codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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Agenda Item 6 **CX/CF 10/4/6**
December 2009

JOINT FAO/WHO FOOD STANDARDS PROGRAMME
CODEX COMMITTEE ON CONTAMINANTS IN FOODS
4th Session
Izmir, Turkey, 26 – 30 April 2010

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

Codex Members and Observers wishing to submit comments at Step 3 on the above matter (**Annex I and Annex II to this document**), including possible implications for their economic interests, should do so in conformity with the *Uniform Procedure for the Elaboration of Codex Standards and Related Texts* (Codex Alimentarius Commission Procedural Manual) before **28 February 2010**. Comments should be directed:

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BACKGROUND

1. At the 3rd CCCF, the Delegation of Brazil introduced the discussion paper which considered the occurrence of aflatoxins in Brazil nuts, taking into account the evaluation performed by JECFA at its 68th session on the impact of different hypothetical limits of total aflatoxins (AFT) in tree nuts, including Brazil nuts, on dietary intake, the results of the Conforcast Project (2005-2009) submitted by the Brazilian Government and proposed maximum levels, as well as a sampling plan. As a result of these considerations, it was proposed to establish four categories of products and corresponding MLs: shelled Brazil nut ready-to-eat; shelled Brazil nut destined for further processing; in-shell Brazil nut ready-to-eat; in-shell Brazil nut for further processing.

2. The 3rd Session agreed to return the proposed draft document to Step 2 for redrafting by the Delegation of Brazil, to circulate for comments at Step 3 and consideration by the 4th Session of the Committee[1].

3. The present document was prepared by Brazil with contributions from the European Community, Sweden, United Kingdom and United States of America.

[1] ALINORM 09/32/41, paras 68 - 78

4. In preparation of the paper, views were expressed that the ML should be established on the basis of the intended use of the nuts (ready-to-eat or intended for further processing), with no distinction between shelled and in-shell nuts. Brazil would like to emphasize that Brazil nuts are a special case and continues to propose a separate ML for in-shell Brazil nut.

5. Furthermore, the use of rotten nut aflatoxin levels in the calculation of the processing factor (PF) was raised. While Brazil argues that consumers will never eat rotten nuts (it is dark and tastes awful), hence never be exposed to very high levels of aflatoxins, some countries are of the opinion that the burden of selecting good nuts should not be left to consumers.

6. It is important to point out the characteristics of Brazil nut. Many initiatives for implementing good collecting practices have been initiated in the country; however, a complete control of the collecting-production chain is impossible due to the characteristics of the Amazon forest. Additionally, currently, there is n o good procedure to sort good and bad in-shell Brazil nuts.

7. It has been pointed out that only a limited part of all Brazil nuts collected is put on the market as in-shell Brazil nut ready-to-eat. However, this market exists and its elimination would have an economic impact on the Amazonian population without bringing additional protection to consumers. Furthermore, the Conforcast project was performed with lots of in-shell Brazil nuts to be marketed.

8. The proposed draft maximum levels for total aflatoxins in Brazil nuts are presented in Annex I, the associated sampling plans in Annex II and background information to support the proposed levels in Annex III to this document.

ANNEX I

The following MLs of total aflatoxins in the Brazil nut commodities are recommended to be used in international trade:

Brazil nut commodity	**AFT ML, µg/kg**
Shelled, ready to eat	10
Shelled, destined for further processing	15
In-shell	20

Considering:

- The existence of international market for both shelled and in-shell Brazil nuts and the need for establishing MLs for both commodities to facilitate international trade;
- the actual technological stage of processing of in-shell Brazil nuts that does not permit the complete segregation of rotten nuts;
- the incidence data of aflatoxins in Brazil nuts evaluated in this paper, including the data reported by the Conforcast project for in-shell Brazil nuts, that showed the impact of rotten nut in the AFT contamination in in-shell Brazil nut.

ANNEX II

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). The aspects related to Brazil nuts that are different from the other nuts will be covered in 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” and “In-shell Brazil nut”, as defined bellow. As a result, maximum levels and sampling plans are proposed for all commercial types of Brazil nuts.

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

Shelled Brazil nuts 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 nuts: are in-shell nuts which can be directly marketed to the final consumer and 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 to the extractive characteristic of the nuts in the rainforest and the technological processing aspects. The results of the Conforcast project show that the segregation of the rotten nuts from the aggregate (analytical sample) before analysis could give an estimation of AFT contamination present in the good nuts, as discussed in the paragraphs 52-57.

AFLATOXIN TEST PROCEDURE AND MAXIMUM LEVELS

Shelled Brazil nuts destined for further processing

Maximum level – 15 µg/kg total aflatoxins

Number of laboratory samples – 1

Laboratory sample size - 20 kg

Sample preparation – samples (KERNELS) shall be finely ground and mixed thoroughly using a process, e.g, or slurry with turrax type mill, which has been demonstrated to provide the lowest sample preparation variance.

Test portion: 100 g test portion (slurry: 50 g kernels: 50 g water)

Decision rule – If the aflatoxin test result is less than or equal to 15 µg/kg 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.

Shelled Brazil nuts ready-to-eat:

Máximum level: 10 µg/kg total aflatoxins

Number of laboratory samples – 2

Laboratory sample size - 10 kg

Sample preparation – samples (KERNELS) shall be finely ground and mixed thoroughly using a process, e.g. slurry with turrax type mill, which has been demonstrated to provide the lowest sample preparation variance.

Test portion: 100 g test portion (slurry: 50 g kernels: 50 g water)

Decision rule – If the aflatoxin test result is less than or equal to 10ng/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 the Annex to these sampling plans.

In-shell Brazil nuts

Maximum level – 20 µg/kg total aflatoxins

Number of laboratory samples – 1

Laboratory sample size - 20 kg (in-shell Brazil nut)

Sample preparation – samples shall be finely ground and mixed thoroughly using a process, e.g. slurry with turrax type mill, which has been demonstrated to provide the lowest sample preparation variance.

Test portion: 100 g test portion (slurry: 50 g kernels: 50 g water)

In case of the "in-shell Brazil nuts", the test portion may be the whole nut, as sometimes the removal of the inedible part (shell) is not feasible. % ratio kernel:shell is 0.5.

Decision rule – If the aflatoxin test result is less than or equal 20 µg/kg 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.

