CODEX ALIMENTARIUS COMMISSION E

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World Health Organization

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CODEX COMMITTEE ON CONTAMINANTS IN FOODS

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DISCUSSION PAPER ON OCHRATOXIN A IN COCOA

BACKGROUND

1. The Codex Committee on Food Additives and Contaminants (CCFAC) at its 38th Session in The Hague (2006) agreed to develop a discussion paper on Ochratoxin A (OTA) in cocoa[1]. An electronic Working Group (eWG) chaired by Ghana presented a discussion paper (CX/CF/07/1/18) on OTA in cocoa in Beijing at its 1st Codex Committee on Contaminants in Foods (CCCF)[2]. This discussion paper was updated and presented at the 2nd CCCF in The Hague as CX/CF/08/2/15, but after some deliberation the Committee suspended consideration of OTA in cocoa due to the need to generate new data[3].

2. At the 4th Session of CCCF, the Delegation of Brazil informed the Committee of a new study carried out in Brazil that could form the basis for the development of a code of practice to reduce or prevent OTA in cocoa. The Committee agreed that a eWG led by Ghana and co-chaired by Brazil, would prepare a discussion paper on OTA in cocoa to assess whether a code of practice should be developed.[4]

3. At the 5th Session of CCCF, the eWG presented an updated discussion paper (CX/CF 11/43/12) highlighting the recommendations of the eWG concerning the future possible development of a code of practice for the prevention/reduction of OTA contamination in cocoa taking into account the knowledge currently available.

4. The 5th Session of CCCF re-established the electronic Working Group led by Ghana to update the discussion paper taking into consideration new available data.[5] As agreed by the CCCF, the eWG prepared the revised discussion paper by incorporating new data with a view to developing a code of practice for consideration at the 6th Session of CCCF. This discussion paper is accompanied by a draft project document proposing new work (as presented in Annex I to this document) and a possible outline of the proposed draft Code of Practice (Annex II). A list of the participants in the eWG is presented in Annex III to this document.

INTRODUCTION

5. Ochratoxin A is a mycotoxin that occurs naturally worldwide in food commodities such as cereals and cereal products, pulses, coffee, beer, grape juice, dry vine fruits and wine as well as cocoa products, nuts and spices (EFSA, 2006). In cocoa, OTA is mostly associated with cocoa bean shells and fat-free cocoa solids (cocoa powder) (Amezqueta et al., 2004; Bastide et al., 2006). Both fungi and OTA can be present in all stages of the production chain: harvest (manual and breaking of pods), fermentation (box fermentation or on-farm fermentation on banana leaves), drying (solar or mechanical), storage (in jute bags), food elaboration and transport (COCOQUAL, 2007; FAO/WHO/UNEP, 1999).

6. The term “cocoa” is derived from the plant *Theobroma cacao* L. belonging to the family *Malvaceae*. The tree originated from the Amazon and other tropical areas of South and Central America and is grown in a 20° belt north and south of the equator. The mean minimum and maximum temperatures in most cocoa growing regions are 18°C and 32°C. A high rainfall of 1000-4000 mm/year is required.

7. The term ‘cocoa’ is used in reference to the beans of commerce and derived products whereas ‘cacao’ is restricted to the cacao tree and its parts, although both terms are used interchangeably in a few places.

8. Cocoa is a dried fermented fruit product. The cocoa beans are not eaten as such; they undergo industrial conversion before consumption. Cocoa is a very important ingredient in pharmaceuticals and several kinds of foods, such as cakes, biscuits, chocolate confectionery, chocolate spread, cocoa drink, infant foods, ice creams and sweets (Tafuri et al, 2004).

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1 ALINORM 05/28/12, paras. 229-230 and ALINORM 06/29/12 para. 145.
2 ALINORM 07/30/41, para. 113.
3 ALINORM 08/31/41, paras. 169-170.
4 ALINORM 10/33/41, para. 115.
5 REP11/CF para. 75.

9. During industrial processing of cocoa, the first steps are cleaning, roasting and mechanical removal of the shell fraction from the nibs. The process of removing shells is not 100% efficient; up to about 2% of the total cocoa nib weight can be due to the presence of shell and germ that has not been possible to remove during the manufacturing process (CODEX STAN141-1983, Rev. 1, 2001). The nib is milled into cocoa mass/liquor for further processing.

10. Around 68% of the world supply of cocoa beans comes from West Africa, especially Cote d'Ivoire, Ghana and Nigeria. Cocoa is also produced in Asia and Latin America (Table 1). Being a crop produced by smallholders, cocoa is a valuable non-perishable cash crop for hundreds of thousands of farmers in the cocoa producing countries, and it is also of great importance to the economies of these countries. Most of the cocoa beans are exported to Europe and North America to be made into cocoa liquor, cocoa butter and cocoa cake, which will be processed into cocoa powder and chocolate (Table 2)(ICCO, 2007).

Table 1. World Production of cocoa beans (2008 – 2010) (thousand tonnes)

Country	**2008/09**		**2009/2010 Estimates**		**2010/2011 Forecast**	
Africa	**2519.4**	**69.9%**	**2482.5**	**68.4%**	**3100.2**	**73.9%**
Cameroon	226.6		205.0		215.0	
Cote d'Ivoire	1223.2		1242.3		1470.0	
Ghana	662.4		632.0		1010.0	
Nigeria	250.0		235.0		240.0	
Others	157.2		168.2		165.2	
America	**485.4**	**13.5%**	**516.7**	**14.2%**	**536.1**	**12.8%**
Brazil	157.0		161.2		195.0	
Ecuador	135.0		149.8		140.0	
Others	193.4		205.7		200.1	
Asia & Oceania	**597.7**	**16.6%**	**632.8**	**17.4%**	**559.0**	**13.3%**
Indonesia	490.0		550.0		470.0	
Papua New Guinea	59.4		38.7		45.0	
Others	48.3		44.1		44.0	
World Total	**3602.5**		**3632.0**		**4195.3**	

(Reference: ICCO Quarterly Bulletin of Cocoa Statistics. Vol. XXXVII No. 3, Cocoa Year 2010/2011)

Note: Totals may differ from sum of constituents due to rounding off.

Table 2. World Consumption/Grindings of cocoa beans (2008-2011) (thousand tonnes)

(Reference: ICCO Quarterly Bulletin of Cocoa Statistics. Vol. XXXVII No. 3, Cocoa Year 2010/2011)

	2008/2009		2009/2010 Estimates		2010/2011 Forecast	
Europe	**1445.8**	**41.3%**	**1494.0**	**40.4%**	**1561.4**	**40.8%**
Germany	341.7		361.1		410.0	
France	154.4		145.0		155.0	
Italy	58.3		63.2		65.0	
Netherlands	460.0		500.0		530.0	
Spain	90.9		87.0		90.0	
United Kingdom	110.0		110.0		70.0	
Others	104.2		105.0		110.0	
Africa	**621.7**	**17.8%**	**684.5**	**18.5%**	**650.9**	**17.0%**
Cameroon	24.0		26.9		27.5	
Cote d'Ivoire	418.6		411.4		340.0	
Ghana	133.1		212.2		250.0	
Nigeria	34.0		25.0		25.0	
Others	12.0		9.0		8.4	
America	**780.4**	**22.3%**	**815.3**	**22.1%**	**846.5**	**22.1%**
Brazil	216.1		226.1		235.0	
Canada	55.4		59.2		62.0	
United States	360.7		381.9		395.0	
Others	148.2		148.1		154.5	
Asia & Oceania	**649.3**	**18.56%**	**704.2**	**19.0%**	**769.1**	**20.1%**
Indonesia	120.0		130.0		180.0	
Malaysia	278.2		298.1		300.0	
Singapore	79.5		83.0		85.0	
Turkey	51.8		65.0		65.0	
Others	119.0		128.1		139.1	
World Totals	**3497.3**		**3698.0**		**3827.9**	

CHEMICAL STRUCTURE

11. OTA(7-(L-β-phenylalanyl-carbonyl)-carboxyl-5-chloro-8-hydroxy-3,4-dihydro-3R-methyl isocumarin) (Figure 1) is a secondary metabolite produced by certain species of *Aspergillus* and *Penicillium* (Pittet and Royer, 2002), which may be present in foodstuff even when visible mould is not seen. OTA is a colourless crystalline compound that is soluble in polar organic solvents and dilute sodium bicarbonate solution and slightly soluble in water (Scott, 1996).

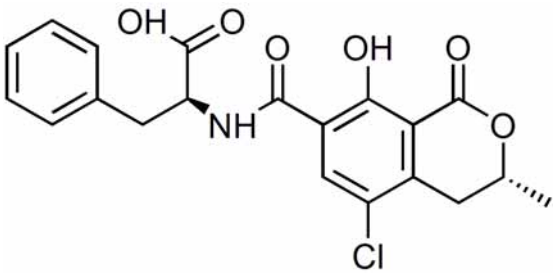

Figure 1. Chemical structure of OTA

12. The mammalian enzyme carboxypeptidase A has the ability to cleave OTA into non-toxic products (ochratoxin alpha and phenylalanine) (Stander et al, 2001).

13. OTA keeps its stability during most food processing stages such as cooking, washing and fermenting and can be detected in manufactured food products (Bakker and Pieters, 2002). Boudra et al (1995) has shown that a maximum of 20% of OTA in wheat was decomposed by dry heat at 100°C for 160 min or 150°C for 32 min. During roasting of cocoa, the final bean temperature reaches 100 – 120°C and the duration is 15-70 min (Minifie, 1982), therefore roasting is not expected to significantly reduce OTA levels.

TOXICOLOGICAL EVALUATION

14. OTA is classified as a possible human carcinogen (group 2B) (CAC, 1998; IARC, 1993) and has been reported to be nephrotoxic, immunosuppressive, carcinogenic and teratogenic in animal studies (JECFA, 1995; JECFA, 2001; O'Brien and Dietrich, 2005; Tsubouchi et al, 1995). OTA is thought to be related to the Balkan Endemic Nephropathy, Chronic Interstitial Nephropathy (in North Africa), and urothelial tumours in humans (O'Brien and Dietrich, 2005). Based on epidemiological associations, the hypothesis linking OTA exposure early in life and testicular cancer has been put forward (Schwartz, 2002). Previous National Toxicology Program (NTP) studies in the United States showed that OTA can induce renal tumours in rodents at high doses (Boorman, 1989).

15. OTA was analyzed in human blood samples in Cote d'Ivoire between 1998 and 2004 (Sangare-Tigore et al, 2006). The results showed that 22 out of 63 healthy participants had OTA blood levels of 0.01 – 5.81 µg/L, with a mean of 0.83 µg/L; the levels found in 8 out of 39 nephropathy patients undergoing dialysis were 0.167 – 2.42 µg/L, with a mean of 1.05 µg/L.

