
24. Johnsson, P, Lindbland, M., Thim, A.M., Jonsson, N., Vargas, E.A., Medeiros, N.L., Brabet, C., Quaresma de Araújo, M., and Olsen, M. Growth of aflatoxigenic moulds and Aflatoxin formation in Brazil nuts. *World Mycotoxin Journal*, 1(2):127-137, 2008.
25. Marklinder, I.; Lindblad, M.; Gidlund, A.; Olsen, M. Consumers' ability to discriminate aflatoxincontaminated Brazil nuts. *Food Add. Cont.* 22 (1): 56-64. 2005.
26. MAPA - Ministério da Agricultura, Pecuária, Abastecimento. LACQSA/LANAGRO-MG. POP 055, Determinação de aflatoxinas por cromatografia liquida de alta eficiencia e em camada delgada, revisão 02, 25 de fevereiro de 2008.
27. Mello F. R. and Scussel V. M. 2007. Characteristic of in-shell Brazil nuts and their relationship to aflatoxin contamination: criteria for sorting. J. of Agric. and Food Chemistry, October, 9305-9310.
28. Olsen M, Johnsson P, Möller T, Paladino R and Lindlblad M. Aspergillus nomius, an important aflatoxin producer in Brazil nuts? World Mycotoxin Journal 1 (2), 123-126, 2008
29. Ozay, G., Seyhan, F., Yilmaz, A.,.Whitaker, T. B., Slate, A. B., Giesbrecht, F. G. Sampling Hazelnuts for Aflatoxin: Effect of Sample Size and Accept/Reject Limit on Reducing the Risk of Misclassifying Lots: Food Chemical Contaminants Journal of AOAC International, 90, 1028-1035, 2007
30. Ozay, G., Seyhan, F., Yilmaz, A.,.Whitaker, T. B., Slate, A. B., Giesbrecht, F. G. Sampling Hazelnuts for Aflatoxin: Uncertainty Associated with Sampling, Sample Preparation, and Analysis : Food chemical contaminants - Journal of AOAC international vol. 89 No. 4, 2006.
31. Pacheco A., Robert F.; Scussel V. Detecção de aflatoxinas em castanha-do-brasil (Bertholletia excelsa H.B.K) na safra de 2005. *Analytica*, 22: 64-65. 2006.
32. Pacheco A; Scussel V. Selenium and aflatoxin levels in raw Brazil nuts from the Amazon basin. *J. Agric. Food Chem.* 55:11087-92, 2007.
33. Pacheco, A.M.; Scussel. V.M. Aflatoxins Evaluation on *In-shell* and *Shelled* Dry Brazil Nuts for Export Analyzed by LC MS/MS - 2006 and 2007 Harvests. World Mycotoxin Journal. *In press*. 2009.
34. Pohland A. E. Mycotoxins in review. *Food Add. Cont*., 10: 17-28. 1993.
35. Salunkhe D. K.; Adsule R. N.; Padule D. N. Aflatoxins in foods and feeds, Metropolitan, Book Co.Pvt. Ltd., New Delhi, India, p. 18. 1987.
36. Sobolev VS. Simple, rapid, and inexpensive cleanup method for quantitation of aflatoxins in important agricultural products by HPLC. *J Agric Food Chem*. 55:2136-41, 2007

37. Souza, J. M. L.; Cartaxo, C. B. C.; Leite, F. M. N.; Reis, F. S. Avaliação microbiológica de castanha do brasil em usinas de beneficiamento no Acre. In: XLIX Reunião Anual da Sociedade Interamericana de Horticultura Tropical. Anais. Fortaleza, p. 201 (Embrapa Agroindústria Tropical. Documentos, 67). 2003.
38. Spanjer M, C., Rensen, P., M.; Scholten, J.M., LC-MS/MS multi-method for mycotoxins after single extraction, with validation data for peanut, pistachio, wheat, maize cornflakes, raisins and figs. Food additives and contaminants, 25(4): 472-489, April 2008.
39. Stroka, J., Anklam, E., Jorissen, U., Gilbert, J. Immunoaffinity column cleanup with liquid chromatography using post-column bromination for determination of aflatoxins in peanut butter, pistachio paste, fig paste and paprika powder: collaborative study. J. AOAC, 83, 2, 320-340, 2000.
40. Thuvander, A.; Möller, T.; Enghardt Barbieri, H.; Jansson, A.; Salomonsson, A.-C.; Olsen, M. Dietary intake of some important mycotoxins by the Swedish population. *Food Add. Cont*. 18 (8): 696-706. 2001.
41. Wadt., L. H. O.; Kainer, K. A.; Gomes-Silva, D. A. P. Population structure and nut yield of a Bertholetia excelsa stand in southwestern Amazonia. *Forest Ecology and Management*. 211: 371-384. 2005.
42. Whitaker, T.B., Slate, A.B., Giesbrecht, F. G Sampling Almonds for Aflatoxin, Part II: Estimating Risks Associated with Various Sampling Plan Designs: Food chemical contaminants - Journal of AOAC International vol. 90, 3, 2007.
43. WHO – World Health Organization. GEMS/Food Custers Diet (Global Environment Monitoring System/ Food Contamination Monitoring and Assessment Program). 2006. Available at http://www.who.int/foodsafety/chem/gems/en/index1.htm.