Annex

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 (particle size, 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 associated with the sampling plan steps for shelled Brazil nut

Data used for variance analysis	Sampling variance	Sample preparation variance	Analytical variance	Total variance
Shelled Brazil Nut From Shelled Study Total aflatoxin, lots < 0.39 omitted, Kernel only 15 lots, 10 kg sample, 185 kernels/kg	$s_s^2 = 4.8616C^{1.889}$ R=0.80	$s_{ss}^2 = 0.0306C^{0.632}$ R = 0.24	Experimental $s_a^2 = 0.0164C^{1.117}$ R = 0.43 FAPAS $s_a^2 = 0.0484C^{2.0}$	$s_t^2 = 5.464C^{1.850}$ 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 15µg/kg 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.

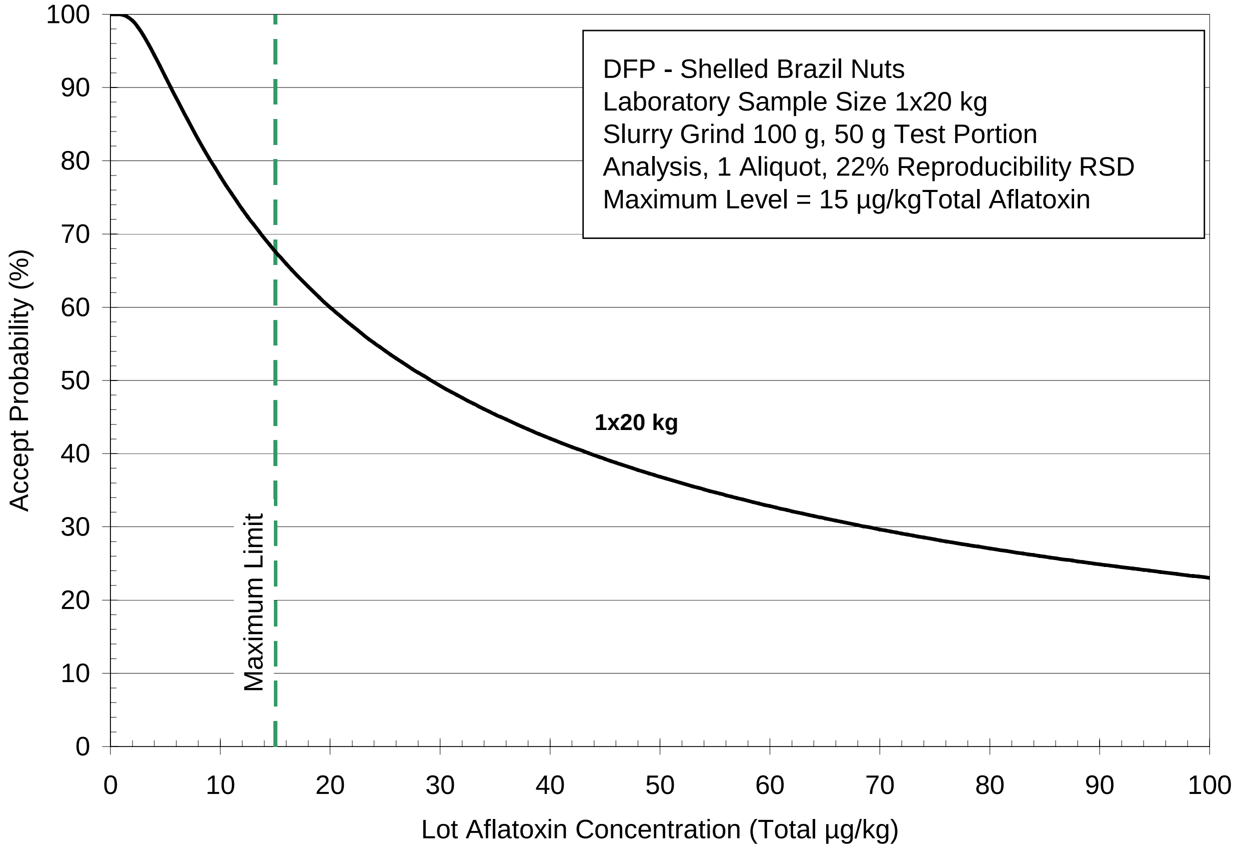

Figure 1. Operation curves for shelled Brazil nuts destined for further processing (DFP) at a maximum limit of 15µg/kg

Operating Characteristic curves 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 10 µg/kg 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.