16. According to the Opinion of the Scientific Panel on Contaminants in the Food Chain from EFSA (European Food Safety Authority) (EFSA, 2006), the site-specific renal toxicity of OTA as well as the DNA damage and genotoxic effects, found in various *in vivo* and *in vitro* studies, are most likely attributable to cellular oxidative damage, with no evidence for the existence of OTA-DNA adducts. On the basis of the lowest observed adverse effect level (LOAEL) of 8 µg/kg bw/day for early markers of renal toxicity in pigs, and an uncertainty factor of 450 for the extrapolation of experimental data derived from animals to humans as well as for intra-species variability, a TWI (Tolerable Weekly Intake) of 120 ng/kg bw was derived for OTA. In 2010, the EFSA addressed the possible co-exposure to ochratoxin A and aristolochic acid of the human population in areas previously identified as having a higher prevalence of Balkan Endemic Nephropathy, but found no reasons to change the conclusions of its previous opinion (EFSA, 2010).

17. At its 68th Meeting, the JECFA reconsidered the PTWI of 100 ng/kg bw in the light of new data and found no reason to change the previous outcome (JECFA, 2007).

SAMPLING

18. Spanjer et al. (2006) indicated that the sample homogenization process is an important factor in OTA determinations in various food matrices. Depending on the type of milling procedure, which ultimately determines the particle size distribution, the amount of OTA that is measured could vary. The relevance of this finding is that sampling plans that are not suitably designed could lead to lots being wrongly rejected or accepted.

19. Sampling procedures and performance criteria for the methods of analysis for mycotoxins in foodstuff have been provided for by the EU Commission Regulation 401/2006 (EC 401/2006, 2006), There are no specific sampling procedures for analysis of OTA in cocoa and cocoa products.

ANALYTICAL METHODS

20. A rapid antibody-based assay involving sequential clean-up and visual detection of OTA in cocoa powder has been described (Lobeau et al., 2007). The screening test has a cut-off level of 2.0 µg/kg and is suitable for use in the field.

21. The validated method for OTA quantification uses reversed-phase HPLC with fluorescence detection (HPLC/FLD) after clean-up by immunoaffinity column (Brera et al. 2003). An inter-laboratory study aimed at assessing the performances of 18 laboratories for OTA determination in cocoa powder samples using this method has been performed (Brera et al., 2005). Satisfactory results were obtained from 10/18, 11/18 and 12/18 participants, at low level (0.19 µg/kg), median level (0.45 µg/kg), and high level (1.45 µg/kg), respectively.

22. Copetti (2009) validated an analytical method for cocoa beans using immunoaffinity column for clean up and HPLC/FLD at levels of 0.49, 1.96 and 9.80 µg/kg. Recovery rates were from 97.5 to 80.0% and the limit of detection was 0.01 µg/kg. Turcotte and Scott (2010) also validated a similar method for cocoa powder and chocolate, with a LOQ of 0.08 ng/g, recovery from 94 - 79% and coefficient of variation < 5%.

23. When a high number of samples have to be screened for OTA production, rapid, inexpensive and easy-to-perform methods are desirable, especially in low-income countries in which surveillance is less available because of economical and technological constraints (Murphy et al., 2006). However the data interpretation must be done carefully and in some case complementary analysis should be performed.

PRIMARY PROCESSING OF COCOA

24. The primary processing of cocoa beans consists of two major steps, namely fermentation and drying. Different methods of fermentation and drying are followed in the cocoa growing countries. Cocoa fermentation begins immediately after the beans embedded in mucilaginous pulp are removed from the pods. The beans and associated pulp are subject to microbial fermentation. Fermentation of cocoa beans depends on production methods, batch sizes, pod ripeness and storage, and environmental conditions.

25. After removal of the beans from the pods, the first step in cocoa processing is a spontaneous 4 to 7-days fermentation of beans with pulp in heaps, boxes, baskets, or trays, and lately propylene bags and black plastic sheeting.

26. Moisture content of fermented cocoa beans is between 55 and 60% (Zahouli *et al.,* 2010). During fermentation the temperature of the beans rises from ambient to about 50-55^{o}C due to exothermic oxidative reactions. Guehi *et al.* 2010, studied the effect of turning beans and fermentation method on the acidity and physical quality of raw cocoa beans. In this study, fermentation trials were conducted in wooden boxes, plastic boxes and in heaps with or without turning. Cocoa fermented in boxes during 4 days without mixing had pH values above 5.0 while cocoa fermented in heaps had pH 4.92. For fermentation with turnings, beans treated in wooden boxes were less acidic than beans fermented in plastic boxes, which recorded pH 4.75. Cocoa obtained from all fermentation methods and fermented for 5 days without mixing showed pH above 5. Cocoa fermented in plastic boxes with turnings became acidic with pH 4.73 while beans fermented in heaps were not acidic. All the beans showed no sign of insect damages and negligible levels of internal molding whatever the turning and the methods of fermentation.

27. Cocoa fermentation occurs largely on the mucilaginous pulp on the outer surface of the cocoa bean. A microbial succession of yeasts, lactic acid bacteria (LAB), and acetic acid bacteria (AAB) occurs during cocoa bean mass fermentation. The starting low pH, together with low levels of oxygen, favour the colonization by yeasts which assimilate citric acid, depectinize and liquefy the pulp and convert sucrose, glucose and fructose present in the pulp to ethanol. LAB ferments sugars and citric acid to lactic acid, acetic acid and mannitol causing a further rise in pH and favouring growth of AAB; AAB, now in an aerobic environment, grow and convert ethanol into acetic acid. The acetic acid permeates and dissolves the internal membranes of the cocoa seeds leading to mixing of cell components and triggering further reactions within the bean leading to degradation of polyphenols and production of metabolites which serve as flavour precursors for chocolate production (Camu et al. 2007)

28. After fermentation, the beans are dried immediately to avoid over fermentation, which could lead to product deterioration. Drying is usually carried out by sun drying and artificial hot air techniques. Smallholders prefer sun drying while in larger plantations the hot air (artificial) method is preferred (Hii *et al.,* 2009). Drying is usually terminated when the dried beans' moisture content reaches 7.5% (wet basis).

29. Storage conditions for cocoa beans in the tropics are generally not optimal, mainly because of high humidity, and therefore storage periods are restricted to at most three months unless special precautions are taken. Dried cocoa beans can absorb moisture if the humidity is high. If the moisture content rises above 8%, mould may develop inside the bean. At 8% moisture content, cocoa beans are in equilibrium with the ambient relative humidity (about 70% and normal temperatures in the tropics). Where the relative humidity exceeds this level for prolonged periods there is danger of internal mould development. The dried cocoa beans are usually put into open-weaved clean jute sacks or appropriate bags and stored. Bagged cocoa is stored in specially constructed buildings with the objective of keeping the moisture content of the beans sufficiently low and within acceptable limit. After drying, the cocoa beans are sorted and packed into appropriate bags and stored. The cocoa bags are usually made of non-toxic materials, preferably food grade hydrocarbon-free bags, which do not attract insects and rodents and are sufficiently strong to resist storage for longer periods. The bagged cocoa beans are placed in storage sheds that are weatherproof, well aerated, and free from damp and insect pests and away from smoke and other smells that would contaminate the cocoa. Bagged cocoa when well stored can keep for periods between 9 and 12 months.

30. Prior to shipment, every consignment of cocoa is fumigated; and the empty container or ships' holds are disinfected prior to stuffing or loading. The consignment is usually accompanied by appropriate documentation.

OCCURRENCE OF OTA AND OTA PRODUCING FUNGI IN COCOA BEANS

31. Several efforts had been made to isolate and identify OTA producing moulds from cocoa beans. In a study conducted in Ghana to assess moldiness in cocoa for a period of one year, 58 fungi species were isolated and identified. These included 26 species of *Aspergillus*, some of them potentially toxigenic (*A. niger*, *A. ochraceus* and *A. flavus*), 5 species of *Penicillium* and 8 species of *Fusarium* (Appiah, 2001). The percentage of ochratoxigenic fungi was not shown.

32. In another study, none of the 66 *Aspergillus* strains isolated during fermentation and drying of cocoa beans from Ghana was able to produce OTA. A total of 13 *Aspergillus* strains from Cote d'Ivoire, 16 from Nigeria and 86 from Ghana were screened for OTA production and only two ochratoxigenic producing A*spergilli* were found (COCOQUAL, 2007).

33. In a study carried out in Ghana (Abrokwa and Sackey, 2010), three types of cocoa fermentation were undertaken at three ecological locations using pods classified as healthy, diseased, diseased and damaged and damaged/broken. Drying of fermented beans were done under different regimes including standard open air, and extended short day drying to simulate improper or rain affected drying. Several fungal species were isolated during the fermenting and drying stages with some species appearing only during the drying stage. Results showed that all samples were positive for the presence of ochratoxigenic and spoilage fungi. The following fungi were isolated: *Aspergillus niger, A. flavus, A. sulphureus, A. ochraceus Trichoderma viride, Fusarium solani, Rhizopus stolonifer* and *Candida albicans*. Only *A. niger* and *A. ochraceus* have the potential to form OTA. Several of the samples were positive for OTA at all the three ecological stations but the levels were generally very low ranging from 0.00 to 0.57 ppb (µg/kg).

34. A study on the incidence of ochratoxigenic fungi and OTA in cocoa was carried out in Brazil during the period of 2006 to 2008 (Copetti et al., 2010). A total of 222 samples of cocoa collected at different processing stages included: samples before fermentation (25), fermentation (51), drying (81) and storage (65). In this study, 271 fungi belonging to potentially ochratoxigenic *Aspergillus* species were isolated and identified as *A. carbonarius, A. niger* aggregate*, A. ochraceus, A. melleus* and *A. westerdijkiae.* Before fermentation, no species capable of producing OTA was found in cocoa pods, either healthy or damaged. During fermentation, only a few isolates belonging to *Aspergillus niger* aggregate were found and the greatest diversity and numbers of species capable of producing OTA were found during sun drying. During storage, an increase in the occurrence of *Aspergillus niger* aggregate and *A. carbonarius* was observed*. Aspergillus niger* aggregate was the most common species isolated with the potential to produce OTA. However, only ten (5.2%) of the 191 isolates were able to produce OTA on YES agar. On the other hand, all 92 isolates of *A. carbonarius* and 10 isolates from *Aspergillus* section *Circumdati* (6 *A. melleus, 2 A. ochraceus* and 2 *A. westerdijkiae*) were able to produce OTA (Table 3). This study concluded that *A. carbonarius* is the main source of OTA in cocoa, although other ochratoxigenic species isolated may also contribute.

Table 3. Isolation frequency of ochratoxigenic species and incidence of infected cocoa beans at different processing stages (Copetti et al., 2010).

	Fermentation (51 samples)		Drying (81 samples)		Storage (65 samples)	
	IF (%)	RI (%)	IF (%)	RI (%)	IF (%)	RI (%)
Aspergillus carbonarius	1.96	0–3	3.70	0–24	7.81	0–66
A. niger aggregate	3.92	0–9	14.8	0–48	26.15	0–51
A. ochraceus	0	0	2.47	0–3	0	0
A. melleus	0	0	2.47	0–6	3.13	0–3
A. westerdijkiae	0	0	2.47	0–6	0	0

[a] IF = isolation frequency % (number of samples contained a fungal species/ total of samples evaluated, %); RI = range of infection % (range of infected beans in a sample, %).

