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CODEX ALIMENTARIUS COMMISSION

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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

(Prepared by Electronic Working Group led by Ghana and co-chaired by Brazil)

BACKGROUND

1. At the 38th Session, the Codex Committee on Food Additives and Contaminants (CCFAC) agreed to develop a discussion paper on Ochratoxin A (OTA) contamination in cocoa. The discussion paper was to form the basis for a decision on the potential need for a code of practice to reduce and manage the incidence of OTA in cocoa. The Electronic Working Group was led by Ghana and assisted by Brazil, European Community, Indonesia, Switzerland, the United Kingdom and the United States of America, and its report was presented at the 1st Session of the Codex Committee on Contaminants in Food (CCCF) held in Beijing, China.

2. At its 1st Session, CCCF agreed that it was premature to initiate the development of a code of practice and a decision in this regard should wait until more data had been collected. After further discussion, CCCF decided to establish an Electronic Working Group, led by Ghana to update the discussion paper with new data and other relevant information, and take into account the comments made at the 1st Session, for consideration at the 2nd Session of CCCF.

3. At its 2nd Session, the Committee suspended the consideration of OTA in cocoa due to the need for generation of new data as the matter would be reconsidered once new data became available.

4. At the 4th Session, the Delegation of Brazil informed the Committee that a new study on the incidence of ochratoxigenic fungi and OTA in cocoa had been carried out in Brazil and that the study could provide the elements for the development of a code of practice to reduce or prevent OTA in cocoa. The Committee agreed that an Electronic Working Group led by Ghana and co-chaired by Brazil, would prepare a discussion paper on the occurrence of ochratoxigenic fungi and OTA in cocoa to assess whether a code of practice should be developed.

5. The electronic working group members include: Argentina, Australia, Brazil, Canada, Ecuador, Egypt, European Commission, Ghana, Greece, Italy, Japan, Malaysia, Switzerland, United Kingdom, United States of America, European Food Law Association (EFLA), International Confectionery Association and the Food and Agriculture Organization. Comments were received from the following members: Brazil, Ghana, Greece, Italy, Japan, United Kingdom, United States of America and the International Confectionery Association.

INTRODUCTION

6. 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).

7. 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^{o} belt north and south of the equator. The mean minimum and maximum temperatures in most cocoa growing regions are 18^{o}C and 32^{o}C. A high rainfall of 1000-4000 mm/year is required.

8. 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.

9. 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).

10. 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; about 2% of the total cocoa nib weight is due to the presence of shell and germ during the manufacturing process (CODEX STAN141-1983). The nib is milled into cocoa mass/liquor for further processing.

11. 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	**2007/08**		**2008/09**		**2009/10 (forecast)**	
Africa	**2693**	**72.1%**	**2520**	**69,9%**	**2459**	**68,4%**
Cameroon	185		227		200	
Cote d'Ivoire	1382		1223		1190	
Ghana	729		662		650	
Nigeria	230		250		260	
Others	166		158		159	
America	**450**	**12,1%**	**487**	**13,5%**	**505**	**14,0%**
Brazil	171		157		155	
Ecuador	113		134		150	
Others	167		196		200	
Asia & Oceania	**591**	**15,8%**	**598**	**16,6%**	**632**	**17,6%**
Indonesia	485		490		535	
Papua New Guinea	52		59		50	
Others	54		49		47	
World Total	**3734**	**100.0%**	**3604**	**100.0%**	**3596**	**100.0%**

Source: ICCO Quarterly Bulletin of Cocoa Statistics, Vol. XXXVI, No.3, Cocoa Year 2009/2010. Published: 26-08-2010.

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

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

	2003/04		2004/05		2005/06	
Europe	**1346**	**41.6%**	**1375**	**41.1%**	**1462**	**42.1%**
Germany	225		235		302	
Netherlands	445		460		470	
Others	676		680		690	
Africa	**446**	**14.4%**	**493**	**14.8%**	**507**	**14.6%**
Cote d'Ivoire	335		364		360	
Others	131		130		147	
America	**852**	**26.3%**	**853**	**25.5%**	**856**	**24.6%**
Brazil	207		209		223	
United States	410		419		426	
Others	235		225		207	
Asia & Oceania	**575**	**17.7%**	**622**	**18.6%**	**651**	**18.7%**
Indonesia	120		115		120	
Malaysia	203		250		250	
Others	252		257		281	
World total	**3238**		**3343**		**3476**	
Origin	**1188**	**36.7%**	**1254**	**37.5%**	**1279**	**36.8%**

Source: ICCO Quarterly Bulletin Cocoa Statistics. Vol. XXXII. 2005/06.