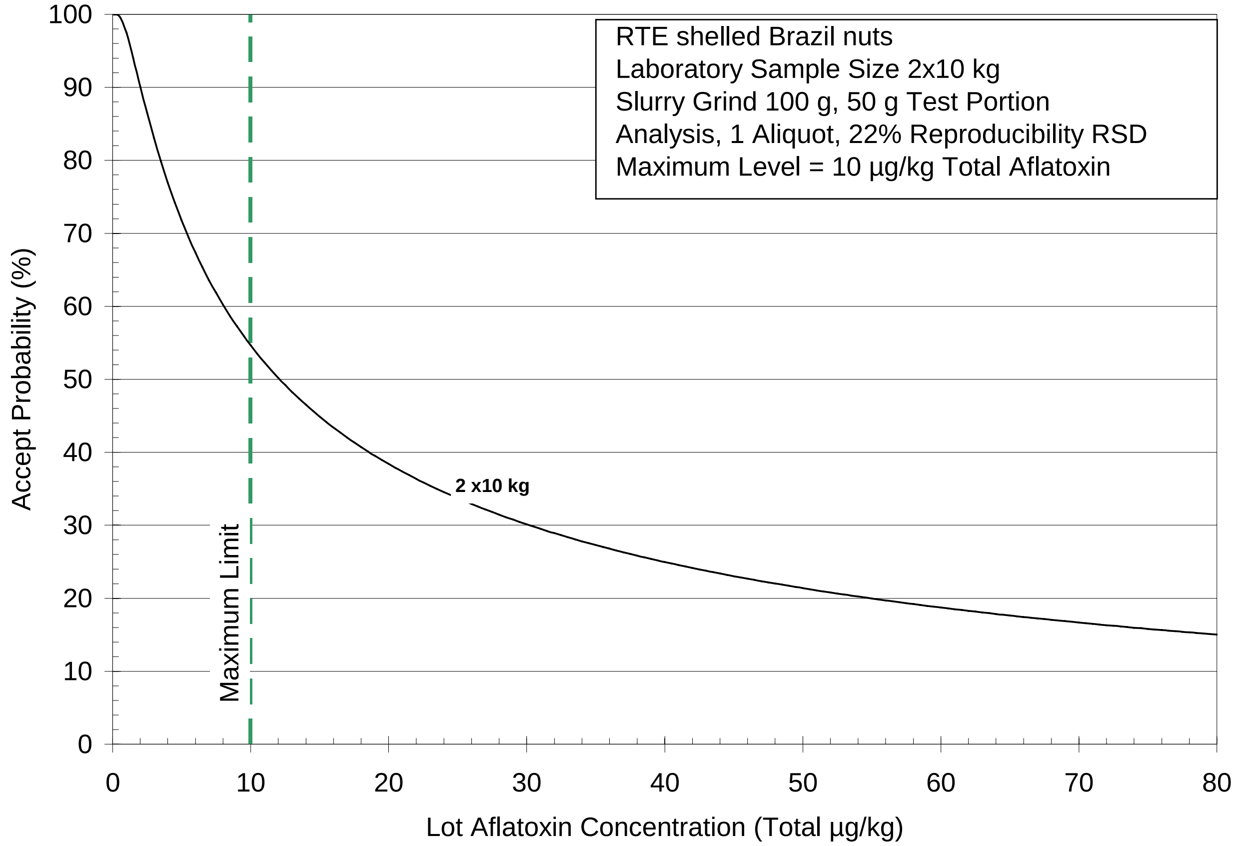

Figure 2. Operation curve for shelled Brazil nuts ready-to-eat (RTE) at a maximum limit of 10 µg/kg

In-shell Brazil nuts

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

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

Data used for variance analysis	Sampling variance	Sample preparation variance	Analytical variance	Total variance
In-shell Brazil Nut (Shell Mass Corrected) From Inshell Study Total aflatoxin All (Good & Rotten) Kernels 10 kg kernel (20 kg in-shell Brazil nut)	$s_s^2 = 0.797C^{1.898}$ *calculated from total	$s_{ss}^2 = 0.0306C^{0.632}$ R = 0.24	experimental $s_a^2 = 0.0164C^{1.117}$ R = 0.43 FAPAS $s_a^2 = 0.0484C^{2.0}$	$s_t^2 = 0.8219C^{1.8913}$ R = 0.88

In-shell Brazil nut operating characteristic curve

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 20µg/kg for total aflatoxins is shown on Figure 3. The operating characteristic curves reflect uncertainty associated with a 20 kg laboratory sample of in-shell Brazil 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.

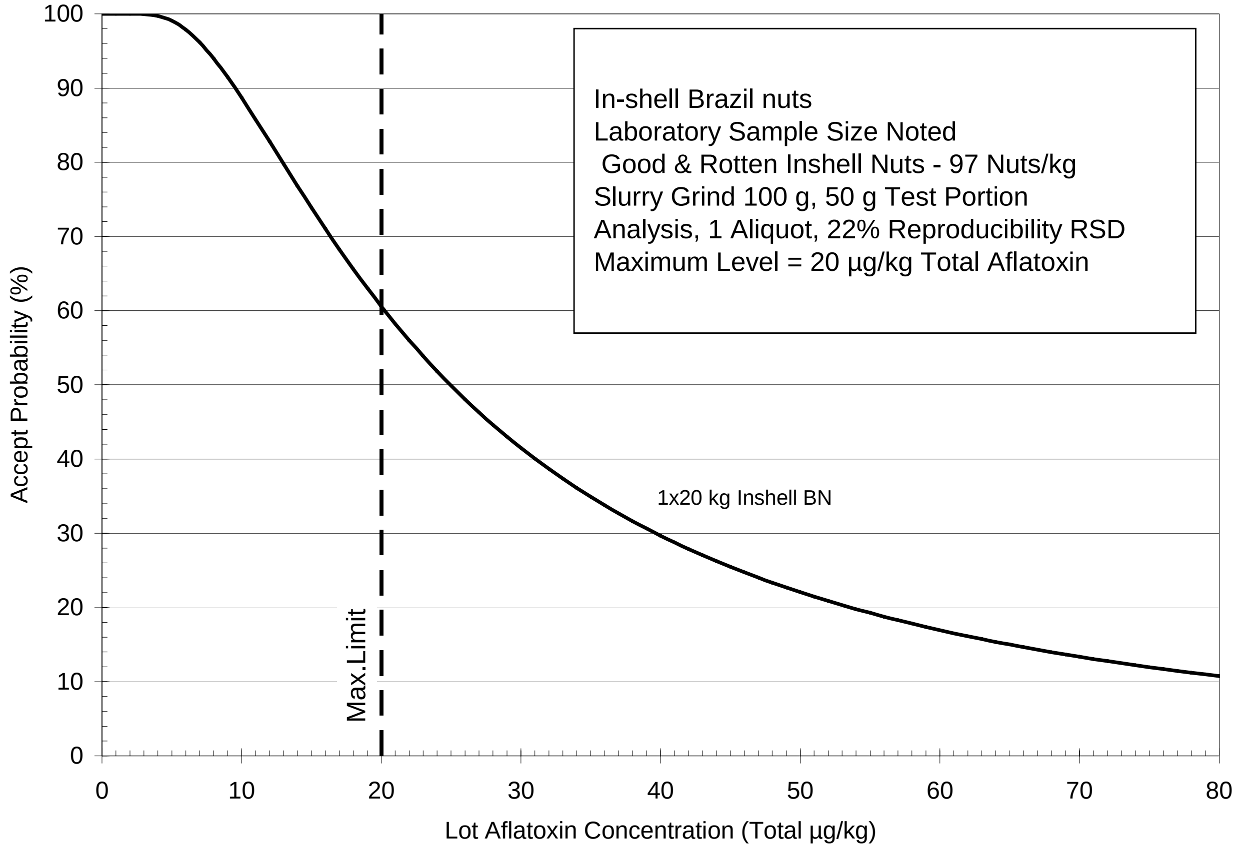

Figure 3. Operation curves for in-shell Brazil nuts at a maximum limit of 20µg/kg

ANNEX III

Background information

INTRODUCTION

1. Aflatoxin (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 primary handling of forest products that are intended for national or international trade (CAC/RCP 59-2005).