## ANNEX I

## SAMPLING PLAN FOR TOTAL AFLATOXINS IN BRAZIL NUTS

This Annex will be part of the SAMPLING PLANS FOR AFLATOXIN CONTAMINATION IN READY-TO-EAT TREENUTS AND TREENUTS DESTINED FOR FURTHER PROCESSING: ALMONDS, HAZELNUTS AND PISTACHIOS (CODEX STAN 193-1995, Schedule I, Annex 2) approved at the 2nd Meeting of the CCCF and adopted by the 31st Session of the Codex Alimentarius Commission. The aspects related to Brazil nuts which are different from the other nuts will be covered by this Annex.

### SAMPLING PLAN DESIGN CONSIDERATIONS

Brazil nuts can be commercialized as “Shelled Brazil nut ready-to-eat”, “Shelled Brazil nut destined for further processing”, “In-shell Brazil nut ready-to-eat” and “In-shell Brazil nut destined for further processing”, as defined bellow. As a result, maximum levels and sampling plans are proposed for all commercial types of Brazil nuts.

Shelled Brazil nut ready-to-eat: are shelled nuts (kernels only) which can be directly marketed to the final consumer

Shelled Brazil nut destined for further processing: are shelled nuts (kernels only) that are intended to undergo a sorting process to reduce aflatoxin levels before being marketed to the final consumer.

In-shell Brazil nut ready-to-eat: are in-shell nuts which can be directly marketed to the final consumer

In-shell Brazil nut destined for further processing: are in-shell nuts that are intended to undergo sorting and/or shelling process to reduce aflatoxin levels before being marketed to the final consumer.

The in-shell Brazil nut lots always include rotten nuts due the extractive characteristic of the nuts in the rainforest. Tables 1 and 2 (Annex III) show that in-shell Brazil nut lots ready to be marketed sampled within the CONFORCAST project contained, in average, 6% of rotten nuts. The results of this project show that the segregation of the rotten nuts from the aggregate sample (analytical sample) before analysis could give an estimation of AFT contamination present in the good nuts only. Based on the results of CONFORCAST Project (Annex III), it is being proposed the removal of 5% of rotten nuts from the sample of in-shell nut ready-to-eat lots, previously to its analysis, according to the following procedures.

### AFLATOXIN TEST PROCEDURE AND MAXIMUM LEVELS

Shelled Brazil nuts destined for further processing

Maximum level – 20 ng/g total aflatoxins

Number of laboratory samples – 1

Laboratory sample size - 20 kg

Sample preparation – turrax type mill, 100 g test portion (slurry: 50 g kernels: 50 g water)

Decision rule – If the aflatoxin test result is less than or equal to 20 ng/g total aflatoxins, then accept the lot. Otherwise, reject the lot.

The operating characteristic curve describing the performance of the sampling plan for the Shelled Brazil nuts destined for further processing is shown in Annex II.

In-shell Brazil nuts destined for further processing

Maximum level – 50 ng/g total aflatoxins

Number of laboratory samples – 1

Laboratory sample size - 20 kg

Sample preparation – turrax type mill, 100 g test portion (slurry: 50 g nut: 50 g water). In case of the FFP “in-shell Brazil nuts”, the test portion may be the whole nut, as sometimes the removal of the inedible part (shell) is not feasible.

Decision rule – If the aflatoxin test result is less than or equal to 50 ng/g total aflatoxins, then accept the lot. Otherwise, reject the lot.

The operating characteristic curve describing the performance of the sampling plan for the In-shell Brazil nuts destined for further processing is shown in Annex II.

Shelled Brazil nuts ready-to-eat:

Máximum level: 15 ng/g total aflatoxins

Number of laboratory samples – 2

Laboratory sample size - 10 kg

Sample preparation – turrax type mill, 100 g test portion (slurry: 50 g kernel: 50 g water)

Decision rule – If the aflatoxin test result is less than or equal to 15 ng/g total aflatoxin in both test samples, then accept the lot. Otherwise, reject the lot.

The operating characteristic curve describing the performance of the sampling plan for the ready-to-eat Shelled Brazil nuts is shown in Annex II.

In-shell Brazil nuts ready-to-eat:

Maximum level – 20 ng/g total aflatoxins

Number of laboratory samples – 2

Laboratory sample size - 10 kg

Sample preparation – cut the nuts in half, segregate the rotten nuts up to 5%, analyse the good nuts (kernels and shells), turrax type mill, 100 g test portion (slurry: 50 g nut: 50 g water). Rotten nuts must be segregated up to 5% (% sample mass-kg) from the analytical sample (2x10 kg) by visual inspection at the laboratory stage by cutting the nuts in half in order to estimate the AFT contamination in the lot. When the sample presents more then 5% of rotten nuts it should be prepared without nut segregation.