CHEMICAL STRUCTURE

12. 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).

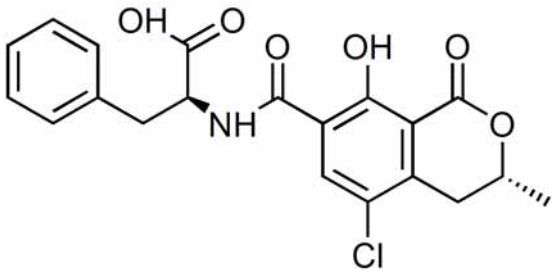

Figure 1. Chemical structure of OTA

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

14. 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

15. 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 Intestitial 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).

16. 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.

17. 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 and 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).

18. 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

19. Spanjer at 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.

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

ANALYTICAL METHODS

21. 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.

22. 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 interlaboratory 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.

23. 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 79-94% and coefficient of variation < 5%.

24. 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.

OCCURRENCE OF OTA AND OTA PRODUCING FUNGI IN COCOA BEANS

25. 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.

26. 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).

27. In an ongoing study 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 drying stage. Those species present during both processes include *A. niger, A. flavus, A. ochraceus, A. sulphureus, and R. stolonifer*. Analysis of OTA showed low levels in beans (up to 0.5 µg/kg) obtained predominantly from diseased and diseased/damaged and damaged/broken pods.

28. 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 wounded. 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, %).

29. 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 221 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%.

30. Mounjouenpou et al. (2008) assessed how filamentous fungi and toxigenesis were affected by the type of cocoa post-harvest treatment (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.

31. 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 mould 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).

32. 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 mouldy 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.

33. 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.

34. 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).

35. A screening of Nigerian ready for sale cocoa beans indicated that 91.5% 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.

36. 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.

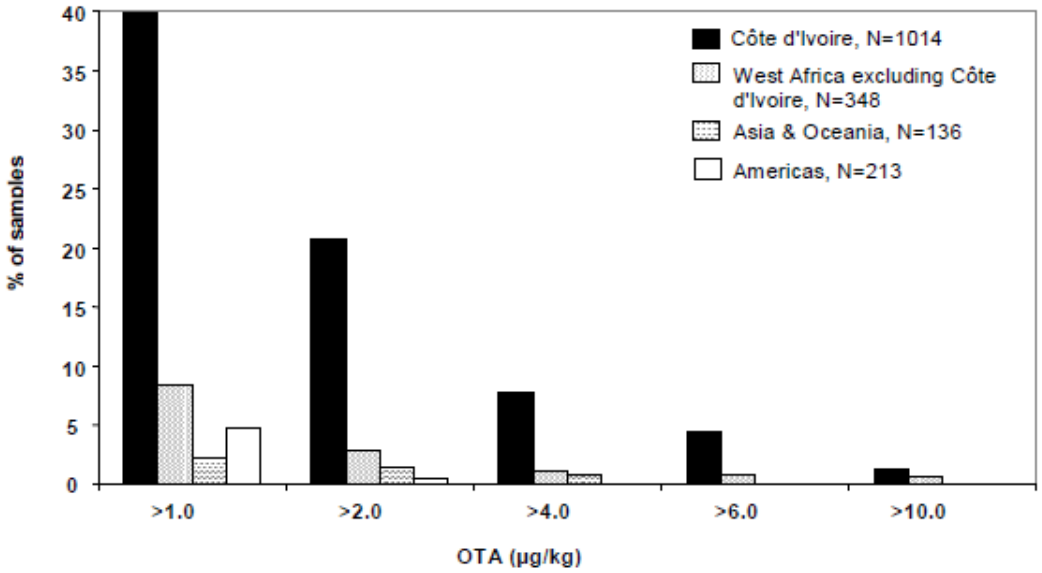

Figure 2 - OTA levels in cocoa beans imported into Europe from different regions of the world 1999-2005 (Gilmour and Lindblom, 2008).