2. The Brazil nuts are the seeds of *Bertholletia excelsa* Humb. & Bonpl, 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).

3. 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%.

4. 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 threatens the ecological balance and the environment. On the contrary, the *Bertholletia Excelsa* trees are essential to the rain forest as they help to maintain the equilibrium in the relationship between flora and fauna.

5. The number of collectors and processors involved in 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, 64 % of which came from Bolivia, 24 % from Brazil and 12 % from Peru (INC, 2007).

6. A Code of Practice for the Prevention and Reduction of AFT Contamination in Tree Nuts was adopted by the CAC at its 28th Session (CAC, 2001); an Appendix specifically for Brazil nuts was adopted by the CAC at its 29th Session. This Appendix is being revised taking into account the results of the SAFENUT Project (STDF, 2009). Information provided by the International Nut and Dried Fruit Foundation (INC) 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

7. Processing/treatment that has been proven to reduce aflatoxin levels in Brazil nuts includes shelling or sorting by size, specific gravity, colour and visual defects. 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 for both ready-to-eat (RTE) and destined for further processing (DFP) and in-shell; each of these commodities is defined as following:

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

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

<u>In-shell Brazil nut</u>: are in-shell nuts that can be directly marketed to the final consumer or that are intended to undergo sorting and/or shelling process to reduce aflatoxin levels before being marketed to the final consumer.

8. The conditions of production of Brazil nuts cannot be controlled as in the case of other nuts, and therefore the distinction between shelled and in-shell nuts requires further consideration.

9. This Paper considers many aspects related to the aflatoxins in Brazil nuts, including occurrence,

dietary intake estimation and maximum levels. In addition to the information provided before, this revised version includes information from the SAFENUT Project (STDF, 2009) and additional data from the CONFORCAST project. A sampling plan for total aflatoxins in Brazil Nuts is proposed in Annex I and II.

TOXICOLOGICAL EVALUATION

10. 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.

11. 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 of liver cancer (FAO/WHO, 1998).

ANALYTICAL METHODS

12. 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 sample 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).

13. 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 these normally are within the 0.1 to 1 µg/kg range (Marklinder et al., 2005, Sobolev, 2007).

14. 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).

15. 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).

16. 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

17. 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).

18. 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. Baymam *et al.* (2002) have shown that effects of surface sterilization in Brazil nuts were minimal: only *A. nidulans* decreased significantly. Unlike the other nuts, most inoculum (including most *A. flavus*) was internal in Brazil nuts.

19. *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)

20. 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 the 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.

21. 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 (4,483 µg/kg) and AFT (6,817 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.

22. 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.

23. The SAFENUT Project, conducted in the Brazilian states of Para and Acre, found that the aflatoxin producing fungi infect the Brazil nuts early in the forest, just after the pods fall from the tree (STDF, 2009). The critical control point in the nut production is the drying step undertaken by extractivist community, which is not always effective bringing the nuts to a safe moisture level (corresponding to water activity less than 0.70). The moisture content can reach the optimal range for aflatoxin production resulting in an increase of aflatoxin content during storage. Hence, the Brazil nut arrives at the processing plants contaminated with aflatoxins and the sorting processes applied at this stage are not effective at removing the contaminated in-shell nuts. Effective sorting can only be achieved after the nuts are shelled.

24. According to Johnsson et al (2008), the aflatoxigenic fungal growth and aflatoxin production increases rapidly between 40-90 days after collection of nuts and before arrival at the processing plant for final drying. In 2009, when the SafeNut project was finalised[2] and substantially more data collected, it was demonstrated that the practices currently implemented in the Brazil nut production chain, which are based on the recommendations of the existing guidelines on good practices, were not effective for reducing aflatoxins. It was concluded that within 10 days after collection, the unshelled Brazil nuts must be dried to a safe moisture level (water activity of the nuts to less than 0.7) in the extractivist communities or transported and dried in the processing industries. After 40 days there is a 20% probability to exceed 10 µg/kg AFT and a 15% probability to exceed 20 µg/kg AFT (Olsen et al, 2009).

[2] http://www.standardsfacility.org/files/Project_documents/Project_Grants/STDF_114_Final_report_Website.pdf

OCCURRENCE OF AFS IN BRAZIL NUTS

25. 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 20µg/kg, and 6 % had levels above 20µg/kg. The maximum level detected was 619µg/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).

26. 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 regulatory limit at that time of 4µg/kg for AFT (Food Standards Agency, 2004).

27. 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. AFB_1 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).

28. 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 AFB_1 (< 0.6 or 1 µg/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).

29. 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 95th 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.

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

31. 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 (200 nuts were selected for each group from a lot of 65 kg collected in a Brazil nut factory in the state of Amazonas, Brazil). Samples from the small size nut group presented a mean AFB_1 level of 5.62 *µ*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.

32. 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). Marklinder et al (2005) have shown that in most cases, AFT levels were lower in shells than in kernels from the same sample.

33. Olsen et al. (2008) have shown that the ratio 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. Furthermore, they isolated and identified *A. nomius*, which is a producer of B and G aflatoxins, and suggested that this species may be an important producer of aflatoxins in Brazil nuts.

34. 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%.

35. Brazil nut samples collected within the STDF project (*data not published*) contained similar % ratio of AFB_1/AFT. When considering only the positive samples (75 % of the 569 samples analysed), AFB1 represented over 55% of AFT levels in about 52 % of the samples.

36. 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. Of the 622 Brazil nuts samples analysed, 56.4% had AFT levels below the LOD (0.1 – 0.2 µg/kg), 22% between the LOD and 4 µg/kg, 2.4 % between 4 and 10 µg/kg and 19.1 % above 10 µg/kg (EFSA, 2007, 2009). It was not clear in the report whether the samples analysed where shelled or in-shell Brazil nuts or included both types of samples.

37. The Brazilian Government – Ministry of Agriculture - submitted the results of the Conforcast Project, presented in a workshop in Belém, Pará, Brazil, from 10^{th} to 12^{th} 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) would 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 nuts (4 to 8 tons) were sampled according to unbalanced sampling designs.

38. 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 Commission Regulation (EC) No. 401/2006.

39. Annex I (Figure 1) shows the unbalanced experimental nested design for shelled Brazil nuts; 25 samples of 200 kg were taken from each of the 25 lots sampled. From each sample, 20 sub-samples of 10 kg were taken for analysis.