35. None of the 25 samples taken before the commencement of fermentation contained OTA. Fourteen (27%) samples from fermentation contained OTA, although most samples were close to the limit of detection of the method (0.01 µg/kg). Only three samples had levels higher than 0.10 µg/kg, with a maximum of 1.70 µg/kg. After fermentation, at the sun drying stage, OTA was detected in 51% of the samples, and most (73%) of the samples had levels lower than 0.10 µg/kg. Only one sample contained 5.54 µg/kg. In storage, both the number of OTA positive samples and the level of contamination were similar to results found during drying (Table 4). Of the 222 samples analyzed, only two had OTA values above 2 µg/kg (Copetti et al., 2010).

Table 4. OTA contamination in cocoa beans at different processing stages (Copetti et al., 2010).

Stage/number of samples evaluated		OTA>LOD n (%)	OTA>2 µg/kg n (%)	OTA (µg/kg)		
				Max.	Median	Mean
Before fermentation	25	0 (0%)	0 (0%)	<0.01	<0.01	<0.01
Fermentation	51	14 (27%)	0 (0%)	1.70	<0.01	0.05
Sun drying	81	41 (51%)	1 (1%)	5.54	0.01	0.13
Storage	65	33 (52%)	1 (2%)	4.64	0.02	0.10

[a] Limit of detection (LOD): 0.01 µg/kg; method mean recovery: 90.8%.

36. Mounjouenpou et al. (2008) assessed how filamentous fungi and toxigenesis were affected by the types of cocoa post-harvest treatments (boxes or heaps). *Aspergillus carbonarius* was the main OTA-producing strain isolated, and very low levels of OTA in unfermented and fermented beans from healthy pods. Filamentous fungi were more abundant at the end of the harvesting season. Factors affecting bean integrity (poor handling, deferred processing) resulted in a qualitative and quantitative increase in contamination, when the total number of filamentous fungi could reach a maximum value of 5.5±1.4×107 CFU/g and black Aspergilli a maximum value of $1.42\pm2.2\times10^7$ CFU/g. Fermented dried cocoa from poor quality pods was the most contaminated by OTA: up to 48 ng/g.

37. Gilmour and Lindblom (2008) also found higher OTA levels in beans from damaged pods after 5 days of pod storage, contamination that began on the first day of fermentation, with a higher contamination levels in the middle of the heap. Three days into the fermentation, the trend was reversed and contamination was clearly greater at the edges. This reversal was accompanied by considerable mold growth on the surface of the heap. Five days into the fermentation, the OTA content increased further. Only traces of OTA were found after fermentation and drying of beans from healthy pods stored for 5 days. After 4 weeks of pod storage the OTA levels were low and there was only a small difference between the levels in beans from healthy and damaged pods. OTA levels in the moldy (~ 7 ng/g), insect damaged (~ 4 ng/g) and mummified (~ 3 ng/g) pods were substantially less than that found in the physically damaged pods (~20 ng/g), but generally greater than that found in the control un-damaged pods (~ 2 ng/g).

38. In a study conducted by Ratters and Matissek (2006), a total of 8 visibly healthy cocoa pods from the growing regions of Dominican Republic (year of cultivation 1999) and Ghana (year of cultivation 2000) and 7 damaged or moldy cocoa pods from Ghana cultivated in 2001 were separated into pulp and beans. OTA was not detected in any cocoa pod, beans or pulp samples analyzed (LOD of 0.02 µg/kg). The authors also showed that the ripening phase of cocoa pods from the tree up to being harvested was not a critical step for the generation of OTA.

39. Amezqueta et al. (2004) analyzed OTA in 46 cocoa bean samples of different origins and batches. A total of 63% of the samples were contaminated (LOD of 0.04 µg/kg), with levels from 0.04 to 14.8 µg/kg, mean and median of 1.71 and 1.12 µg/kg respectively.

40. In a study in Cote d'Ivoire, cocoa arriving at the ports of Abidjan and San Pedro were evaluated for OTA contamination. Samples of dried cocoa beans were taken for analysis according to the Commission Regulation (EC) No 401/2006. Out of 150 samples tested in Abidjan, 23 had OTA levels >2.0 µg/kg, and 10 out of 150 samples collected in San Pedro had levels>2.0 µg/kg (Dembele, 2009).

41. A screening of Nigerian ready for sale cocoa beans indicated that less than 90% of the 59 samples tested were positive for OTA, with concentrations ranging between 1.0 and 277.5 µg/kg (Dongo et al., 2008). An indirect competitive ELISA, much less sensitive than the HPLC method was used for the determination.

42. The European industry has analyzed samples of imported cocoa beans from different origins since 1999 (Figure 2). The results show that OTA contaminated cocoa beans are found in all cocoa producing regions (Gilmour and Lindblom, 2008). Additional data on the incidence of OTA in cocoa beans from various producing countries is shown in Table 5.

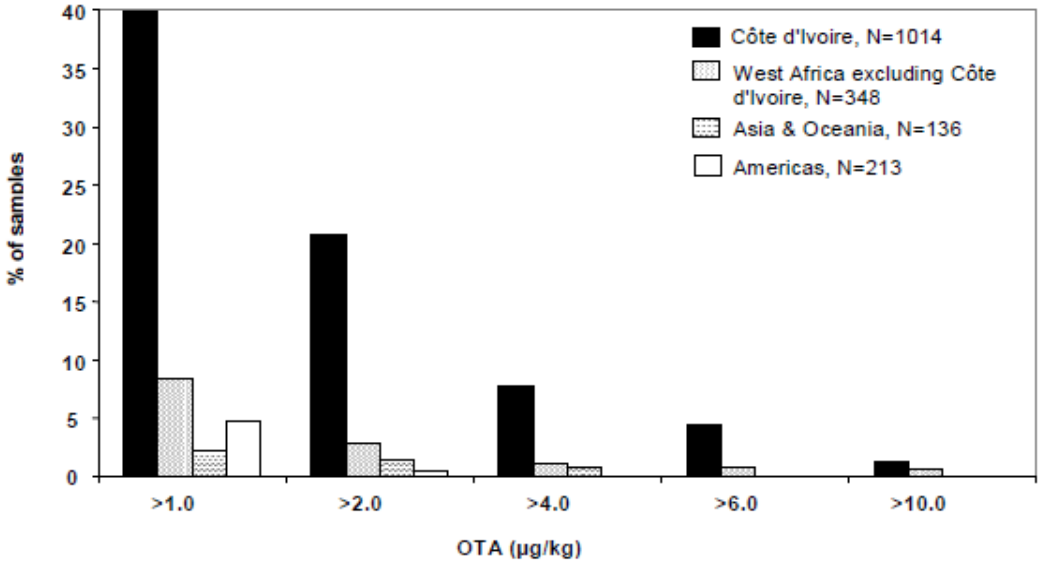

Figure 2 - OTA levels in cocoa beans imported into Europe from different regions of theworld1999-2005 (Gilmour and Lindblom, 2008).

Table 5. Additional data on occurrence of OTA in cocoa beans from various producing countries

Origin	Year	Number of samples			%	References
		Total	>LOQ	>2 µg/kg	>2 µg/kg	
Abidjan	2005	147		23	16	Dembele et al., 2009
San Pedro	2005	151		10	7	Dembele et al., 2009
Cote d'Ivoire		33	24	5	15	Amazqueta et al., 2004
Cameroon		7	3	1	14	Amazqueta et al., 2004
Equitorial Guinea		6	2	0	0	Amazqueta et al., 2004
Africa		21	16	1	5	Bonvehi, 2004
Brazil	2006-2008	222	88	2	1	Copetti et al., 2010

EFFECTS OF PROCESSING ON OTA LEVELS IN PRODUCTS

43. Cocoa beans must undergo industrial conversion before consumption. During this industrial processing, the a_w is < 0.8, which is too low for OTA production. The first steps in processing are roasting and removal of the shell (Gilmour and Lindblom, 2008).

44. OTA was analyzed in 15 pairs of cocoa shell and nib samples taken at the same time from industrial winnowers (Gilmour and Lindblom, 2008). Based on the results for the shell and nib fraction the OTA content in the beans used for processing was calculated. The calculated OTA content in the whole beans was between 0.3 and 3.0 ng/g. An average of 48% (range 25-72%) of the OTA in the beans was removed with the shell fraction.

45. In a study where cocoa shells were removed by hand, Amezqueta et al., (2005) observed a reduction in OTA content by >95% in 14/22 samples, 65-95% in 6/22 samples and only one sample showed a reduction of less than 50%.

46. In a study conducted by Bonvehi (2004), the highest levels of OTA were detected in roasted cocoa shells (mean value 111 µg/kg) followed by cocoa cake (mean value 2.79 1 µg/kg). Only minor levels were found in the other cocoa products.

47. The nibs are milled to form cocoa mass/liquor, a viscous liquid containing ~ 50% fat. The cocoa mass/liquor can be mixed with other ingredients to produce chocolate or it can be “pressed” to produce cocoa butter and cocoa powder. After pressing all of the OTA originally present in the nibs is recovered in the cocoa powder. This result is expected since cocoa powder is a concentrated cocoa solids fraction. OTA has not been found in the cocoa butter fraction (Gilmour and Lindblom, 2008).

48. Sixteen large samples of dried cocoa beans, specially stored under conditions which favored mould growth for 4 months, were processed into cocoa butter and chocolate to determine the effect of processing on OTA content of contaminated beans. The shells were removed by hand. Out of the 16 samples processed, levels of OTA varied between 3.37 and 46.15 µg/kg, with an average of 24.0 µg/kg. Shells of unroasted beans were the most heavily contaminated, with a mean value of 91.0 µg/kg. Chocolates contained 1.86 µg/kg on the average, and butter was free of OTA (see Figure3). On the average about 70% of the OTA was removed with the shell fraction (Dembele et al. 2009).

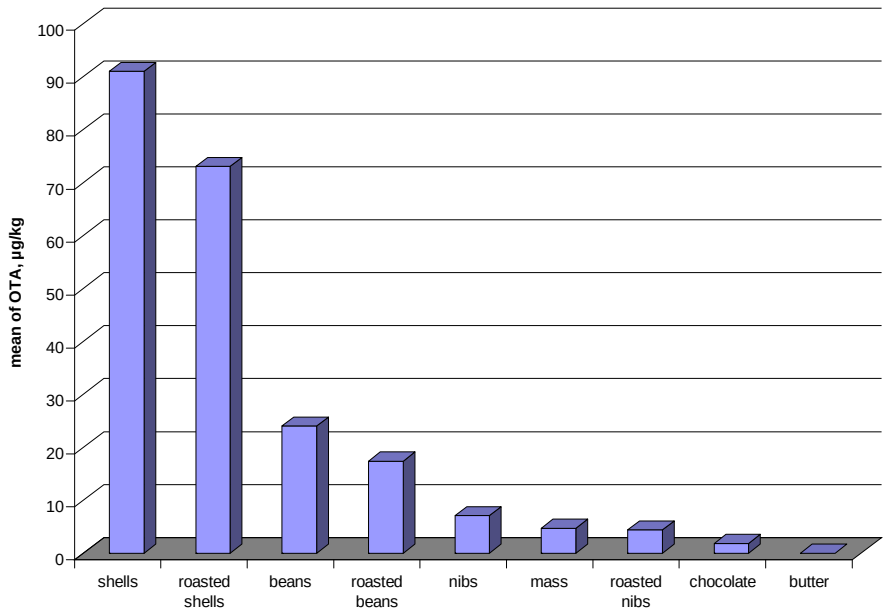

Figure 3. Mean level of OTA in different processing products of contaminated cocoa beans. Data from Cote d'Ivoire (Dembele et al., 2009).