Decision rule – If the aflatoxin test result is less than or equal to 20 ng/g total aflatoxins, then accept the lot. Otherwise, reject the lot.

The operating characteristic curve describing the performance of the sampling plan for the in-shell Brazil nuts ready-to-eat is shown in Annex II.

OBS: The CONFORCAST Project has shown that the AFT levels found in in-shell good nuts was mostly below 5µg/kg.

## Annex II

### Operating Characteristic Curves describing the performance of draft aflatoxin sampling plans for Brazil nuts

For each lot, the observed distribution among the 40 sample aflatoxin results (total aflatoxins) for shelled Brazil nut was compared to three positively skewed theoretical distributions, the negative binomial, compound gamma, and lognormal theoretical distributions. The power divergent method was used to measure the goodness of fit (GOF) between the observed and theoretical distributions. The negative binomial was chosen to simulate the distribution among sample test results for a given lot concentration and sample design. The negative binomial was also chosen to simulate the distribution among sample test results for almonds, hazelnuts, and pistachios.

A plot of acceptance probabilities versus lot concentration is called an operating characteristic (OC) curve. The shape of the OC curve is uniquely defined by the sampling plan design. A sampling plan is defined by an accept/reject limit and the aflatoxin test procedure. An aflatoxin test procedure is defined by number and size samples, sample preparation method (type mill and test portion size), and analytical procedure. The OC curve is used to predict the good lots rejected (seller's risk or exporter's risk) and the bad lots accepted (buyer's or importer's risk).

### Shelled Brazil nuts

The estimation of the variances associated with each step of the sampling plan for shelled Brazil nut is shown on Table 1.

**Table 1.** Variances in the sampling plan steps for shelled Brazil nut

| Data Used For Variance Analysis | Sampling Variance | Sample Preparation Variance | Analysis Variance | Total Variance |
|---|---|---|---|---|
| Shelled Brazil Nut<br>- From Shelled Brazil Nut Study I<br>- Total aflatoxin, lots < 0.39 ug/kg omitted<br>- Kernels only<br>- 15 Lots, 10 kg Sample, 185 kernels/kg | $s_s^2 = 4.8616C^{1.889}$<br>R = 0.80 | $s_{ss}^2 = 0.0306C^{0.632}$<br>R = 0.24 | experimental<br>$s_a^2 = 0.0164C^{1.117}$<br>FAPAS<br>$s_a^2 = 0.0484C^{2.0}$<br>R = 0.43 | $s_t^2 = 5.464C^{1.850}$<br>R = 0.73 |

**Shelled Brazil nuts destined for further processing**

Operating Characteristic curve describing the performance of the aflatoxin sampling plan for shelled Brazil nut destined for further processing using a single laboratory sample of 20 kg and a maximum level of 20 ng/g for total aflatoxins is shown in Figure 1. The operating characteristic curves reflect uncertainty associated with a 20 kg laboratory sample of shelled nuts, turrax type mill, 100 g test portion (slurry: 50 g kernel,: 50 g water), and quantification of aflatoxin in the test portion by HPLC - FL with pos-column derivatization with Kobra Cell.

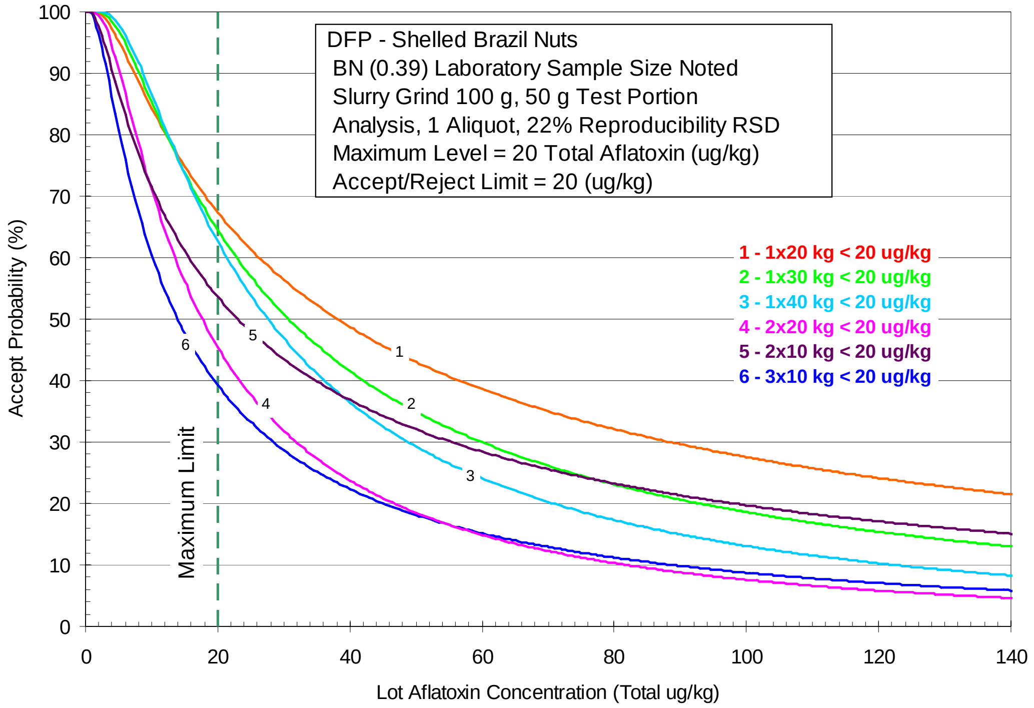


**Figure 1 –** Operation curves for shelled Brazil nuts destined for further processing (DFP) at a maximum limit of 20 µg/kg