40. Table 2 shows the summary of the results obtained in the 500 sub-samples of shelled Brazil nuts analyzed for AFT within the Conforcast Project (from Figure 1). In most sub-samples (67.8 %), the levels of aflatoxins were below 0.39 µg/kg AFT and 4 % of them had levels higher than 10µg/kg. The mean and median levels were 2.07 and 0.17 µg/kg, respectively, with the highest level at 100.9µg/kg.

Table 2. Distribution (%) of 500 sub-samples of shelled Brazil nuts from 25 lots classified by discrete range of contamination of AFT.

	Range of total aflatoxin concentration (AFT)*, 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
	96					4				

*LOD = 0.11 µg/kg (MAPA, 2008)

41. Annex I (Figure 2) shows the diagram of the sampling design protocol for in-shell Brazil nuts. Thirteen (13) lots were sampled and four hundred (400) kg were taken from each lot. From each of the 13 lots, 10 sub-samples of 40 kg were taken, the nuts were shelled and, after visual inspection by trained personnel, segregated into groups of good kernels and respective shells (with kernel residue and without kernel residue) and damaged rotten kernels and respective shells. Damaged rotten kernels were segregated as if they could be easily identified by consumers. For 5 lots, this process resulted in 20 sub-samples of good kernels (~10kg), 10 sub-samples of respective shells (~20.kg), 10 sub-samples of damaged rotten kernels and 10 sub-samples of respective shells (three sub-samples were excluded later due to technical problems) of variable mass. For each of the remaining 7 lots, this process resulted in 20 sub-samples of good kernels (~10 kg) and 1 sub-sample of respective shells (~20 kg) (with kernel residue and without kernel residue), 10 sub-samples of damaged rotten kernels of variable sample mass and 10 sub-samples of respective shells of variable mass (Conforcast project).

42. The mean proportion of shell: kernel ratio (kg/kg) of the Brazil nut was estimated by shelling and weighting individual kernels and shells from 100 nuts obtained from the 13 lots sampled within the Conforcast Project. Kernel and shell contributed, in average, 50% each of the Brazil nut weight, confirming studies conducted since 1999 by the Ministry of Agriculture Laboratory.

43. Table 3 shows the mass of nut (kg) and the level of AFT obtained in the 54 sub-samples, obtained according to the design described in Figure 2. Although the total mass (kg) of good nuts (shell and good kernels) was almost 25 times that of rotten nuts (rotten shells and kernels), the contribution of AFT in rotten nuts to the total AFT mass (76.6%) was over 3 times higher than that for good nuts (23.4%). All shell and all kernels categories contributed each with approximately 50% of the total nut mass (kg) (as previously found) and AFT mass (µg).

44. Mean AFT concentration in rotten nuts (302.3µg/kg) was 77 times higher than what was found in good nuts (3.92 µg/kg) (Table 3). All defects, which included rotten kernels and all shells (55.3% of total mass, kg), contributed about 92.2 % of the AFT mass (µg). Similar results were reported by Whitaker et al. (1998) that found that a small mass of defects (18%) contributed to 93% of aflatoxin mass in peanuts.

45. The data presented on Table 3 clearly show that aflatoxins are found in both shell and kernel parts of the Brazil nut. The levels found in good kernels represent about 57 % of that found in the shells from good nuts.

Table 3. Sample mass (kg), aflatoxin mass (µg), and aflatoxin concentration (µg/kg) in all samples of in shell Brazil nuts by category – 54 samples analysed.

Grade Category	Mass (kg)	Mass (% of total)	AFT Mass (µg)	AFT Mass (% of total)	AFT[1] Concentration (µg/kg)
Good nuts (kernel & shell)	1,987.15	95.94	7,787.86	23.44	3.92
Good kernels	926.37	44.72	2,582.11	7.77	2.79
Shells from good nuts[2]	1,060.78	51.21	5,205.75	15.67	4.91
Rotten nuts (kernel & shell)	84.13	4.06	25,433.79	76.56	302.32
Rotten kernels	40.35	1.95	14,322.39	43.11	354.95
Shells from rotten nuts	43.78	2.11	11,111.40	33.45	253.80
All defects (rotten kernels & all shells)	1,144.91	55.28	30,639.55	92.23	26.76
All kernels (good and rotten)	966.72	46.67	16,904.50	50.88	17.49
All shells (good and rotten)	1,104.56	53.33	16,317.15	49.12	14.77
All Categories[3]	**2,071.28**	**100.00**	**33,221.66**	**100.00**	**16.04**

[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 (Annex 1. Figure 2); for the AFT concentration, the mean was taken. [3] all nut fractions (shells and kernels)

46. Table 4 shows the distribution of AFT levels found in the 54 sub-samples of in-shell Brazil nuts. When all nuts are considered (good and rotten kernels and respective shells), 79.7 % and 42.7 % of the sub-samples had AFT above 10 µg/kg and 15 µg/kg, respectively, meaning that a large percentage of Brazil nuts would be non-compliant if these limits are applied to in-shell nuts. If a limit of 20 µg/kg and 30 µg/kg is

applied, 22.3% and 5.6% of the samples would be non-compliant. Removing the rotten nuts drastically reduces the percentage of non-compliant samples to 1.9%, 3.8% and 5.7% for levels of 20µg/kg, 15µg/kg, and 10µg/kg, respectively. If only good nuts are considered (kernels and respective shells), the percentages of non-compliant samples would be 5.6% and 1.9% respectively for levels of 10 and 15 µg/kg. Only 4% of good kernels sub-samples had aflatoxin levels above 15µg/kg. The mean and median AFT contaminations determined for all nuts were 15.3µg/kg and 11.7µg/kg, respectively with the highest contamination of 102.6µg/kg. The distribution found of all nuts is more close to those reported by EFSA (2007, 2009).