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

37. 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).

38. 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.

39. 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%.

40. Analysis of 170 samples of cocoa products of different geographical origins indicated that highest levels of OTA were detected in cocoa shell and cocoa cake (0.1 to 23.1µg/kg) and only a minor level in the cocoa products such as cocoa cake, cocoa mass, roasted ribs, cocoa butter, cocoa powder and chocolate (Bonvehi, 2004).

41. 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).

42. Sixteen large samples of dried cocoa beans, specially stored under conditions which favoured 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 Figure 3). On the average about 70% of the OTA was removed with the shell fraction (Dembele et al. 2009).

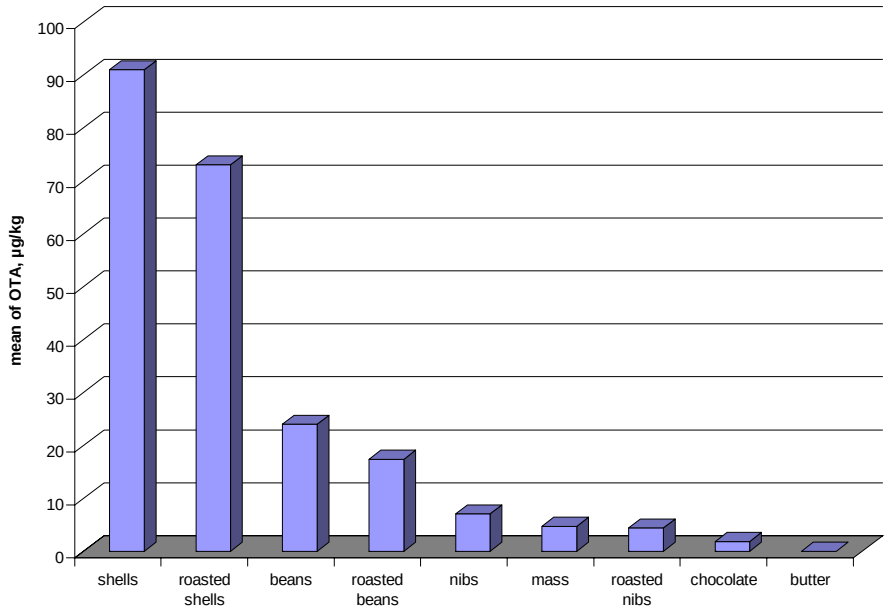

Figure 3. Mean level of OTA in different processing products of contaminated cocoa beans. Data from Cote d'Ivoire (Dembele et al., 2009).

OCCURRENCE OF OTA IN COCOA PRODUCTS

43. Ten cocoa powder and 9 chocolate samples from the open Belgium market were analyzed in 2005 (Christine 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.

44. 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).

45. 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).

46. 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.

47. 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 in Table 6.

48. Turcotte and Scott (2010) 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.

49. 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., 2010).

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. 2010
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]
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. 2010
Dark chocolate	Italy	120/92		0.74		0.20	Brera et al. 2010
Dark chocolate	Brazil	25/25	0.01	0.87		0.34	Copetti, 2009
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/	-	11/8	0.1	1.59		0.63	Bonvehi, 2004

Product	Origin	Total / positive samples*	LOQ or LOD, µg/kg	Max, µg/kg	Median, µg/kg	Mean, µg/kg	References
chocolate cream							
Easter egg	Italy	15/5		0.50		0.20	Brera, et al. 2010
Liquor	Brazil	25/5	0.01	1.09		0.34	Copetti, 2009
Milk chocolate	Brazil	25	25	0.45		0.15	Copetti, 2009
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. 2010
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
White chocolate	Brazil	25/23	0.01	0.05		0.03	Copetti, 2009
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

50.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 an 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.