Operating Characteristic curve describing the performance of the aflatoxin sampling plan for ready-to-eat shelled Brazil nut using two laboratory samples of 10 kg each and a maximum level of 15 ng/g for total aflatoxins, turrax type mill, 100 g test portion (slurry: 50 g kernel: 50 g water), and quantification of aflatoxin in the test portion by HPLC - FL with pos-column derivatization with Kobra Cell is shown on Figure 2.

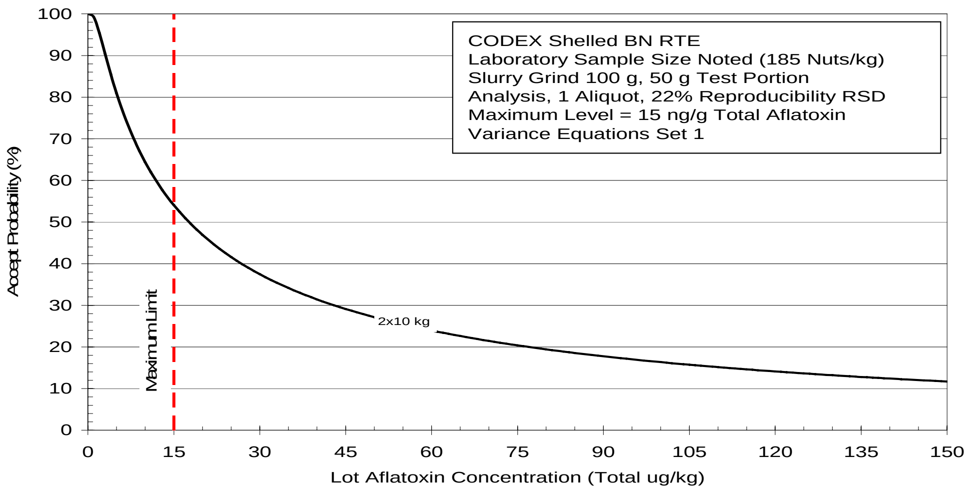


**Figure 2 -** Operation curve for shelled Brazil nuts ready-to-eat (RTE) at a maximum limit of 15 µg/kg

**In-shell Brazil nuts**

The estimation of the variances associated with each step of the sampling plan for shelled Brazil nut is shown on Table 2.

**Table 2.** Variances in the sampling plan steps for in-shell Brazil nut

| Data Used For Variance Analysis | Sampling Variance | Sample Preparation Variance | Analysis Variance | Total Variance |
|---|---|---|---|---|
| Inshell Brazil Nut<br>- From Inshell Study II (Set 5)<br>- Total aflatoxin<br>- 10 kg Sample, 97 nuts/kg | $s_s^2 = 5.855C^{1.092}$<br>*calculated from total | $s_{ss}^2 = 0.0306C^{0.632}$<br>R = 0.24 | experimental<br>$s_a^2 = 0.0164C^{1.117}$<br>FAPAS<br>$s_a^2 = 0.0484C^{2.0}$<br>R = 0.43 | $s_t^2 = 5.922C^{1.090}$<br>R = 0.90 |

**In-shell Brazil nuts destined for further processing**

Operating Characteristic curve describing the performance of the aflatoxin sampling plan for in-shell Brazil nut destined for further processing using a single laboratory sample of 20 kg and a maximum level of 50 ng/g for total aflatoxins is shown on Figure 3. The operating characteristic curves reflect uncertainty associated with a 20 kg laboratory sample of in-shell nuts, turrax type mill, 100 g test portion (slurry: 50 g kernel: 50 g water), and quantification of aflatoxin in the test portion by HPLC - FL with pos-column derivatization with Kobra Cell.