OCCURRENCE OF OTA IN COCOA PRODUCTS

49. Ten cocoa powder and 9 chocolate samples from the open Belgium market were analyzed in 2005. (Vinkx, 2007). Five cocoa powder samples were below the LOQ (0.3 µg/kg) and the remaining 5 samples had OTA levels from 0.60 to 0.81 µg/kg. All the 9 chocolates samples contained OTA levels below the LOQ.

50. In a study conducted in 2005 in Japan, 14 of the 41 retail chocolates samples analyzed had OTA levels ranging from <0.10 µg/kg (14 samples) to 0.94 µg/kg (MHLW, 2006).

51. The report on the Task for Scientific Cooperation 3.2.7 showed that 81.3% of the cocoa-derived products analyzed were contaminated with OTA. This means out of the 547 cocoa product samples analyzed 445 were positive. The contamination level varied from 0.01 to 3.8 µg/kg, with 0.23 µg/kg average (Table 6) (Miraglia and Brera, 2002).

52. Vecchio and Finoli (2007) detected OTA levels of 0.1 to 3 µg/kg in 82% of cocoa powder marketed in Italy; two samples had levels > 2 µg/kg.

53. Burdaspal and Legarda (2003) evaluated the occurrence of OTA in 296 samples of different types of chocolate and cocoa powder purchased in Spain and other 15 countries. OTA was detected in all except one sample (99.7% positive samples). Details are shown on Table 6.

54. Turcotte and Scott (2011) detected OTA in cocoa and cocoa products available on the Canadian retail market at an incidence of 100%. OTA concentrations in alkalized cocoa (n=16) ranged from 0.57-7.8 µg/kg, while concentrations in natural cocoa (n=16) ranged from 0.25-2.6 µg/kg. Six samples of cocoa (5 alkalized and 1 natural) had an OTA content > 2 µg/kg. Concentrations in baking chocolate (n=7), dark chocolate (n=14) and milk chocolate (n=7) ranged from 0.12-1.4, 0.17-0.88 and 0.05-0.19 µg/kg, respectively.

55. In Italy, 60% of the 300 samples of cocoa powder and chocolate products purchased had OTA levels above the LOQ (0.08 µg/kg). All cocoa powder samples were contaminated and the highest OTA level was found in a dark chocolate bar sample (Table 6). The mean concentrations were below the former Italian legal limit (0.5 µg/kg for chocolate products and 2.0 µg/kg for cocoa powder) (Brera et al., 2011).

56. Copetti (2011) investigating the co-occurrence of aflatoxin and ochratoxin A in chocolate products, including powdered, bitter, dark, milk and white chocolate on the Brazilian market (125 samples) found that ochratoxin A was the most common mycotoxin in the evaluated samples and 98% of purchased chocolate was contaminated. The highest levels of ochratoxin A were found in cocoa powder, dark and bitter chocolate respectively: 0.39; 0.34 and 0.31 µg/Kg. The average of aflatoxin in bitter, powdered and dark chocolate were 0.66, 0.53 and 0.43 µg/ Kg, respectively. There was a weak correlation between contamination by aflatoxins and ochratoxin A in the products tested and it was not possible to conclude about the co-occurrence of these contaminants.

Table 6. Ochratoxin A content of various cocoa products

Product	Origin	Total / positive samples*	LOQ or LOD, µg/kg	Max, µg/kg	Median, µg/kg	Mean, µg/kg	References
Chocolate		41/27		0.94			MHLD, 2006
Chocolate		40[2]					MAFF, 1999
Chocolate	Germany	352/297	0.01	3.6	0.06	0.1	Miraglia and Brera, 2002
Chocolate	UK	40/18	0.1	0.6	0.1	0.38	Miraglia and Brera, 2002
Chocolate	Spain	35	0.01		0.12		Burdaspal&Legarda, 2003
Chocolate	not Spain	52	0.01		0.268		Burdaspal&Legarda, 2003
Chocolate candies	Italy	47/21		0.42		0.15	Brera, et al. 2011
Cocoa shell	Brazil	19/19	0.01	2.01		1.13	Copetti, 2009
Cocoa butter	Brazil	25/5	0.01	0.06		0.03	Copetti, 2009
Cocoa butter	various	4/0	0.1				Bonvehi, 2004
Cocoa butter	Netherlands	6/0	0.25				Miraglia and Brera, 2002
Cocoa spread	Netherlands	8/0	0.25				Miraglia and Brera, 2002
Cocoa cake	Brazil	26/19	0.01	3.18		0.97	Copetti, 2009
Cocoa cake	various	80/74	0.1	9		2.79	Bonvehi, 2004
Cocoa drink powder		247/101	0.1-0.5[3]			0.2	Gilmour &Lindblom, 2008
Cocoa mass	various	8/4	0.1	3.5		1.07	Bonvehi, 2004
Cocoa mass	Netherlands	1/0	0.25				Miraglia and Brera, 2002
Cocoa powder	Brazil	44/44	0.01	5.13		1.09	Copetti, 2009
Cocoa powder	various	31/29	0.1	4.4		2.41	Bonvehi, 2004
Cocoa powder	Spain	21			0.24		Burdaspal&Legarda, 2003
Cocoa powder	not Spain	5			0.17		Burdaspal & Legarda, 2003
Cocoa powder		1189/1094	0.1-0.5[3]			1	Gilmour &Lindblom, 2008
Cocoa powder	Italy	18/9	9	0.77		0.43	Tafuri et al., 2004[1]

Product	Origin	Total / positive samples*	LOQ or LOD, µg/kg	Max, µg/kg	Median, µg/kg	Mean, µg/kg	References
Cocoa powder		20/19		2.4		0.68	MAFF, 1999
Cocoa powder		20/20				1.67	MAFF, 1999
Cocoa powder	Germany	96/91	0.01	1.8	0.3	0.38	Miraglia and Brera, 2002
Cocoa powder	UK	40/39	0.2	2.4		1.2	Miraglia and Brera, 2002
Cocoa powder	Netherlands	6/0	0.25				Miraglia and Brera, 2002
Cocoa powder	Italy	40/40		1.82		0.55	Brera, et al. 2011
Dark chocolate	Italy	120/92		0.74		0.20	Brera et al. 2011
Dark chocolate	Brazil	25/25	0.01	0.87		0.34	Copetti, 2009, 2011
Dark chocolate	Spain	35			0.25		Burdaspal & Legarda, 2003
Dark chocolate	not Spain	52			0.27		Burdaspal and Legarda, 2003
Dark chocolate		536/300	0.1-0.5[3]			0.26	Gilmour and Lindblom, 2008
Chocolate/ chocolate cream	-	11/8	0.1	1.59		0.63	Bonvehi, 2004
Easter egg	Italy	15/5		0.50		0.20	Brera, et al. 2011
Liquor	Brazil	25/5	0.01	1.09		0.34	Copetti, 2009, 2011
Milk chocolate	Brazil	25	25	0.45		0.15	Copetti, 2009, 2011
Milk chocolate	Spain	47			0.12		Burdaspal & Legarda, 2003
Milk chocolate	not Spain	122			0.1		Burdaspal & Legarda, 2003
Milk chocolate	Italy	78/21		0.26		0.15	Brera et al. 2011
Milk chocolate		228/52	0.1-0.5[3]			0.16	Gilmour &Lindblom, 2008
Chocolate powder	Brazil	25/25	0.01	0.92		0.39	Copetti, 2009, 2011
White chocolate	Brazil	25/23	0.01	0.05		0.03	Copetti, 2009, 2011
White chocolate	Spain	5			0.03		Burdaspal & Legarda, 2003
White chocolate	not Spain	9			0.03		Burdaspal & Legarda, 2003

[1]results are corrected for recovery; [2]30 samples <0.6 µg/kg; samples were analyzed by different laboratories with LODs of 0.1, 0.2, or 0.5 µg/kg; * including samples between LOD-LOQ

FACTORS AFFECTING THE PRESENCE OF OTA IN COCOA

57. Gilmour and Lindblom (2008) reported a study conducted between 1999 and 2004 in West Africa (Figure 4). The objective of the study was to identify critical control points in the cocoa chain intended to form the basis for the formulation of prevention strategies to be instituted in a HACCP framework to minimize consumer exposure.

The conclusions were:

a) the contamination starts between the on-tree/harvesting to pre-fermentation stages, and that damaged pods are a major part of the problem;

b) an indication that the initial inoculation occurred before or during the fermentation;

c) the drying procedure for cocoa beans may play a role in OTA development, but does not seem to be the main source of contamination;

d) poor drying appears to allow further increase in toxin levels in already-contaminated beans;

e) OTA levels may vary within the cropping season;

f) further increases in OTA levels are not found in samples of cocoa beans taken at stages later in the supply chain; and

g) ~50% of the contaminating toxin is physically removed when the shells are removed from the beans.

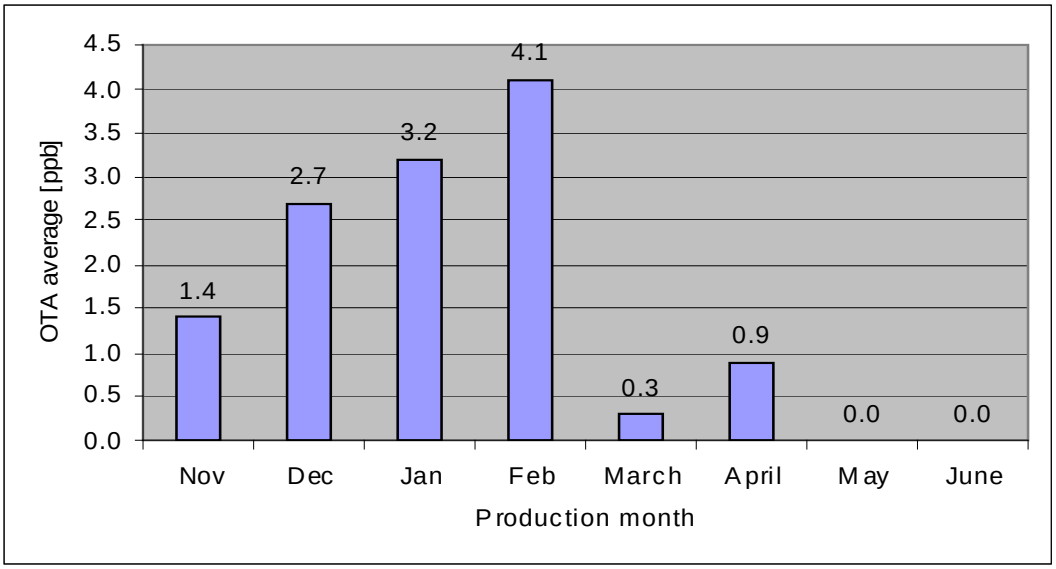

Figure 4. Variation of OTA levels as a function of month of production (Gilmour and Lindblom, 2008)

58. Experiments in large commercial farms in Cote d'Ivoire indicated that very little OTA was produced during well controlled fermentations. The fermentation was conducted in bags placed in the middle and on the top of wooden fermentation boxes. The boxes had no visible mold contamination when the fermentation was complete; the content of the boxes was dried at two different depths (3 and 8 cm) in drying beds. No OTA was detected in any of the samples (Gilmour and Lindblom, 2008).