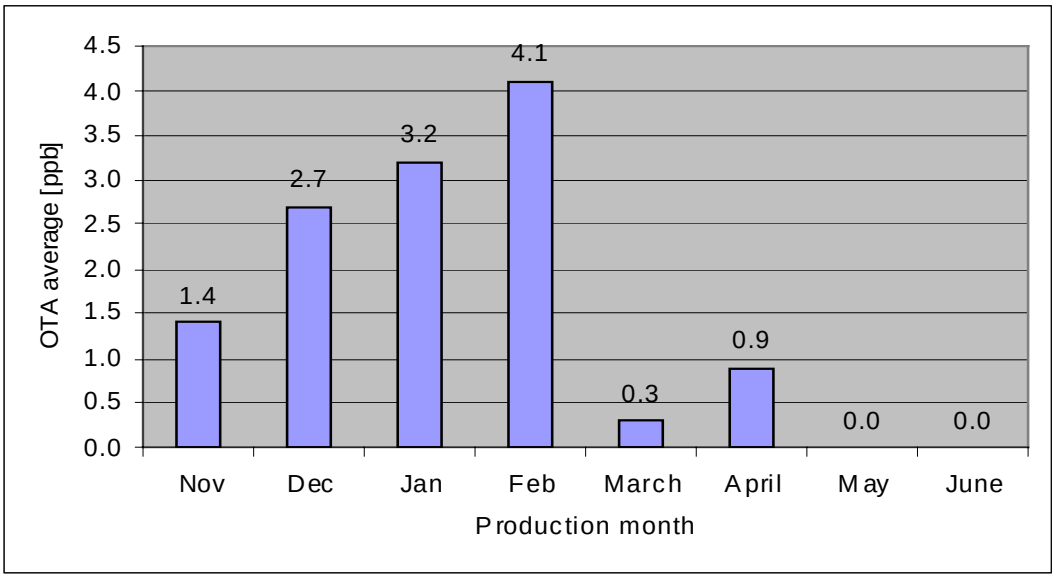

Figure 4. Variation of OTA levels as a function of month of production (Gilmour and Lindblom, 2008)

51. 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 mould 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).

52. 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 mould growth and OTA production (Gilmour and Lindblom, 2008).

53. In an ongoing OTA programme (Dembele et al., 2009) in Cote d'Ivoire, studies were conducted to determine the critical points of contamination at the farm level. 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).