Table 4. Distribution (%)[1] of 54 sub-samples of in-shell Brazil nuts* classified by discrete range of contamination

AFT distribution (%)	**Range of total aflatoxin concentration**, µg/kg**						
in categories	**≤5**	**>5 ≤10**	**> 10 ≤15**	**> 15 ≤20**	**> 20 ≤30**	**> 30 ≤50**	**>50**
All nuts (%)	1.9	18.5	37.0	20.4	16.7	3.7	1.9
Cumulative distribution (%) ≥ 10			79.7				
Cumulative distribution (%) ≥15				42.7			
Cumulative distribution (%) ≥20					22.3		
Cumulative distribution (%) ≥30						5.6	
Only good nuts (%)	85.2	9.3	3.7	0.0	0.0	1.9	0.0
Cumulative distribution (%) ≥ 10			5.6				
Cumulative distribution (%) ≥ 15				1.9			
Cumulative distribution (%) ≥ 20					1.9		
Cumulative distribution (%) ≥ 30						1.9	
Only good kernels (%)	90.7	3.7	1.9	1.9	0.0	0.0	1.9
Cumulative distribution (%) ≥ 10			5.7				
Cumulative distribution (%) ≥ 15				3.8			
Cumulative distribution (%) ≥ 20					1.9		
Cumulative distribution (%) ≥ 30						1.9	
All kernels*** (%)	0.0	20.4	31.5	22.2	13.0	11.1	1.9
Cumulative distribution (%) ≥ 10			79.7				
Cumulative distribution (%) ≥ 15				48.2			
Cumulative distribution (%) ≥ 20					26.0		
Cumulative distribution (%) ≥ 30						13.0	

[1] Conforcast Project; *Nuts = good and/or rotten kernels and respective shells. **LOD = 0.11 µg/kg (MAPA, 2008); *** from good and rotten nuts

47. The possibility to predict the aflatoxin concentration in in-shell Brazil nuts sampled from a lot was also investigated in the Conforcast project. The best correlation was found between aflatoxin concentration in in-shell Brazil nut and the aflatoxin concentration in all defects (R^2=0.93; regression equation: $C = 0.5188M + 0.9591$ where C is the aflatoxin concentration in in-shell Brazil nut and M is the aflatoxin concentration in all defects). The correlation between the AFT concentration and aflatoxin mass found in all defects and rotten nuts were also high (R^2=0.90 and 0.88, respectively) (Table 5). These correlations show that AFT levels in

Brazil nuts are strongly related to the presence of rotten kernels and shells.

Table 5. Correlation from a regression between the AFT concentration in in-shell Brazil nut sample in a lot (µg/kg) and various parameters, where C is the aflatoxin concentration in in-shell brazil nut and M is the parameter listed below (x axis).

Parameter	**Regression equation**	**R^2**	**R**
Aflatoxin mass, rotten nuts (µg)	C = 0.0287M + 2.9245	0.88	0.94
Aflatoxin concentration, all defects (µg/kg)	C = 0.5188M + 0.9591	0.93	0.96
Aflatoxin mass, all defects (µg)	C = 0.0296M - 0.3059	0.90	0.95
Aflatoxin mass, rotten kernels (µg)	C = 0.0314M + 6.6494	0.51	0.7
Aflatoxin concentration, rotten nuts (µg/kg)	C = 0.024M + 7.9767	0.35	0.59
Aflatoxin concentration, rotten kernels (µg/kg)	C = 0.0227M + 6.8246	0.32	0.57
Mass of rotten nut (% of total mass)	C = 1.4754M + 8.5754	0.17	0.41
Mass of rotten kernels (% of total mass)	C = 3.0948M + 8.5362	0.17	0.41
Mass of all defects (% of total mass)	C = -0.3648M + 24.753	0.01	0.12
Mass of rotten nut (% of total mass)	C = -0.0523M + 3.7695	0.002	0.04

48. In many countries, when an in-shell Brazil nut lot enters the market, the nuts are sampled and analysed as such, without any type of further processing. The results from the Conforcast project show that the levels found in the in-shell nuts should not be used to assess the human exposure to aflatoxins from the consumption of Brazil nut, as the shells and the rotten kernels, which are not eaten, can be highly contaminated. In the laboratory, good in-shell Brazil nuts can be segregated from the rotten nuts by visual inspection after cutting the nut in half. This procedure is routinely performed by the exporters when evaluating the nuts coming from the producers and when sorting a lot to be exported as ready-to-eat.

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 (raw commodity, that contains the inedible portion) is to estimate the processing factor (PF), a common procedure accepted by JMPR/CCPR (CX/PR/07/39/8). 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. This variability found on PFs is especially true in the case of aflatoxins in Brazil nut, as the environmental conditions from harvest to market highly influence the levels of fungal contamination and of aflatoxin in the kernel and the shell. For Brazil nut, the processing procedures of interest are shelling and sorting. Visual sorting after shelling can be performed at the processing plant or by the consumers.

50. A processing factor of aflatoxins in Brazil nuts can be estimated by dividing the concentration of aflatoxin found in good kernels (edible fraction, as the consumer does not eat shells or rotten kernels) by the level found in in-shell Brazil nut. Using the data shown on Table 3, the estimated PF was 0.17 (2.79µg/kg/16.04 µg/kg). Individual results from the 54 sub-samples of in-shell Brazil nuts gave PFs ranging from 0.0 to 0.62, with median of 0.03. This processing factor can be used to evaluate the safety of the regulatory levels of aflatoxin in in-shell Brazil nut. It is recognized that the estimated PF reflects the situation found within the Conforcast project, where Brazil nut lots contained, in average, 4 % of rotten nuts in mass. In lots with a higher or lower percentage of rotten nuts, PF will be lower or higher, respectively.

51. Figure 1 shows the impact of PF on the levels of AFT in each of the 54 sub-samples analyzed within the Conforcast Project.