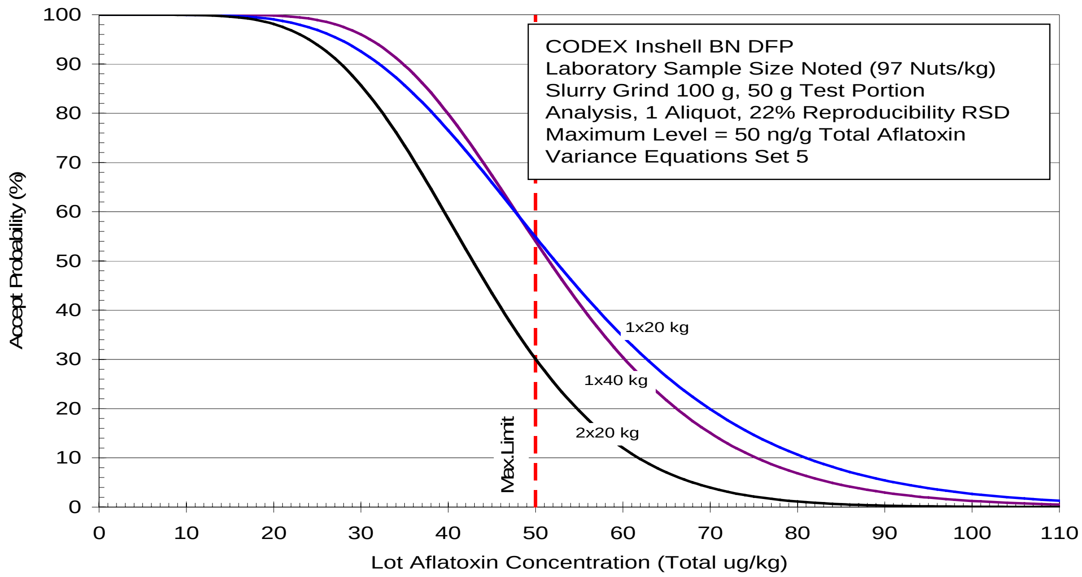


**Figure 3** - Operation curves for in-shelled Brazil nuts destined for further processing (DPF) at a maximum limit of 50 µg/kg

**In-shell Brazil nuts ready-to-eat**

Operating Characteristic curve describing the performance of the aflatoxin sampling plan for ready-to-eat in-shell Brazil nut using two laboratory samples of 10 kg each and a maximum level of 20 ng/g for total aflatoxins, turrax type mill, 100 g test portion (slurry: 50 g kernel: 50 g water), and quantification of aflatoxin in the test portion by HPLC - FL with pos-column derivatization with Kobra Cell is shown on Figure 4.

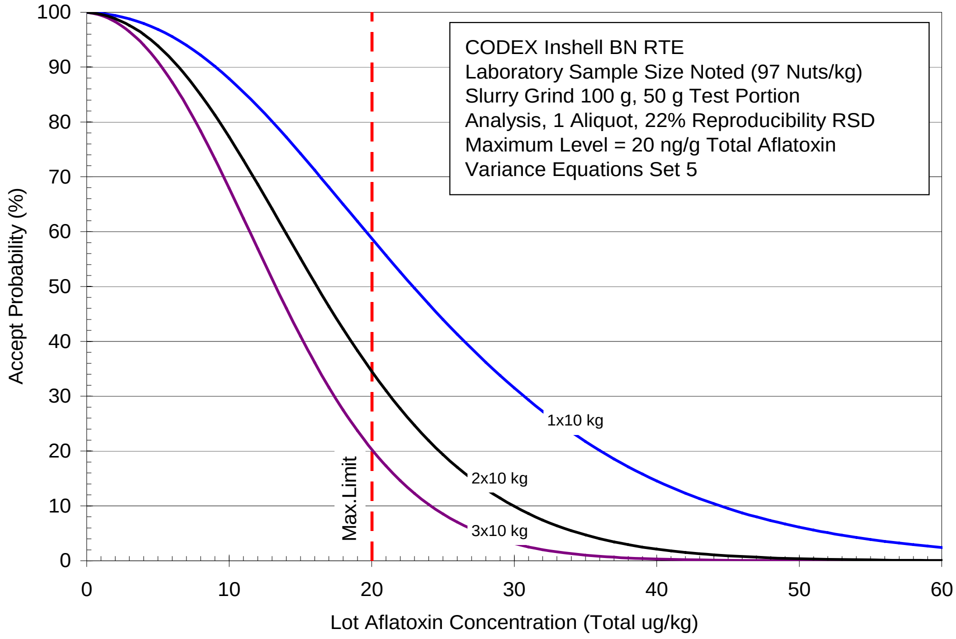


**Figure 4** - Operation curve for in-shelled Brazil nuts ready-to-eat (RTE) at a maximum limit of 20 µg/kg

## ANNEX III

**Table 1.** Component mass contribution (% of sample mass - kg) by all categories in all samples from 12 lots of Brazil nuts by category).