59. Unlike the results from industrial scale fermentation studies in large boxes, the beans produced under small holder conditions (heap fermentation, small batches) contained OTA in many of the samples. The OTA level was > 0.5 ng/g in 24 of the samples (39%) and > 2 ng/g in 11 samples (18%). The authors concluded that drying conditions alone are not responsible for the OTA level, which depends on interactions between harvesting, fermentation and drying conditions and the dry season appears to be the most critical period for OTA contamination. Thus, the more difficult fermentation conditions that occur at that time, due to climatic conditions and the nature of the mucilage could facilitate mold growth and OTA production (Gilmour and Lindblom, 2008).

60. Dembele et al., 2009 conducted studies to determine the critical points of contamination at the farm level in Cote d'Ivoire. The results showed that beans from physically damaged pods were the most contaminated, with levels between 2.49 to 2.8 µg/kg, however, partially rotten pods showed a contamination level of 0.3 to 0.74 µg/kg whilst beans from good pods had 0.22 to 0.37 µg/kg OTA content (Figure 5). The results show that OTA development is significantly higher in beans from damaged pods than in beans from healthy pods and confirm the results reported by Bastide (Bastide et al 2006).

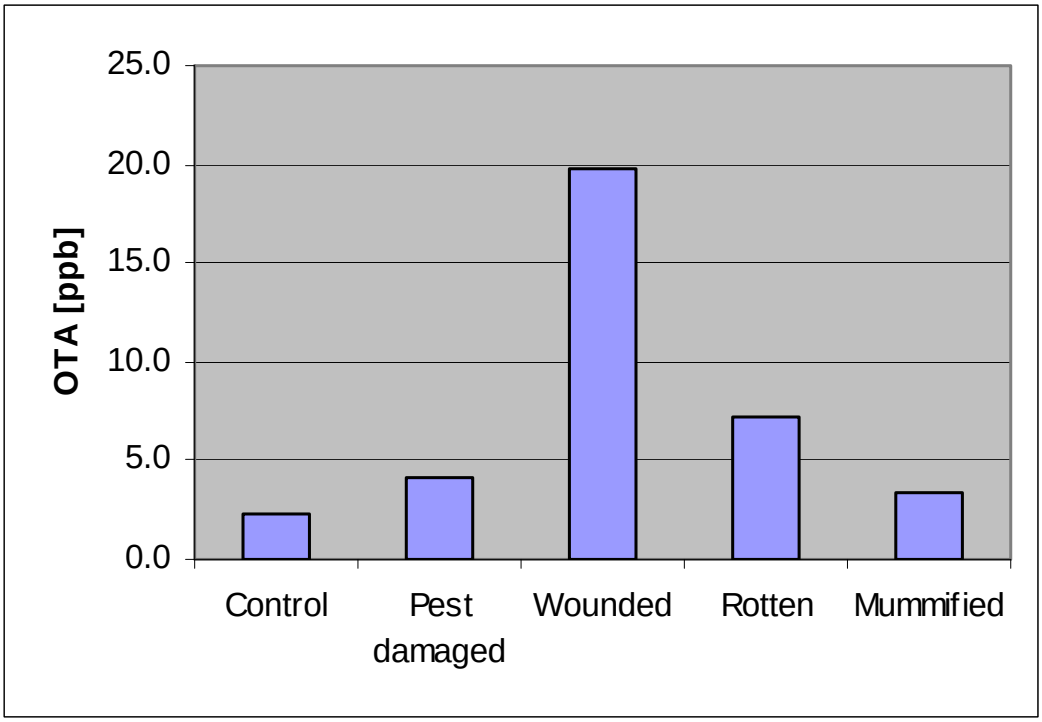

Figure 5. Effect of phytosanitary condition of the cocoa pods on levels of OTA found in the dried cocoa beans (Bastide et al, 2006)

61. Out of 37 samples collected from fermentation heaps, drying beans, drying mats, plantain leaves and air samples, only one OTA producing *A. niger* was found. Also, from other OTA-positive cocoa bean samples taken during drying and storage, only one OTA- producer, *A. carbonarius* was found. This limited work on the mycoflora of cocoa beans and farm environment showed that fungi capable of producing OTA were present in bean samples and in the on-farm environment and equipment (COCOQUAL, 2007)

62. Studies using cocoa media demonstrate the potential of *A. ochraceus* and other moulds that can be isolated from cocoa (*A. carbonarius, A. niger, A. tubingensis)* to grow and produce OTA. OTA production was shown to strongly depend on temperature, pH and water activity of the substrate. Water activity of 0.97 was found to be optimal for OTA biosynthesis. *A. niger* BFE 632 showed highest OTA production at 30ºC on malt glucose agar whereas *A. carbonarius* BFE 640 produced more OTA on cocoa agar at 25ºC (COCOQUAL, 2007).

DIETARY INTAKE

63. The Scientific Panel on Contaminants in the Food Chain from EFSA estimated that the current levels of exposure to OTA in EU member states ranges from 15 to 60 ng/bw/week (5th RTD Framework Programme project OTA-Risk Assessment – QLK1-2001-01614). This rate of exposure is below the TWI value of 120 ng/kg bw as derived by the Panel. However, as current EFSA consumption databases do not include infants and children, the Panel concluded that more data would be needed to assess exposure rates of this segment of consumers and of those that consume large amounts of certain regional specialty foods containing OTA (EFSA, 2006).

64. The SCOOP Task 3.2.2 presented data which indicated that the daily consumption of cocoa was 31 g/day/person corresponding to an OTA intake of 21 ng/kg/wk/person, contributing to 5% of total OTA intake. The consumption of cereals contributed with 55% of the total intake. The follow up SCOOP Task 3.2.7 (Miraglia and Brera, 2002) confirmed that cereal was still the main contributor of total OTA intake.

65. To estimate the dietary exposure to OTA, the Food and Environmental Hygiene Department (FEHD, 2006) of Hong Kong completed a study in February of 2006 which covered 8 major food groups including chocolate and cocoa products. It was found that dietary exposure to OTA was 4 and 9 ng/kg bw/week for the average secondary school student and above average consumer respectively. The main dietary source of OTA was cereal and cereal products (61% of total exposure), chocolates contributed 6% of the total dietary exposure.

66. In the Netherlands, the average OTA intake was estimated to be 1.0 ng/kg bw/day, of which 5% from the consumption of cocoa products and over 50% from the consumption of cereals. Other contributors include coffee, red wine and meat (Baker and Pieters, 2002).

67. In Canada, the estimated exposure to OTA ranged from 1.15-1.76 ng/kg bw/day for adults and from 2.6-4.38 ng/kg bw/day for children, with cereals and cereal-based foods as the main contributors to the exposure. OTA exposures resulting from cocoa and chocolate were not included in the assessment (Kuiper-Goodman et al., 2010).

68. In Italy, the highest weekly intake of OTA was referred to the consumption of Easter eggs by the children (group aged 0-10 years) (Brera et al., 2011). Assuming that the cocoa and chocolate based products represent 4% of the diet (Miraglia and Brera, 2002), the estimated intake was 4.8 ng/kg bw/week, much lower than the PTWI set by EFSA (120 ng/kg bw/week).

69. In Spain, the estimated daily intake of OTA through the consumption of chocolate and cocoa products (mean consumption of 8.6 g, 60 kg bw) was 0.036 ng/kg bw/day), which represents 0.26% of the provisional tolerable daily intake (PTDI) established by JECFA (Burdaspal and Legarda, 2003).

REGULATORY STATUS

70. In the EU, the Commission Regulation (EC) No. 1881/2006, 2006 established maximum levels for OTA in raw cereal grains, all products derived from cereals and dried vine fruit (currants, raisins and sultanas), roasted coffee, soluble coffee, wine, grape juice, baby foods, processed cereal based foods for infant and young children and dietary foods or special medical purposes intended specifically for infants. Some of the maximum levels are already in application since April 2002 and others since April 2005.

71. The European Community (Commission Regulation 105/2010), stated that "On the basis of the information available, it does not appear necessary for the protection of public health to set a maximum level of OTA in dried fruit other than dried vine fruit, cocoa and cocoa products, meat products, including edible offal and blood products and liqueur wines as they are not a significant contributor to OTA exposure and high levels of OTA have been found only seldom in those commodities. In the case of green coffee and beer, the presence of OTA is already controlled at another more appropriate stage of the production chain (respectively roasted coffee and malt)".

72. In 2003, the Italian Ministry of Health, claiming the precautionary principle, set a legal limit of 2.0 µg/kg and 0.5 µg/kg for cocoa powder and chocolate products respectively. On the basis of the risk assessment carried out in the Brera et al. (2010), which showed no health concerns, and to align with the EU regulation, the Italian Superior Council of Health decided to remove the Italian legal limit for OTA in cocoa and chocolate based products.

73. Brazil has established 5 µg/kg as maximum level for OTA in cocoa products, including chocolate (ANVISA Resolução nº7/2011).

74. Health Canada is currently in the process of proposing maximum limits for OTA in a variety of foodstuff, as a result of a conducted health risk assessment (Turcotte and Scott, 2010). At this time, maximum limits for OTA in cocoa are not being considered.

75. The US FDA has not set advisory limits or action levels for Ochratoxin A in any commodity.

PREVENTION AND REDUCTION OF OTA IN COCOA

76. The European chocolate and cocoa industry and producing countries are engaged in studies to understand the sources of OTA contamination and appropriate remedial actions.

77. Research backed by the European chocolate and cocoa industry in some producing countries has shown that OTA can be found in beans from most producing countries and that the practice during early processing steps at the cocoa farm is critical. This means that interventions have to be made at the farm level for a significant reduction of OTA contamination to happen (Gilmour and Lindblom, 2008). The preventive actions could include segregation of damage pods, control of fermentation and drying processes.

78. A study conducted in small farms in Cote d'Ivoire and Togo suggested that OTA is linked to post-harvest processing practices such as pod defects and to climatic conditions related to month of harvest (Bastide et al., 2006).

79. Coppetti et al. (2010) assessed the OTA levels during the cocoa processing in the farm and concluded that the drying stage is the critical point.

80. Some quality management systems exist in the primary processing of cocoa. Dahl (2006), working under the EU-funded Cocoqual Project, has developed a Quality Management System based on ISO 22000 for the primary processing of cocoa for the purpose of ensuring good quality cocoa including prevention of OTA.

81. The finding that some lactic acid bacteria inhibited the growth of ochratoxigenic mould has profound food safety implications which could be exploited for prevention of OTA in cocoa. This finding can possibly be exploited in a future development of starter cultures for fermentation of cocoa (COCOQUAL, 2007).