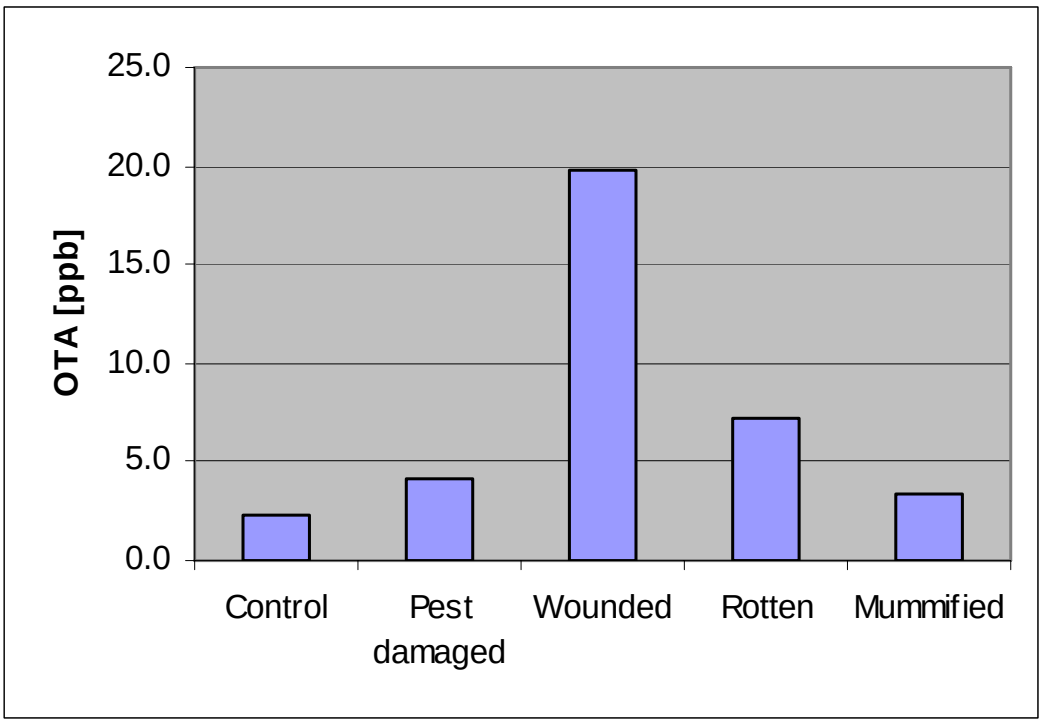

Figure 5. Effect of phytosanitary condition of the cocoa pods on levels of OTA found in the dried cocoa beans (Bastide et al, 2006)

54. 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)

55. 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

56. 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 120ng/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).

57. The SCOOP Task 3.2.2 presented data which indicated that the daily consumption of cocoa was 31g/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.

58. 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.

59. 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).

60. 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 to the main contributors to the exposure. OTA exposures resulting from cocoa and chocolate were not included in the assessment (Kuiper-Goodman et al., 2010).

61. 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., 2010). 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).

62. 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

63. In the EU, the Commission Regulation (EC) No. 1881/2006 (Commission Regulation 401/2006, 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.

64. 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)".

65. In 2003 the Italian Ministry of Health, claiming the precautionary principle, set a legal limit for cocoa powder and chocolate products considering that no sufficient exposure data was available for not considering the OTA as a risk for the population. Legal limits for OTA in cocoa (2.0 µg/kg) and chocolate products (0.5 µg/kg) were set. 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.

66. The above mentioned Regulation foresees that "the appropriateness of setting a maximum level for OTA in foodstuffs such as dried fruit other than dried vine fruit, cocoa and cocoa products, spices, meat products, green coffee, beer and liquorice, as well as a review of the existing maximum levels, in particular for OTA in dried vine fruit and grape juice, will be considered in the light of the EFSA opinion".

67. Brazil has a proposal for establishing a maximum level for OTA in cocoa beans and cocoa products at 10 µg/kg and 5µg/kg, respectively (ANVISA CP100/2009).

68. Health Canada is currently in the process of proposing maximum limits for OTA in a variety of foodstuffs, 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.

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

PREVENTION AND REDUCTION OF OTA IN COCOA

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

71. 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.

72. 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).

73. 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.

74. 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 including prevention of OTA.

75. 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). .

76. Data indicate 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).

77. Essential oils of *Aframomum danielli* has 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.

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

79. 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, 2001).

CONCLUSIONS AND RECOMMENDATIONS

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

(a) Cocoa production represents an important economic activity for all the cocoa producing countries in Africa, Asia and Latin America.

(b) Available data indicates that if cocoa beans are not properly handled in order to minimize ochratoxigenic fungi colonization, high levels of OTA could develop in cocoa products.

(c) There is insufficient data, but there are some well known critical points that could be controlled in order to minimize OTA formation.

(d) Cocoa is a minor component of the human diet and has a small contribution to total dietary intake of OTA. However most studies did not include infants and children in their assessment.

(e) A major part of OTA originally present in cocoa beans is found in the shell fraction, which is not consumed. Codex Alimentarius already provides recommendation for cocoa shell and germs in cocoa mass and cocoa cake.

Recommendations:

81. Codex member states as well as the cocoa processing industry should be encouraged to:

a) Monitor the levels of OTA in cocoa and cocoa products over a period of several years,

b) Promote studies in order to develop a sampling plan specific to OTA in cocoa and cocoa products;

c) Develop studies directed to small holder farms to generate data on levels of OTA over several years.

d) Conduct dietary intake studies of OTA in cocoa and cocoa products for infants and children.

82. Taking into account the knowledge currently available, a Code of Practice could be considered for future development. The Code of Practice could include provisions on: primary processing steps (Pre harvest, Harvest, Post harvest -transport, selection and washing, pods breaking, fermentation, drying, storage) and secondary processing steps (transport, roasting and shelling, packaging, storage). Therefore producers should be helped with relative training material or experience or with a suitable course to understand the procedures they should follow to be able to reduce OTA contamination of their products. Comprehensive training has to be developed, where it is necessary

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