| **Lot** | Good kernels (%) | Shells with and without kernel residue (%) | Good kernels shells with and without kernel residue (%) | Rotten shells and kernels (%) | Good kernels - aflatoxin (µg/kg) | Good nuts aflatoxin (µg/kg) | Rotten nuts aflatoxin (µg/kg) | Total contamination (all fractions) (µg/kg) | % rotten nuts (%mass) | Average aflatoxin in good nuts | Average aflatoxin in all categories **l** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 66.4 | 31.6 | 98.0 | 2.0 | 6.0 | 5.7 | 416.0 | 13.8 | < 2% | 5.7 | 13.8 |
| **5** | 62.5 | 34.6 | 97.1 | 2.9 | 0.5 | 1.9 | 860.4 | 26.6 | <4% | 3.0 | 13.9 |
| **1** | 65.5 | 31.6 | 97.1 | 2.9 | 0.3 | 2.0 | 631.5 | 20.5 | | | |
| **1** | 66.8 | 30.1 | 96.9 | 3.1 | 3.9 | 4.4 | 456.7 | 18.2 | | | |
| **4** | 64.3 | 32.4 | 96.8 | 3.2 | 0.5 | 2.9 | 232.3 | 10.3 | | | |
| **1** | 63.4 | 33.0 | 96.4 | 3.6 | 3.7 | 4.8 | 489.9 | 22.2 | | | |
| **4** | 63.5 | 32.8 | 96.3 | 3.7 | 0.4 | 2.5 | 187.6 | 9.3 | | | |
| **8** | 52.1 | 44.2 | 96.3 | 3.7 | 0.0 | 0.7 | 436.8 | 16.8 | | | |
| **11** | 53.7 | 42.5 | 96.3 | 3.7 | 0.0 | 0.9 | 102.8 | 4.7 | | | |
| **10** | 52.8 | 43.3 | 96.1 | 3.9 | 0.2 | 4.9 | 69.1 | 7.3 | | | |
| **12** | 53.3 | 42.8 | 96.1 | 3.9 | 0.0 | 2.8 | 125.8 | 7.6 | | | |
| **3** | 69.7 | 26.3 | 96.0 | 4.0 | 0.4 | 2.4 | 189.7 | 9.9 | | | |
| **7** | 51.3 | 44.6 | 95.9 | 4.1 | 0.0 | 1.9 | 633.4 | 28.0 | <5% | 2.8 | 15.5 |
| **1** | 66.4 | 29.3 | 95.7 | 4.3 | 0.2 | 1.3 | 310.0 | 14.4 | | | |
| **5** | 63.0 | 32.7 | 95.7 | 4.3 | 0.4 | 1.9 | 360.6 | 17.3 | | | |
| **6** | 55.7 | 40.0 | 95.7 | 4.3 | 2.1 | 3.0 | 422.1 | 21.0 | | | |
| **5** | 66.4 | 29.3 | 95.7 | 4.3 | 0.9 | 1.8 | 234.3 | 11.8 | | | |
| **4** | 61.8 | 33.9 | 95.6 | 4.4 | 0.3 | 2.7 | 207.1 | 11.6 | | | |
| **1** | 62.2 | 33.4 | 95.6 | 4.4 | 0.2 | 2.3 | 326.2 | 16.6 | | | |
| **4** | 66.2 | 29.4 | 95.6 | 4.4 | 2.6 | 4.4 | 352.5 | 19.8 | | | |
| **5** | 62.9 | 32.4 | 95.3 | 4.7 | 1.7 | 3.0 | 229.0 | 13.6 | | | |
| **4** | 63.1 | 32.2 | 95.3 | 4.7 | 0.5 | 2.1 | 215.7 | 12.2 | | | |
| **3** | 66.4 | 28.8 | 95.3 | 4.7 | 7.1 | 5.7 | 250.5 | 17.3 | | | |
| **9** | 52.6 | 42.5 | 95.0 | 5.0 | 0.2 | 1.3 | 408.8 | 21.5 | | | |