82. The presence of lactic, acetic and citric acid was described during cocoa fermentation (Petithuguenin, 2002; Jinap and Dimick 1990). It was verified that they have an inhibition effect on ochratoxigenic fungi growth and ochratoxin A production (Copetti et al, 2011b). These authors evaluated *A. carbonarius* and *A. niger* growth and ochratoxin A production in culture media by those three organic acids in its formulation and concluded that acetic acid was the most inhibitory against two species, also on OTA production. The authors also recommended the fermentation practice to prevent ochratoxin A due to the presence of acetic acid.

83. Data indicates that phenolic antioxidant compounds, gallic acid, vanillic acid, 4-hydroxybenzoic acid, catechin, caffeic acid (some of these found in cocoa beans), generally suppress OTA production and growth of several ochratoxigenic *Aspergillus* species. The effect of each compound on OTA production and growth differed among strains and generally was variable, suggesting that species-specific OTA production and response to phenolic compounds may be influenced by different ecological and developmental factors. The information regarding genetic and physiological responses to antioxidant compounds could lead to targeted intervention strategies for the reduction of economic losses by OTA contamination (Palumbo et al, 2007).

84. Essential oils of *Aframomum danielli* have been shown to reduce OTA levels in spiked cocoa powder with a reduction efficiency of 64 – 95% (Aroyeun and Adegoke, 2007). The relevance of this work is the potential of using *A. danielli* as a step in procedures aimed at reducing OTA in grossly contaminated samples.

85. Sixty five lactic acid bacteria isolates of cocoa or*igin were te*sted using a spot method for their ability to inhibit grow*th of 12 OTA producing moulds. Most tested L. fermentum* and *L. plantarium* strains inhibited mould growth (COCOQUAL, 2007).

86. A major part of OTA originally present in cocoa beans is found in the shell fraction, which is removed during processing. Other processing steps from cocoa beans to finished products do not lead to removal or destruction/degradation of OTA. Thus a well-controlled shelling process could achieve a very significant reduction in OTA levels in cocoa-derived products (Amézqueta et al., 2005). The Codex standard description of the cocoa (cacao) mass or cocoa/chocolate liquor is the product obtained from cocoa nib, which is obtained from cocoa beans of merchantable quality which have been cleaned and freed from shells as thoroughly as is technically possible (Codex Stan 141-1983, Rev. 1-2001).

CONCLUSIONS AND RECOMMENDATIONS

87. This Discussion Paper on OTA in Cocoa leads to the following broad conclusions and recommendations for consideration at the Sixth Session of the Codex Committee on Contaminants in Foods:

    a) The CCCF may consider commencement of new work for the development of a Code of Practice for the Prevention and Reduction of OTA in Cocoa. The CCCF may consider the proposed draft project document as presented in Annex I for submission to the 35th Session of the Commission, through the Critical Review of the Executive Committee, for approval of new work.

    b) This code, subject to the approval of new work by the Commission, should be developed along similar lines as the current Code of Practice for the Prevention and Reduction of Ochratoxin A Contamination in Coffee (CAC/RCP 69-2009)**.** The proposed outline of the Code presented in Annex II to this discussion paper may also be used as a basis.

    c) The necessity of setting a maximum level for OTA in cocoa should be assessed after the development and implementation of the Code of Practice and it should consider:

        (i) The significant differences between the level of OTA in the shells, unroasted beans, roasted beans, roasted nibs and chocolate & cocoa products containing additives from industrial processing.

        (ii) The implementation of the Code of Practice by all producing countries.

        (iii) The necessity of obtaining reliable data on worldwide exposure

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ANNEX I

PROJECT DOCUMENT

PROPOSAL FOR A "CODE OF PRACTICE FOR THE PREVENTION AND REDUCTION OF OCHRATOXIN A CONTAMINATION IN COCOA"

1. Purpose and Scope of the new work

The purpose of the proposed new work is to provide to member countries and the cocoa industry a guidance to prevent and reduce Ochratoxin A (OTA) contamination in cocoa. The scope of the new work encompasses the development of a draft Code of Practice for the prevention and reduction of OTA Contamination in Cocoa, which will cover all the stages of the cocoa chain. It is anticipated that this new work would be undertaken based on FAO Guidelines for the Prevention of Mould Formation in Coffee and in line with the current Code of Practice elaborated for Coffee.

2. Relevance and timeliness

The toxicity of OTA has been reviewed by the International Agency for Research on Cancer (IARC), that has classified OTA as a possible human carcinogen (group 2B), and by the Joint FAO/WHO Expert Committee on Food Additives (JECFA).

Ochratoxin A is a mycotoxin that occurs naturally worldwide in food commodities including cocoa beans and cocoa products. In cocoa, OTA is mostly associated with cocoa bean shells and fat free cocoa solids (cocoa powder). The cocoa beans are not eaten as such; they undergo industrial conversion into cocoa products before consumption. Cocoa products are very important ingredients in pharmaceuticals, cakes, biscuits and chocolate confectionery. Around 71% of the world supply of cocoa beans comes from West Africa. Cocoa beans are also produced in Asia and Latin America. Being a crop produced by smallholders, cocoa is a valuable cash crop for hundreds of thousands of farmers in the cocoa producing countries, and it is also of great importance to the economies of these countries.

The most effective way to prevent and reduce OTA in cocoa beans and cocoa products is the use of Good Agricultural Practices (GAP) along the cocoa value chain.

3. Main aspects to be covered

The proposed new work will focus on good practices that will control infection of cocoa with OTA producing fungi, growth of the fungi and OTA production. The code will cover all stages of the cocoa value chain (pre-harvest, primary processing, storage and transportation practices) to develop strategies to prevent and reduce OTA contamination of cocoa.

4. Assessment against the criteria for the establishment of work priorities

a) Consumer protection from the point of view of health, food safety, ensuring fair practice in the food trade and taking into account the identified needs of the developing countries.

The code will provide additional guidance for countries in order to improve cocoa quality, preventing and reducing OTA contamination and consequently minimize consumer dietary exposure to OTA from cocoa products.

b) Diversification of national legislations and apparent resultant or potential impediments to international trade.

The code would provide internationally recognized scientific guidance in order to improve the enhancement of international trade.

c) Work already undertaken by other organizations in this field

Not much work has been done by other international organizations on OTA in cocoa; however, FAO has produced some guidelines for the Prevention of mould formation in coffee. Codex has also developed Code of Practice for the prevention and reduction of Ochratoxin A contamination in coffee (CAC/RCP 69-2009).

5. Relevance to Codex Strategic Goals

The work proposed falls under all five Codex Strategic Goals:

Goal 1: Promoting Sound Regulatory Frameworks

The result of this work will assist in promoting sound regulatory frameworks in international trade by using scientific knowledge and practical experience for prevention and reduction of OTA contamination in cocoa.

This work will harmonize procedures for developed and developing countries with a view to promoting maximum application of Codex Standards for fair trade.

Goal 2: Promoting widest and consistent application of scientific principles and risk analysis.

This work will help in establishing risk management options and strategies to control OTA in cocoa.

Goal 3: Strengthening Codex work-management capabilities

By establishing a general framework for the management of food safety risks associated with the prevention and reduction of OTA contamination in cocoa will provide a general document that can be referenced by CCCF and it can be used by many countries

Goal 4: Promoting cooperation between seamless linkages between Codex and other multilateral bodies.

The involvement of FAO in Codex activities has already formed a close link and the work developed by FAO on this issue will be the base of this new Codex work

Goal 5: Promoting maximum application of codex standards

Due to the international nature of this problem, this work will support and embrace all aspects of this objective by requiring participation of both developed and developing countries to conduct the work

6. Information on the relationship between the proposal and other existing Codex documents

This new work is recommended in the Discussion Paper on OTA in cocoa to be presented and discussed at the 6th Session of Codex Committee on Contaminants in Foods.

7. Identification of any requirement for and availability of expert scientific advice

Additional scientific advice is not necessary at this moment, as FAO has already published the Guidelines for the Prevention of Mould Formation in Coffee. Mould formation in coffee and cocoa is caused by similar mycoflora.

8. Identification of any need for technical input to the standard from external bodies

Currently, there is no need for additional technical input from external bodies

9. The proposed timeline for completion of the new work, including the starting date, proposed date of adoption at Step 5 and the proposed date for the adoption by the Commission, the timeframe for developing a standard should not normally exceed 5 years.

If the Commission approves, the draft Code of Practice will be circulated for comments at Step 3 and consideration by the 7th session of CCCF at Step 4 in 2013. Adoption at Step 5 by the Commission is planned for 2013 and adoption at Step 8 by the Commission can be expected by 2014.

ANNEX II

Proposed Draft CODE OF PRACTICE FOR THE PREVENTION AND REDUCTION OF OCHRATOXIN A CONTAMINATION IN COCOA OR CACOA

TABLE OF CONTENTS

1. INTRODUCTION

1. This document is intended to provide guidance for all interested parties producing and handling cocoa beans for human consumption. All cocoa beans should be prepared and handled in accordance with the Recommended International Code of Practice – General Principles of Food Hygiene[6], which are relevant for all foods being prepared for human consumption. These codes of practice indicate the measures that should be implemented by all persons that have the responsibility for assuring that food is safe and suitable for consumption.

2. Ochratoxin A (OTA) is a toxic fungal metabolite classified by the International Agency for Research on Cancer as a possible human carcinogen (group 2B). JECFA established a PTWI of 100 ng/kg bodyweight for OTA. OTA is produced by a few species in the genera *Aspergillus* and *Penicillium*. In cocoa beans, the studies have shown that only *Aspergillus* species, specifically *A. carbonarius* and *A. niger* agregate, with lower numbers of *A. westerdijkiae, A. ochraceus* and *A. melleus* are involved. OTA is produced when conditions of water activity, nutrition and temperature required for growth and biosynthesis are present.

3. The fruit of cocoa derived from the cocoa tree, *Theobroma cacao L*., is composed of pericarp, tissue that arises from the ripened ovary wall of a fruit, and the ovary. When the fruit is ripe this external tissue, also known as the pod, consisting of thick and hard organic material, could be used as compost, animal feed and source of potash. The ovary contains numerous seeds embedded in an aqueous, mucilaginous and acidic pulp. This white and off-white edible pulp is composed of about 12% of sugars and present at low pH (3.3 – 4.0) due to its high citric acid content. The pulp contains up to 10% pectin. The pulp might be used for making jams and jellies as well as alcoholic beverages and vinegar.

4. The main commercial use resides in the seeds, also known as cocoa beans. The cocoa bean is composed of an episperm or integument, embryo and cotyledon. The integument, the protective layer of the seed, is also called shell when it is dried. During fermentation the embryo dies and upon drying, the fat content of the cocoa bean ranges between 34% and 56%.

5. After proper fermentation and drying processes the cocoa beans are further processed industrially to produce various commercial cocoa products.

6. Since the cocoa beans are extracted from a fruit, contamination by microorganisms may occur and the development of OTA producing fungi could begin when conditions become appropriate for growth. Generally the fermentation and drying processes could create this favorable condition when these processes are not properly done.

7. It is important to emphasize that the next manufacturing steps involve removing shells, roasting, liquoring and refining. However, it is only the removal of the shell that can significantly reduce OTA levels. Although this code of practice focuses on reduction and prevention of OTA contamination in cocoa beans, it is recommended that the farm processors and food industry establish food safety specific programmes related to these processes in order to reduce the OTA level in the processed cocoa products for human consumption.