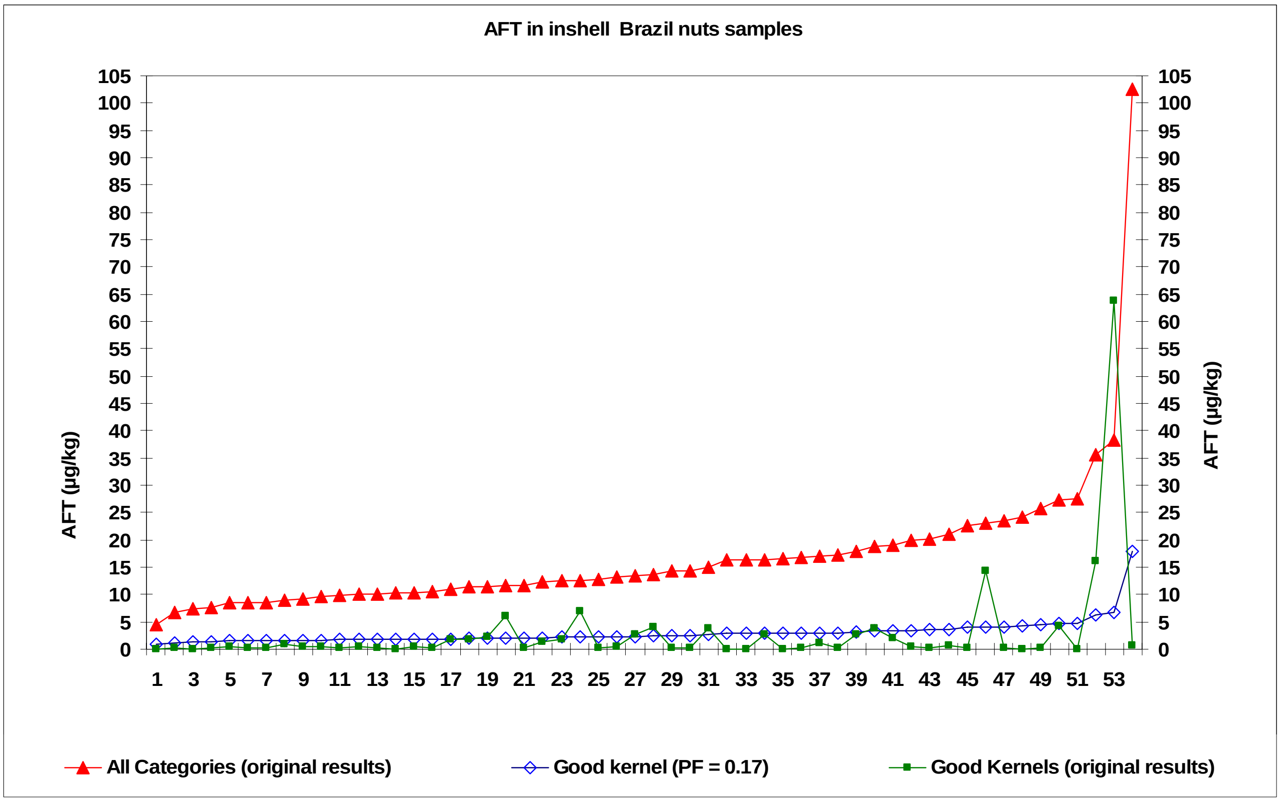

Figure 1. Impact of the use of processing factor PF in the AFT contamination in in-shell Brazil nuts samples.

52. 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 the edible portion of Brazil nut at the time of consumption. Hence, the consumer can achieve the ALOP when shelling and sorting the Brazil nut.

DIETARY INTAKE

53. 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).

54. 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 20µg/kg AFB_1 to 10µg/kg AFB_1 would not result in any observable difference in rates of liver cancer (FAO/WHO, 1998).

55. The dietary intake of AFs by the Swedish population was estimated to be 0.6 and 0.7ng/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 70kg b.w consumer and consumption of Brazil nuts of 0.3 kg during Christmas time, the median AFs intake was 0.73ng/kg bw and the 95^{th} percentile 110ng/kg bw. Smoothing the consumption over a full year, the figures would be 0.002 and 0.3ng/kg bw (Marklinder et al., 2005).

56. 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.

57. 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 is 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.

58. 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.

59. 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).

60. If fully enforced, a ML at 20µg/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.

61. 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).

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

63. The JECFA concluded that enforcing an ML of 15, 10, 8 or 4µg/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 20µg/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 20µg/kg for Brazil nuts was 11 %. This value increased to 17 % for a ML of 4µg/kg. However, according to Conforcast project the enforcement of a ML at 20µg/kg for in-shell Brazil nuts would imply of having 22.3% of Brazilian production out of the international market.

64. 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. In a more recent document, the Scientific Panel concluded that public health would not be adversely affected by increasing the levels for total aflatoxins from 4 µg/kg to 10 µg/kg for all tree nuts (EFSA-Q-2009-00675)

CONCLUSIONS & RECOMMENDATIONS:

65. The present Paper on AFs in Brazil Nuts leads to the following conclusions and recommendations for consideration at the 4th Session of the CCCF:

I) Brazil nuts production represents an important economic and social 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. Unlike for other tree nuts, the extractivist characteristics of the Brazil nut limit a high increase in production and consumption, even if there is an increase in demand by the market.

III) The JECFA has concluded that enforcing a maximum limit (ML) of 15, 10, 8 or 4µg/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 20µg/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, with 96% of the samples containing up to 10µg/kg.

V) Results from the Conforcast project have shown that the levels of AFT in in-shell Brazil nuts ready to be exported were higher than 20, 15 and 10 µg/kg in 22.3, 42.7 and 79.7 % of the sub-samples analyzed, respectively. This means that a high percentage of Brazil nuts in-shell would be out of market if any of these limits were applied to in-shell Brazil nuts and would have a great economic and social impact on the Amazonian population that survives on the Brazil nut extractivism activity

VI. The Conforcast project has shown that the removal of rotten nuts reduces drastically the percentage of samples out of the market to 1.9%, 3.8% and 5.7% for levels of 20 µg/kg, 15 µg/kg, and 10 µg/kg , respectively.

VII. The processing factor of 0.17 determined in this paper (paragraph 63) can be applied to estimate the impact of the ML recommendation for in-shell Brazil nut on dietary intake of aflatoxins from the consumption of the edible part of the in-shell Brazil nut. At a ML of 20µg/kg and 30µg/kg, the consumers would be exposed to AFT level of 3.5µg/kg and 5.2µg/kg in good kernels, respectively. According to the JECFA evaluation, it is unlikely that at these MLs (especially considering the aflatoxins level in the edible part) the dietary AFT exposure will be affected.

VIII) 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. Rotten (inedible, highly contaminated) kernels have a yellowish to dark colour.

IX) The practices for the prevention and reduction of aflatoxin in Brazil nuts are essential for bringing the aflatoxin level to as low as possible. For this purpose, the Code of Practice for Brazil Nuts is being updated under Agenda Item 7 (CX/CF 10/4/4).

X) Sampling plans to support the recommended limits are included in Annex II above.