| **5** | 64.2 | 30.6 | 94.8 | 5.2 | 0.3 | 2.1 | 189.8 | 11.9 | <6% | 2.6 | 15.5 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **5** | 62.3 | 32.5 | 94.8 | 5.2 | 0.2 | 1.8 | 210.0 | 12.6 | | | |
| **1** | 65.6 | 29.1 | 94.7 | 5.3 | 0.6 | 3.0 | 431.6 | 25.7 | | | |
| **4** | 60.6 | 34.1 | 94.7 | 5.3 | 0.2 | 2.1 | 141.8 | 9.5 | | | |
| **3** | 65.4 | 29.1 | 94.5 | 5.5 | 0.2 | 0.9 | 137.7 | 8.5 | | | |
| **1** | 64.0 | 30.4 | 94.5 | 5.5 | 0.2 | 1.6 | 254.8 | 15.6 | | | |
| **4** | 60.6 | 33.5 | 94.1 | 5.9 | 1.2 | 3.4 | 287.3 | 20.1 | | | |
| **3** | 64.8 | 29.3 | 94.1 | 5.9 | 0.3 | 1.6 | 147.9 | 10.3 | | | |
| **3** | 67.3 | 26.7 | 94.0 | 6.0 | 2.6 | 2.9 | 376.1 | 25.2 | | | |
| **5** | 64.1 | 29.9 | 94.0 | 6.0 | 0.3 | 2.6 | 542.3 | 35.1 | | | |
| **3** | 65.6 | 28.3 | 94.0 | 6.0 | 0.3 | 3.2 | 292.7 | 20.7 | | | |
| **1** | 64.7 | 29.3 | 94.0 | 6.0 | 0.2 | 1.7 | 446.2 | 28.6 | | | |
| **3** | 68.3 | 25.6 | 93.9 | 6.1 | 1.7 | 3.0 | 185.2 | 14.1 | | | |
| **4** | 62.1 | 31.7 | 93.7 | 6.3 | 14.4 | 13.2 | 244.1 | 27.7 | <7% | 2.9 | 16.6 |
| **4** | 62.5 | 31.0 | 93.5 | 6.5 | 2.7 | 4.0 | 187.7 | 15.9 | | | |
| **5** | 61.3 | 32.0 | 93.3 | 6.7 | 1.7 | 3.5 | 175.9 | 15.1 | | | |
| **3** | 61.9 | 31.3 | 93.2 | 6.8 | 1.4 | 2.7 | 183.1 | 14.9 | | | |
| **2** | 58.5 | 34.6 | 93.2 | 6.8 | 0.1 | 0.7 | 156.8 | 11.3 | | | |
| **3** | 65.9 | 26.8 | 92.7 | 7.3 | 4.1 | 4.4 | 189.3 | 17.9 | | | |
| **2** | 58.2 | 34.4 | 92.7 | 7.3 | 0.1 | 1.4 | 230.2 | 18.2 | <8% | 2.8 | 16.9 |
| **2** | 60.5 | 32.0 | 92.6 | 7.4 | 0.1 | 0.7 | 366.5 | 27.8 | | | |
| **2** | 59.9 | 31.7 | 91.6 | 8.4 | 0.4 | 1.3 | 121.3 | 11.3 | <9% | 2.8 | 17.0 |
| **2** | 58.2 | 33.4 | 91.6 | 8.4 | 0.3 | 1.2 | 299.3 | 26.3 | | | |
| **5** | 61.3 | 29.7 | 91.0 | 9.0 | 0.2 | 1.6 | 196.2 | 19.1 | | | |
| **2** | 57.9 | 33.0 | 90.8 | 9.2 | 0.0 | 1.5 | 180.3 | 17.9 | <10% | 2.9 | 17.5 |
| **2** | 57.4 | 33.0 | 90.4 | 9.6 | 16.1 | 12.5 | 292.7 | 39.4 | | | |
| **2** | 58.5 | 31.4 | 89.9 | 10.1 | 2.2 | 2.3 | 108.3 | 12.9 | <11% | 2.9 | 17.6 |
| **2** | 57.7 | 32.0 | 89.6 | 10.4 | 4.2 | 3.6 | 266.0 | 30.8 | | | |
| **All** | 61.5 | 33.0 | 94.4 | 5.6 | 1.7 | 2.9 | 268.7 | 17.7 | | | |