[6] Recommended International Code of Practice- General Principles of Food Hygiene (CAC/RCP 1- 1969, Rev. 4-2003)

2. DEFINITIONS

Parts of cocoa fruit (figure 1)

Cocoa bean: The seed of the cocoa fruit composed of episperm (integument), embryo and cotyledon.

Cocoa pod: The cocoa fruit pericarp that arises from the ripened ovary wall of a fruit.

Episperm or integument: The protective layer of the seed also called shell when it is dried.

Pulp: Aqueous, mucilaginous and acidic substance in which the seeds are embedded.

Dry cocoa: A commercial term designating cocoa beans which have been evenly dried throughout and which the moisture content corresponds to the requirements of this standard.

Mouldy bean: A cocoa on the internal parts of which mould is visible to the naked eye.

Slaty bean: A cocoa bean which shows a slaty colour over half or more of the surface exposed by the method described in ISO/R 1114.

Insect Damaged Bean: A cocoa bean with the internal parts of which contains insects at any stage of development, or has been attacked by insects which have caused damage visible to the naked eye.

Germinated bean: A cocoa bean with the shell pierced, slit or broken by the growth of seed germ.

Flat bean: A cocoa bean of which the two cotyledons are so thin that it is not possible to obtain a cotyledon surface by cutting.

Smoky bean: A cocoa bean which has a smoky smell or taste or which shows signs of contamination by smoke.

Broken bean: A cocoa bean of which a fragment is missing, the missing part being equivalent to less than half the bean.

Fragment: A piece of cocoa bean equal to or less than the original bean.

Piece of shell: Part of the shell without any of the kernel

Adulterations: Adulteration of the composition of a parcel of cocoa beans by means whatsoever so that the resulting mixture or combination does not conform to the contractual description.

Foreign matter: Any substance other than cocoa beans or residue.

Contamination: Cocoa which has a smoky, hammy or other off-flavor taste or smell, or which contains a substance not natural to cocoa.

Harvesting and opening the fruits: Fruits are manually harvested and opened using a sickle, machete or wooden baton.

Fermentation: Process intended to degrade the pulp and initiate biochemical changes in the cotyledon by inherent enzymes and micro-organisms from the farm environment.

Drying process: Drying of cocoa beans either under sunlight or in mechanical or solar dryers in order to reduce the moisture content to make them stable for storage.

Sorting: Technological operation intended to remove foreign matter, fragments of dried cocoa beans and dried pulp; and defective beans from dried cocoa beans.

Roasting: Heat treatment that produces fundamental chemical and physical changes in the structure and composition of cocoa beans and brings about darkening of the beans and the development of the characteristic chocolate flavor of roasted cocoa.

3. PROCESSING OF COCOA

8. Harvesting involves removing ripe fruits from the trees. The fruits are harvested manually by making a clean cut through the stalk with a cleaned and well sharpened blade.
9. The pods are opened to remove the cocoa beans with the pulp as soon as possible or within a few days after harvesting.
10. The cocoa beans with pulp removed from the pod are heaped together or put in boxes, trays or platforms to allow micro-organisms to develop and initiate the fermentation process.
11. The fermented cocoa beans are usually sun dried in an open drying yard, or on suspended tables with many variations and technological innovations. Sun and mechanical drying can be combined and used together.
12. When the beans are appropriately dried to target moisture levels, they must be sorted to remove flat beans, shriveled beans, black beans, mouldy beans, small and fused beans, beans with insect damage, and others defects.
13. Once the drying and sorting out process are completed, the dried cocoa must be put into appropriate bags and stored. Appropriate bagging and storage of the processed beans is just as important as proper fermentation and drying.
14. The industrial processing of removing cocoa shells (dried episperm or integument of cocoa seed) before the roasting can reduce OTA levels significantly.

15. The industry for cocoa product should implement monitoring and controlling system, designed to prevent and reduce the level of OTA in subsequent manufacturing steps.

4. RECOMMENDED PRACTICES

4.1 PRE-HARVEST

16. The pulp and the cocoa beans are microbiologically sterile in relation to OTA producing fungi while inside the healthy cocoa pod. The contamination by spores of fungi that can produce OTA occurs during the opening process of cocoa pod and in subsequent processes.

17. Consequently the cocoa plantation should be properly maintained to ensure a low level of mould infestation as possible, in order to avoid inoculation by OTA producing fungi spores during opening of the cocoa pod.

18. Recommended practices to reduce the development and spore load from OTA-producing fungi on cocoa beans are:

    a) Keep cocoa plants healthy, through the appropriate use of good agricultural practices (GAP) such as weeding, improving soil texture, pruning, fertilization, pest and disease control, and irrigation.

    b) Do not use overhead irrigation during the flowering and fruit development period. This could augment normal spore dispersal rates and increase the chance of infection of beans by OTA producers.

    c) Avoid disposal of uncomposted organic wastes from cocoa or any other source, in or around the plantation. Cocoa seeds and seed associated material, such as dust, earth, and other seed can allow proliferation of OTA producing fungi.

4.2 HARVEST

19. Cocoa fruits should be harvested as soon as they are ripe. Harvesting should be done every two weeks if there are not many ripe pods, and every week during peak periods. Likewise, it is important to do a separate round of the farm sanitation every week to remove diseased cocoa fruits with a cocoa hook that is used only for that purpose.

20. Discard mummified fruits because they are more likely to be infected.

21. Avoid harvesting unripe fruits. Beans inside unripe pods do not readily ferment. The unripe cocoa beans have a solid pulp, without mucilage, hence they are difficult to separate from the pod and do not ferment properly.

22. The harvester should avoid unnecessary cutting of the cocoa pods to prevent inoculation and development of OTA producing fungi in the cuts in the pod.

23. Harvesting must be carried out using specific techniques and tools. The tools and baskets used to transport the fruits should be clean and the tools sharpened regularly.

4.3 STORAGE AND POD OPENING

24. Once a sufficiently large quantity of fruits has been harvested, the pods must be opened, manually (using wooden batons or machetes) or mechanically (using cocoa pod breaking machines) and beans extracted. It is recommended opening the fruits as soon as possible or within a few days after harvesting in order to avoid fungal proliferation

25. Wounded or damaged fruits should not be stored longer than one day before fermenting.

26. During the opening process any defective parts of the cocoa pod, mouldy beans, diseased beans, and damaged beans should be removed and appropriately disposed off.

4.4 FERMENTATION OF COCOA BEANS

27. The cocoa beans with pulp should be placed in reasonably clean and dry suitable boxes, trays or platforms for the fermentation.

28. The mucilaginous mass should be turned frequently to ensure uniform heat in the heaps, to allow aeration enter, to break up any lumps and to prevent fungi proliferation. The frequency depends on the method of fermentation.

29. The duration of fermentation is usually 4 to 7 days which will also depends on the method of fermentation. It is however recommended that fermentation beyond 7 days is avoided as this could lead to fungal proliferation.

30. Fermentation is recommended to avoid ochratoxigenic fungi growth and ochratoxin A production because acetic, lactic and citric acid produced by bacteria during fermentation can compete and inhibit these undesirable fungi species. Research has shown that fermentation carried out during drying on a drying mat; and partially depulped cocoa also being fermented directly on the drying mat can increase OTA production in cocoa beans.

4.5 DRYING PROCESS

31. After fermentation, the cocoa beans must be removed and immediately spread on appropriate surfaces to dry, preferably under direct sunlight. If the drying is not started immediately, the cocoa beans will keep fermenting and over-ferment leading to loss of cocoa flavour.

32. The drying process could be done by direct sunlight or artificial drying or a combination of both. Levels of 6-8% of cocoa beans moisture content is safe to avoid growth of microorganisms and good for storage.

33. The drying area should be located away from contaminant sources. and should receive maximum sun exposure and air circulation during most times of the day, to speed up the drying process of cocoa beans. Shady areas should be avoided.

34. In rainy or wet regions, cocoa beans must be covered and re-spread once the surface has dried. Ensure that the drying surface is clean and located away from contaminants sources.

35. The layer of drying cocoa beans should not exceed 6 cm thick, which corresponds to 40 kg of wet cocoa beans per square meter of drying area to avoid slow or inadequate drying that may lead to mould growth.

36. Rake over the cocoa bean layer frequently during the day time to allow faster drying and reduce the risk of fungi growing (5-10 times per day).

37. Protect cocoa beans during drying from rain and dew. The cocoa beans should be heaped and covered at night or during rainy weather to avoid re-wetting.

38. Do not mix cocoa beans at different drying stages. Use specific identification for each one of them to identify each drying stage.

39. Re-wetting of cocoa beans should be avoided because cocoa beans with a certain level of moisture above 8% can allow rapid growth of the mycelium and the possibility of OTA production. Mouldy cocoa bean should be discarded.

40. Protect the cocoa beans during drying from domestic animals, which can be a source of biological contamination.

4.6 STORAGE, TRANSPORTATION AND TRADING OF DRIED COCOA BEANS

41. Before storage of dried cocoa beans, they must be sorted to remove flat beans, shrivelled beans, black beans, mouldy beans, small and/or fused beans, beans with insect damage, etc.

42. Ensure the facilities and equipment that are related with sorting process are regularly inspected, maintained and cleaned, in order to avoid physical damage to cocoa beans that make them more susceptible to contamination and deterioration and to prevent the introduction of new contamination and undesirable materials.

43. The dried cocoa beans that will be stored should be properly identified by lots, at the farm level or in out-of-farm warehouses, in bulk or in clean bags under appropriate storage conditions.

44. Cocoa beans should be packaged in clean bags which are sufficiently strong and properly sewn or sealed. The bags should be made of non-toxic materials, preferably food grade hydrocarbon-free bags that do not attract insects and rodents and are sufficiently strong to resist storage for longer periods.

45. The bagged cocoa beans must be placed in warehouses or storage sheds that are weatherproof, well aerated, cleaned, free from damp and insect pests and away from smoke and other odoriferous materials that could contaminate the cocoa.

    a) The design and structure of the warehouses or storage sheds should be adequate to maintain dryness and uniformity of the stored dried cocoa beans.

    b) Arrange the cocoa bags on pallets and away from walls, to allow good air circulation.

    c) Do not expose stored cocoa beans to direct sunlight nor store near heating sources, to avoid the possibility of temperature differentials and water migration.

    d) Implement cleaning and maintenance programs and ensure that storage facilities are periodically inspected, cleaned and repaired.

46. During the entire process, the cocoa beans must also be protected from re-wetting, degradation and cross-contamination. In long term storage conditions, humidity should be kept under strict control (less than 70% RH). Appropriate storage facilities, the use of good storage practice and regular monitoring can prevent or reduce mould growth..

47. The moisture content of the stored cocoa beans should be periodically checked and kept below 8%.

48. Any infestation must be dealt with by proper and approved methods of fumigation. Appropriate documentation accompanying the cargo should state in clear and correct terms the fumigants and the quantities that were used.

49. From the production areas, cocoa may be conveyed by various means to the trading points. The main aspect of concern here is to avoid rewetting of cocoa beans, due to possible climatic changes between different regions, and taking the necessary control measures.