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ANNEX

Sampling design protocols

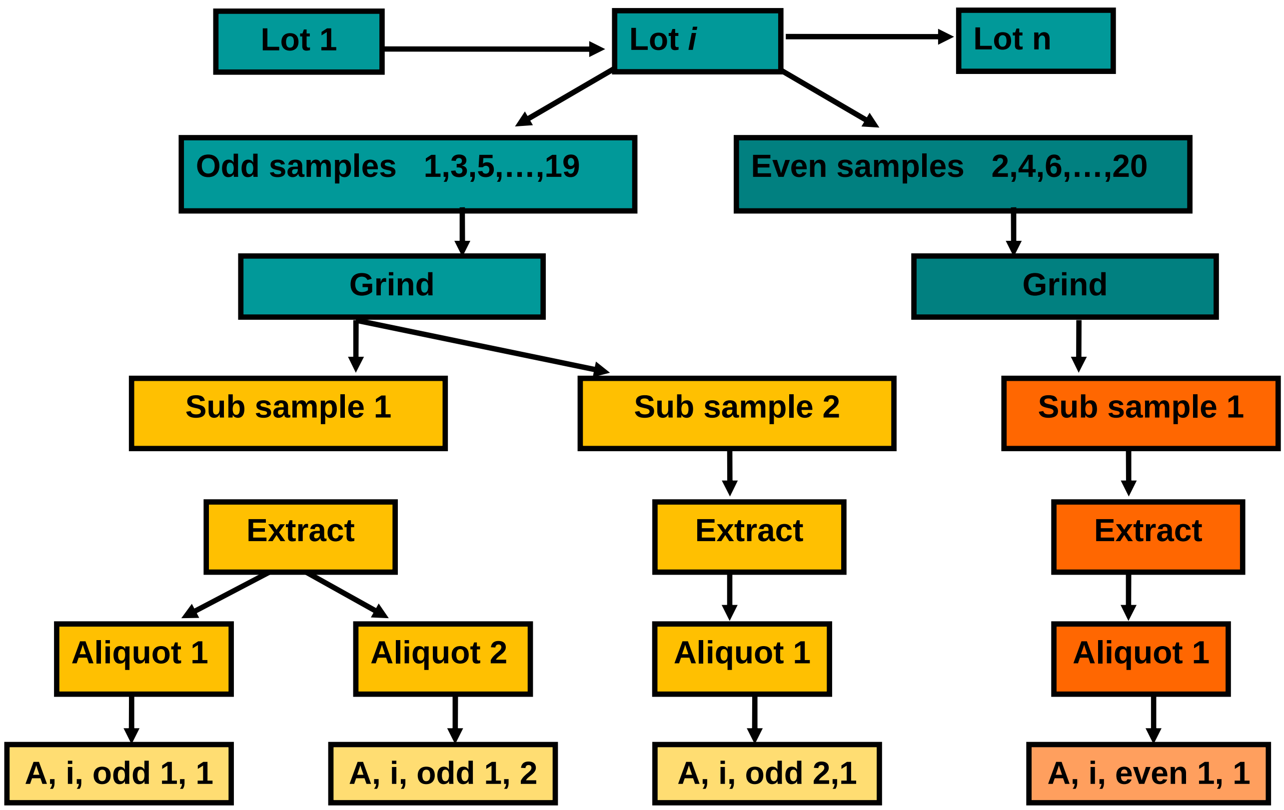

Figure 1: Unbalanced Experimental Nested Design for Shelled Brazil nuts; i = lot number = 1, 2, 3, ...25; j = sample number = 1, 2, 3, …20; k = subsample number = 1 or 2; l = aliquot number = 1 or 2; $A_{i,j,k,l}$ = aflatoxin concentration for the ith lot, jth sample, kth subsample, and lth aliquot.

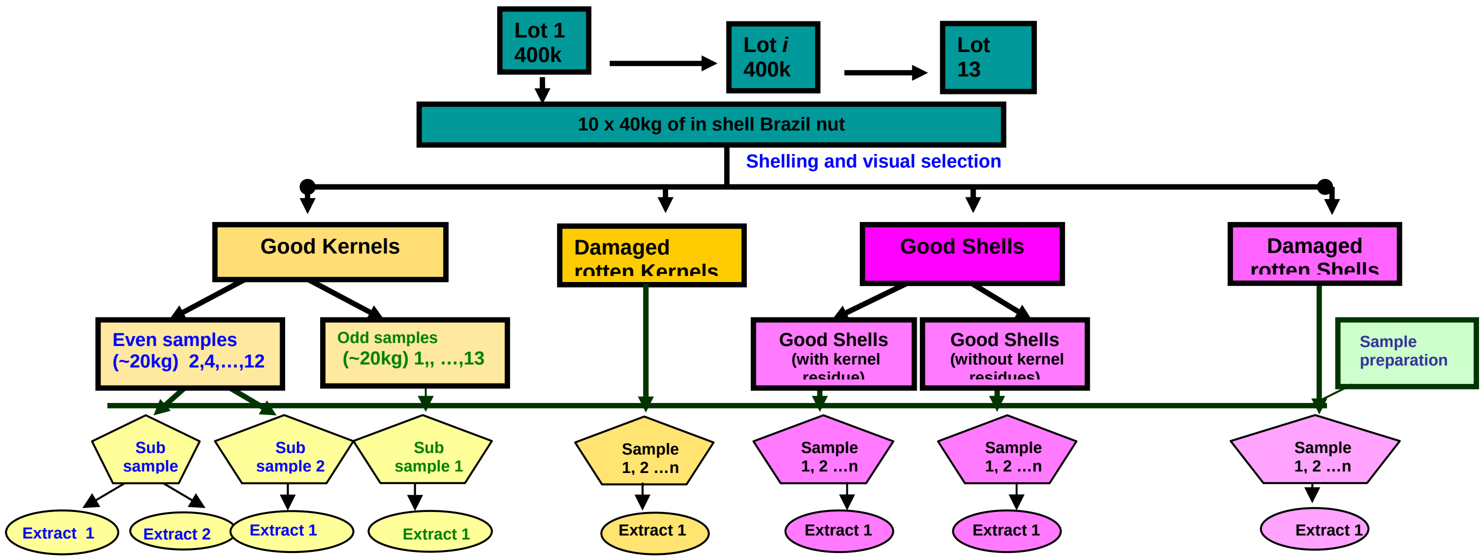

Figure 2. Sampling design protocol for in-shell Brazil nut