* Rotten nuts =  %rotten kernels and shells

**OBS 1:** Lot 1, Sample 2 and Lot 5 Sample 6 deleted as outliers **OBS 2:** Data sorted by % rotten nuts (kernels and shells

**Table 2.** Component mass contribution (% sample mass - kg) by component in all samples from 12 lots of Brazil nuts by category

| Lot | Good kernels (%) | Shells With kernel residue (%) | Shells without kernel residue (%) | Rotten kernels (%) | Rotten shells (%) | Good kernels aflatoxin (µg/kg) | All categories aflatoxin (µg/kg) | % rotten kernels (% mass) | Average aflatoxin in good kernels | Average aflatoxin in all categories |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 66.4 | 15.1 | 16.6 | 1.0 | 0.9 | 6.0 | 13.8 | | | |
| 1 | 66.8 | 16.7 | 13.4 | 1.2 | 1.9 | 3.9 | 18.2 | | | |
| 5 | 62.5 | 20.5 | 14.2 | 1.4 | 1.5 | 0.5 | 26.6 | | | |
| 1 | 65.5 | 20.5 | 11.0 | 1.4 | 1.5 | 0.3 | 20.5 | | | |
| 4 | 64.3 | 21.8 | 10.6 | 1.4 | 1.8 | 0.5 | 10.3 | | | |
| 10 | 52.8 | 20.7 | 22.7 | 1.5 | 2.4 | 0.2 | 7.3 | | | |
| 12 | 53.3 | 15.2 | 27.6 | 1.7 | 2.2 | 0.0 | 7.6 | <2% | 1.2 | 15.4 |
| 8 | 52.1 | 21.6 | 22.6 | 1.7 | 2.0 | 0.0 | 16.8 | | | |
| 11 | 53.7 | 23.3 | 19.2 | 1.7 | 2.1 | 0.0 | 4.7 | | | |
| 4 | 63.5 | 22.0 | 10.8 | 1.9 | 1.8 | 0.4 | 9.3 | | | |
| 7 | 51.3 | 23.1 | 21.6 | 1.9 | 2.3 | 0.0 | 28.0 | | | |
| 1 | 63.4 | 18.4 | 14.7 | 2.0 | 1.6 | 3.7 | 22.2 | | | |
| 1 | 66.4 | 14.0 | 15.3 | 2.0 | 2.3 | 0.2 | 14.4 | | | |
| 5 | 66.4 | 15.5 | 13.8 | 2.0 | 2.3 | 0.9 | 11.8 | <3% | 1.1 | 16.6 |
| 3 | 69.7 | 19.3 | 7.0 | 2.1 | 1.9 | 0.4 | 9.9 | | | |
| 5 | 63.0 | 18.9 | 13.7 | 2.1 | 2.2 | 0.4 | 17.3 | | | |
| 1 | 62.2 | 16.9 | 16.5 | 2.1 | 2.3 | 0.2 | 16.6 | | | |
| 6 | 55.7 | 24.1 | 15.9 | 2.1 | 2.2 | 2.1 | 21.0 | | | |
| 5 | 64.2 | 20.7 | 9.9 | 2.2 | 3.0 | 0.3 | 11.9 | | | |
| 5 | 62.9 | 21.9 | 10.5 | 2.3 | 2.4 | 1.7 | 13.6 | | | |
| 4 | 61.8 | 21.8 | 12.0 | 2.4 | 2.0 | 0.3 | 11.6 | | | |
| 9 | 52.6 | 22.9 | 19.5 | 2.4 | 2.6 | 0.2 | 21.5 | | | |
| 3 | 66.4 | 18.9 | 10.0 | 2.5 | 2.3 | 7.1 | 17.3 | | | |
| 5 | 62.3 | 19.3 | 13.2 | 2.5 | 2.7 | 0.2 | 12.6 | | | |
| 4 | 66.2 | 20.0 | 9.4 | 2.5 | 1.9 | 2.6 | 19.8 | | | |
| 1 | 65.6 | 18.2 | 10.9 | 2.6 | 2.7 | 0.6 | 25.7 | | | |
| 1 | 64.0 | 19.1 | 11.4 | 2.6 | 2.9 | 0.2 | 15.6 | | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 4 | 63.1 | 17.6 | 14.6 | 2.7 | 2.1 | 0.5 | 12.2 | | | |
| 3 | 64.8 | 15.0 | 14.3 | 2.7 | 3.3 | 0.3 | 10.3 | | | |
| 5 | 64.1 | 14.9 | 15.0 | 2.7 | 3.3 | 0.3 | 35.1 | | | |
| 4 | 60.6 | 24.8 | 9.3 | 2.7 | 2.6 | 0.2 | 9.5 | | | |
| 3 | 65.4 | 13.0 | 16.1 | 2.7 | 2.8 | 0.2 | 8.5 | | | |
| 3 | 67.3 | 17.8 | 9.0 | 2.8 | 3.1 | 2.6 | 25.2 | | | |
| 1 | 64.7 | 17.8 | 11.5 | 2.9 | 3.2 | 0.2 | 28.6 | | | |
| 4 | 60.6 | 20.6 | 12.9 | 2.9 | 3.0 | 1.2 | 20.1 | | | |
| 3 | 65.6 | 16.3 | 12.0 | 2.9 | 3.1 | 0.3 | 20.7 | | | |
| 4 | 62.1 | 17.1 | 14.6 | 3.1 | 3.2 | 14.4 | 27.7 | | | |
| 2 | 58.5 | 16.5 | 18.1 | 3.1 | 3.7 | 0.1 | 11.3 | | | |
| 5 | 61.3 | 18.7 | 13.2 | 3.3 | 3.5 | 1.7 | 15.1 | | | |
| 3 | 68.3 | 10.8 | 14.8 | 3.3 | 2.8 | 1.7 | 14.1 | | | |
| 4 | 62.5 | 20.4 | 10.6 | 3.3 | 3.2 | 2.7 | 15.9 | <4% | 1.4 | 16.7 |
| 3 | 61.9 | 17.3 | 14.1 | 3.3 | 3.4 | 1.4 | 14.9 | | | |
| 2 | 60.5 | 21.3 | 10.8 | 3.5 | 3.9 | 0.1 | 27.8 | | | |
| 3 | 65.9 | 14.2 | 12.7 | 3.5 | 3.8 | 4.1 | 17.9 | | | |
| 2 | 58.2 | 17.4 | 17.1 | 3.6 | 3.8 | 0.1 | 18.2 | | | |
| 2 | 59.9 | 17.7 | 14.0 | 3.9 | 4.5 | 0.4 | 11.3 | | | |
| 2 | 58.2 | 21.5 | 11.9 | 4.0 | 4.4 | 0.3 | 26.3 | | | |
| 5 | 61.3 | 14.9 | 14.8 | 4.3 | 4.7 | 0.2 | 19.1 | | | |
| 2 | 57.9 | 13.6 | 19.3 | 4.3 | 4.9 | 0.0 | 17.9 | <5% | 1.7 | 17.6 |
| 2 | 57.4 | 16.2 | 16.8 | 4.5 | 5.2 | 16.1 | 39.4 | | | |
| 2 | 58.5 | 21.7 | 9.6 | 4.8 | 5.3 | 2.2 | 12.9 | | | |
| 2 | 57.7 | 19.0 | 12.9 | 4.9 | 5.4 | 4.2 | 30.8 | | | |
| All | 61.5 | 18.7 | 14.3 | 2.7 | 2.9 | 1.7 | 17.7 | | | |

**OBS 1:** Lot 1, Sample 2 and Lot 5 Sample 6 deleted as outliers **OBS 2:** Data sorted by % Rotten kernels.