50. Transport of cocoa beans also requires the adoption of practices to avoid re-wetting, to maintain temperature as uniform as possible and to prevent contamination by other materials. The main requirements here are:

    a) Cover cocoa beans loading and unloading areas to protect against rain.

    b) Before receiving a new cargo, the vehicles must be cleaned from residues of previous cargo.

c) The vehicles must have floor, side walls and ceilings (in closed vehicles) checked for the presence of points where exhaust fumes or water from rain can be channeled into the cocoa cargo. Tarpaulins and plastic canvas used to cover the cargo should also be regularly checked to ensure that they are clean and without holes. The vehicles should also receive regular maintenance and should be kept in good condition.

d) Reliable transport service-providers that adopt the recommended good transportation practices should be selected by operators.

4.7 SHIP TRANSPORTATION

51. Cocoa beans are transported from producing to consuming countries in bags or in bulk, usually in 18 to 22 tonnes capacity containers. Temperature fluctuations, during the transportation time, can cause condensation of the remaining water (present even in well-dried beans) and local re-wetting. The redistribution of water can lead to fungal growth, with the possibility of OTA production. The recommended practices during transportation in the port are:

a) Cover cocoa loading and unloading areas to protect against rain.

b) Check cocoa lots to ensure that they are uniformly dried and below 8% moisture content, free of foreign matter and conforming to the established defect levels.

c) Check containers before loading to ensure they are clean, dry and without structural damage that could allow water entrance into the container.

d) Bags should be well stacked and crossed over for mutual support in order to avoid the formation of empty vertical columns (chimneys). The top layer and sides of bags should be covered with materials that can absorb condensed water, such as silica gel or cardboard for protection against the growth of fungi that could result in OTA production. For cocoa in bulk, a sealable plastic liner (e.g. big bag which allows aeration) is desirable and this should be kept away from the roof of the container.

e) Choose an appropriate place, not directly exposed to the weather, and aboard the ship to store the cocoa to reduce the possibility of undesirable situations mentioned that can lead to OTA contamination.

f) Keep the ventilation holes in the containers free.

g) Avoid unprotected stowage on the deck (top layer) and stow away from boilers and heated tanks or bulkheads.

h) The moisture content should not exceed 8% anywhere, from the point where the cocoa beans leaves the loading area to the point at which the cocoa is unloaded, stored and/or subjected to other processing procedures such as roasting.

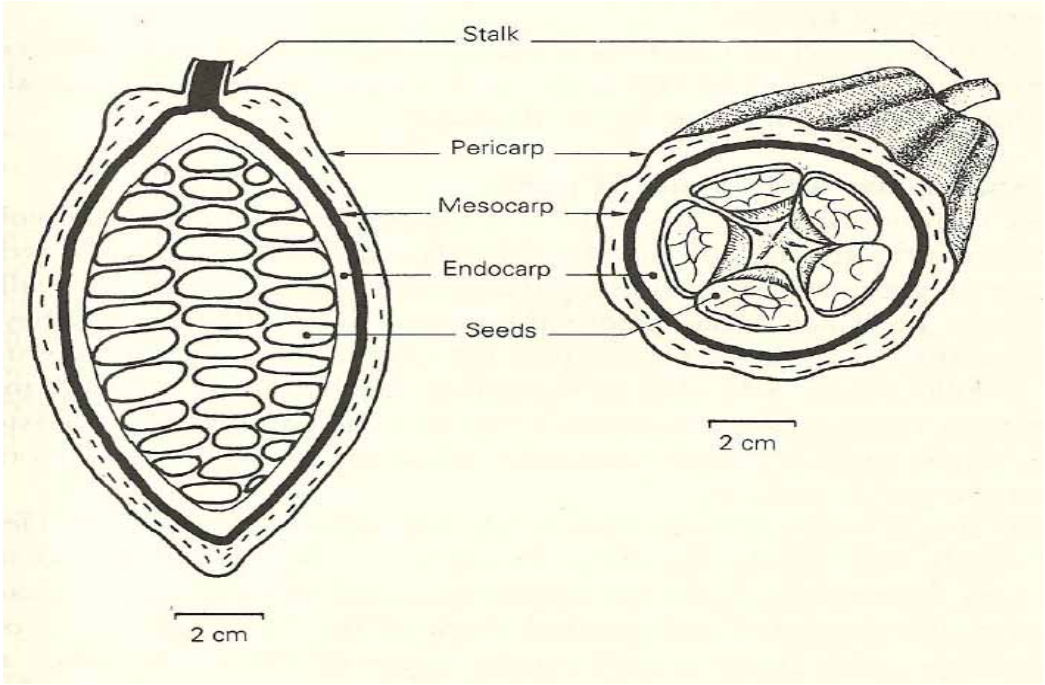


Figure 1a. Longitudinal and transverse sections of a cocoa pod (Mossu, 1992)

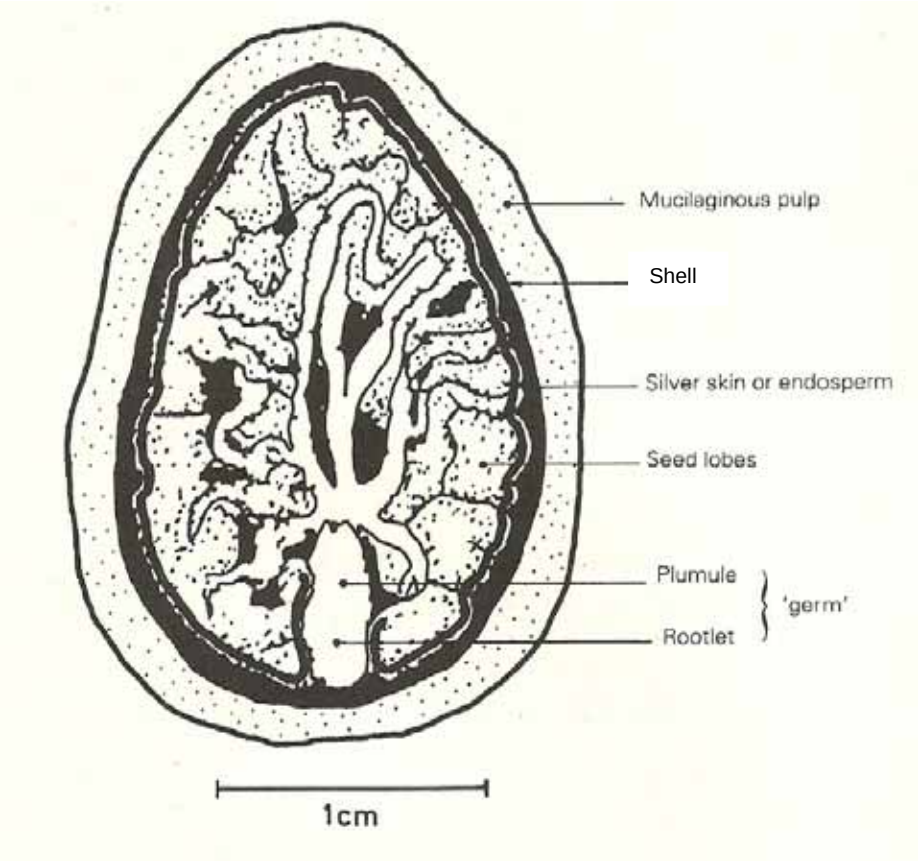
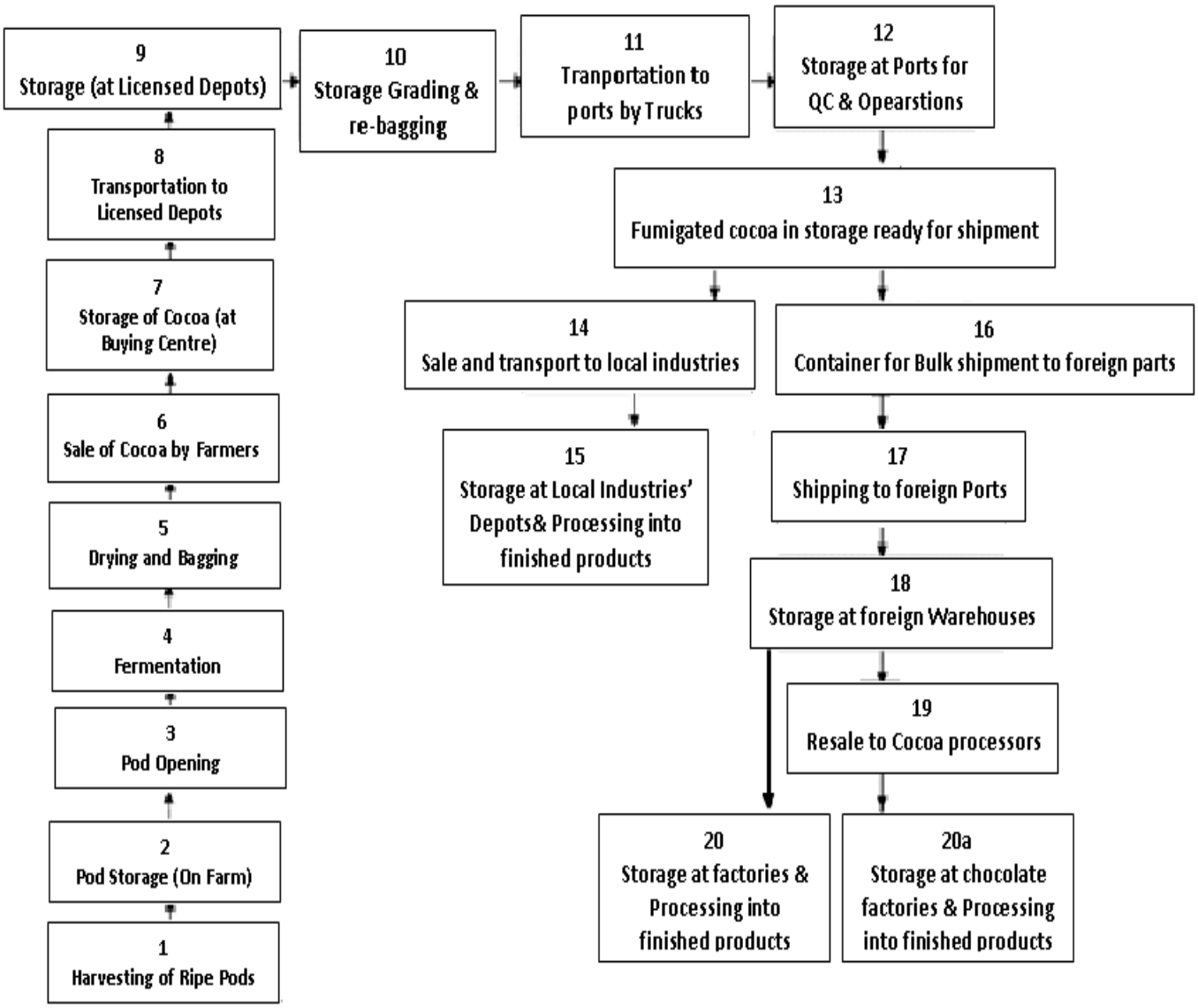


Figure 1b. Longitudinal section of a cocoa seed (Mossu, 1992)

FIG. 2 COCOA VALUE CHAIN



ANNEX III

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